Nonmedicinal interventions in nonalcoholic fatty liver disease

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Unhealthy diet and lack of physical exercise are responsible for fat accumulation in the liver, which may lead to liver disease. Histologically, the severity of the disease has two stages: nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH). NAFLD is defined by the presence of steatosis with no evidence of cellular injury such as hepatocyte ballooning. NASH is a distinct entity from NAFLD, and is characterized by the presence of inflammation with hepatocytes damage, with or without fibrosis. While several therapeutic strategies have been proposed to improve this condition, the present review aims to discuss nonmedicinal interventions used to reduce liver involvement or to prevent the disease altogether. The authors investigated dietary patterns and vitamin deficiencies associated with NAFLD, and their role in enhancing disease severity. Additionally, they reviewed the role of exercise and the use of interventions, such as as intragastric balloon and bariatric surgery, for improving disease progression. The authors propose monitoring disease progression or repair by following changes in cytoadipokine levels.

Key Words: *Adipokine; Bariatric surgery; Cytokeratine 18; Intragastric balloon placement; Nonalcoholic fatty liver disease; Nonalcoholic steatohepatitis; Noninwasive biomarkers*

Les interventions non médicales en cas de stéatose hépatique non alcoolique

Un régime malsain et le manque d'exercice physique sont responsables de l'accumulation de graisse dans le foie et peuvent favoriser une maladie hépatique. Sur le plan histologique, la gravité de la maladie se divise en deux phases : la stéatose hépatique non alcoolique (SHNA) et la stéatohépatite non alcoolique (SNA). La SHNA se définit par la présence de stéatose, sans manifestation de lésions cellulaires comme l'hypertrophie hépatocytaire. La SNA est une entité distincte de la SHNA et se caractérise par la présence d'inflammation et de lésions hépatocytaires, accompagnées ou non de fibrose. Il existe plusieurs stratégies thérapeutiques pour soigner cette maladie, mais la présente analyse porte sur les interventions non médicales utilisées pour réduire l'atteinte hépatique ou prévenir la maladie. Les auteurs ont exploré les profils diététiques et les carences en vitamines associées à la SHNA ainsi que leur rôle dans l'aggravation de la maladie. Ils ont également analysé le rôle de l'exercice et le recours à des interventions comme la chirurgie intragastrique à ballonnet et la chirurgie bariatrique pour en limiter la progression. Les auteurs proposent de surveiller la progression ou la réparation de la maladie après des modifications au taux de cytoadipokines.

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 lipidosis, 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).

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 signalling 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 perivenular 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 necroinflammation/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

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TABLE 1
Mean serum 25-hydroxyvitamin D levels reported in selected studies

Author (reference)	Study sample	Association with NAFLD
Targher et al (15)	60 patients with biopsy-proven NAFLD; 60 healthy controls	51.0±22 nmol/L in NAFLD versus 74.5±15 nmol/L in control (P<0.001)
Pirgon et al (16)	87 obese adolescents (45 NAFLD patients and 43 non-NAFLD obese controls); 30 lean controls	29.5±18.4 in obese NAFLD versus 41.0±17.9 in obese non-NAFLD versus 48.1±22.2 ng/mL in lean controls
Dasarathy et al (17)	148 NAFLD patients; 39 healthy controls	21.2±10.4 ng/mL in NAFLD versus 35.7±6.0 ng/mL in control (P=0.002)
Hao et al (18)	514 subjects with normal liver function tests and with body mass index ≥18.5 kg/m^2 to <25 kg/m^2 (76 NAFLD patients and 438 non-NAFLD controls)	13.46±4.65 ng/mL in NAFLD versus 15.65±5.89 ng/mL in non-NAFLD controls (P=0.002)
Küçükazman et al (19)	211 subjects undergoing NAFLD examination (154 NAFLD patients and 57 non-NAFLD controls)	12.3±8.9 ng/dL in NAFLD versus 20±13.6 ng/dL in non-NAFLD controls (P<0.001)

NAFLD Nonalcoholic fatty liver disease

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)D_3 levels may independently predict NAFLD (18,20). The association between lower serum 25(OH)D_3 levels and increased NAFLD incidence was maintained after controlling for age, sex, body mass index (BMI), creatinine, calcium, homeostasis model assessment-insulin resistance and the presence of the metabolic syndrome (15). Decreased serum 25(OH)D_3 levels further predicted histological severity of hepatic steatosis, hepatocyte ballooning, necroinflammation 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)D_3 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)D_3 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=0.0001). 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=0.0001) (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 1.17to 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 individual 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, nondiabetic 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
Physical activity

Study; sample population	Physical activity regimen	Outcome
Bae et al (29); 72,359 healthy Korean adults without diabetes	Physical exercise (30 min/day, 3 times/week) for 3 months (n=12,967)	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])
	No exercise (n=59,392)	
Hallsworth et al (30); 19 sedentary adults with clinically defined NAFLD	RT for 8 weeks (n=11)	RT: significant reduction in liver lipids (P<0.05), improvements in lipid oxidation, glucose control and HOMA-IR
	Continued normal treatment (n=8)	
Bhat et al (31); 42 NAFLD patients	Regular AT (30 min/day for at least 5 days/week)	AT: decreased insulin resistance, BMI, waist circumference and ALT levels (P<0.01 for all), and improved NASH scores
de Piano et al (32); 58 obese adolescents (28 with NAFLD)	Interdisciplinary weight-loss therapy for 1 year	AT: reduced body mass, BMI and fat mass in non-NAFLD patients
		AT: reduced body mass, BMI, fat mass and visceral fat in NAFLD patients
	AT alone (n=29)	AT + RT: improvement in body mass, BMI, fat mass, glycemia, total cholesterol and low-density lipoprotein-cholesterol in non-NAFLD patients
	AT + RT (n=29)	AT + RT: improvement in body mass, BMI, fat mass, glycemia, total cholesterol and low-density lipoprotein-cholesterol, and subcutaneous fat in NAFLD patients
		AT + RT: higher magnitude of changes in total cholesterol, low-density lipoprotein-cholesterol, ALT and adiponectin compared to AT alone in NAFLD
Fealy et al (33); 13 obese NAFLD patients	Treadmill walking for 60 min/day on 7 consecutive days	AT: decreased CK-18 (558.4±106.8 vs 323.4±72.5 U/L; P<0.01) and ALT levels (30.2±5.1 vs 24.3±4.8 U/L; P<0.05)
		AT: increased whole body fat oxidation (49.3±6.1 mg/min vs 69.4±7.1 mg/min; P<0.05) and circulating sFasL levels (66.5±6.0 pg/mL vs 63.0±5.7 pg/mL; P<0.06)
Grønbæk et al (34); 117 obese NAFLD children	Moderate exercise (1 h/day) with restricted energy intake for 10 weeks	Training: weight loss and improvement in ultrasonographic liver steatosis, liver fat content and insulin resistance
Sullivan et al (35); 18 obese NAFLD patients	Exercise training (0–60 min for 5 days/week) for 16 weeks (n=12)	Exercise training: decreased intrahepatic triglyceride content (10.3±4.6%; P<0.05), with no influence on body weight, percent body fat, and very low density lipoprotein triglyceride and apolipoprotein B-100 secretion rates
	No intervention (n=6)	
Al-Jiffri et al (36); 100 type 2 diabetes male patients with NAFLD	Physical training (3 times/week for 12 weeks) combined with dietary measures	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)
	Dietary measures only	Dietary measures only: no changes
Bacchi et al (37); 31 sedentary adults with NAFLD and type 2 diabetes	Physical exercise for 4 months	Both AT and RT equally effectively reduced hepatic fat content (P<0.001 vs baseline), led to disappearance of hepatic steatosis (defined as hepatic fat content >5.56%) in almost a quarter of patients, increased insulin sensitivity during euglycemic clamp, and decreased total body fat mass, visceral adipose tissue, superficial subcutaneous abdominal adipose tissue and hemoglobin A1c
	AT (n=14)	
	RT (n=17)	
Haus et al (38); 17 obese NAFLD patients	Short-term AT program (60 min/day of treadmill walking at 85% of maximal heart rate) for 7 consecutive days	AT increased the liver polyunsaturated lipid index (P<0.05), insulin sensitivity (P<0.05), high molecular weight adiponectin levels (P<0.05) and maximal oxygen consumption (P<0.05)
		AT reduced reactive oxygen species production during oral glucose tolerance test
Khaoshbaten et al (39); 90 NAFLD patients	Medical treatment after AT (30 min/day, 3 times/week for 3 months) (n=45)	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)
	Medical treatment alone (n=45)	
Malin et al (40); 13 obese NAFLD patients	AT for 7 days (60 min/day at 85% maximum heart rate)	AT: reduced insulin resistance (P<0.05) and circulating fetuin-A levels (P<0.02)
Oh et al (41); 212 obese, middle-age men (19.8% had abnormal liver function and suspicious liver fibrosis)	Exercise training program without any dietary restriction for 12 weeks (n=108)	Both regimens reduced body weight, waist circumference and visceral adipose tissue area, serum ALT and γ-GTP levels, and insulin resistance; dietary restriction program superior
		Exercise training increased adiponectin levels
	Dietary restriction program (n=104)	Exercise training reduced serum levels of inflammation and oxidative stress markers such as ferritin and thiobarbituric acid reactive substances in subjects with suspected liver fibrosis
Oh et al (42); 169 obese NAFLD patients	MVPA weight reduction for 12 weeks	The degree of hepatic steatosis decreased more significantly in the ≥250 min/week group compared to the <250 min/week groups
	<150 min/week (n=40)	
	150–250 min/week (n=42)	≥250 min/wk MVPA associated with more pronounced decreases in abdominal visceral adipose tissue, levels of ferritin and lipid peroxidation, along with a significant increase in adiponectin levels than <250 min/week MVPA
	≥250 min/week (n=87)	
Zelber-Sagi et al (43); 64 NAFLD patients without secondary liver disease	RT 3 times/week for 3 months (n=33)	RT reduced hepatorenal-ultrasound index (P=0.017), total, trunk and android fat, serum ferritin and total cholesterol levels
	Home stretching (n=31)	RT increased lean body mass
		No difference in AST, ALT and γ-GTP levels

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

tracked pediatric NAFLD population (47). Another multidisciplinary intervention consisting of personalized diet, physical exercise and behaviour therapy for three months led to a mean weight loss of 8%, improvement in liver function tests and a decrease in the liver fat content in 12 NAFLD patients included in an open-label nonrandomized study (48). Weight loss >10% was an additional predictor of disease remission in a sample of NAFLD patients undergoing an intervention program designed to increase energy expenditure and reduce caloric intake using lifestyle behavioural changes (49).

A recent study assessed the degree of weight loss necessary to improve the markers of hepatic function and insulin resistance in 100 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 ($P<0.01$), triglycerides ($P<0.05$), fasting plasma glucose ($P<0.01$), 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 adiponenctin, 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 NAFLD 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 nonsteatotic 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 ($F\geq2$), 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

Study and sample population	Changes in adipokines	Associations and diagnostic performance
de Piano et al (32) 58 postpubertal obese adolescents randomly assigned to AT or AT + RT AT group: 15 patients without NAFLD and 14 with NAFLD AT + RT group: 15 patients without NAFLD and 14 with NAFLD	Change in adiponectin at 1 year: −0.13±1.73 in AT without NAFLD, −0.13±2.78 in AT with NAFLD, 2.57±2.50 in AT + RT without NAFLD (P<0.05 vs AT), 2.69±2.54 µg/mL in AT + RT with NAFLD (P<0.05 vs AT) Change in leptin at 1 year: −7.13±12.68 in AT without NAFLD, −12.17±17.22 in AT with NAFLD, −9.19±13.61 in AT + RT without NAFLD (P<0.05 vs AT), −11.95±13.08 µg/mL in AT + RT with NAFLD Change in adiponectin/leptin at 1 year: 0.72±1.56 in AT without NAFLD, 1.02±1.19 in AT with NAFLD, 0.68±1.30 in AT + RT without NAFLD, 0.40±1.85 in AT + RT with NAFLD	n/a
Boyraz et al (57) Group I: 63 obese children with liver steatosis Group II: 12 obese children with elevated serum ALT activity from group I Group III: 85 obese children without liver steatosis	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<0.001 vs group I and P<0.001 vs group II) Mean resistin: 8.5±3.2 in group I, 8.5±3.2 in group II and 15.0±3.9 ng/mL in group III (P<0.001 vs group I and P<0.001 vs group II) Mean RBP4: 33.2±7.5 in group I, 35.4±3.3 in group II and 35.4±3.3 µg/mL in group III (P<0.001 vs group I and P<0.001 vs group II) 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)	Adiponectin: sensitivity of 84.21% and specificity of 63.64% for advanced liver steatosis at cut-off 2.56 µg/mL Resistin: sensitivity of 36.8% and specificity of 95.5% for advanced liver steatosis at cut-off 5.2 ng/mL RBP4: sensitivity of 84.20% and a specificity of 68.20% for advanced liver steatosis at cut-off 35 µg/mL Adiponectin: sensitivity of 100% and specificity of 83.53% to differentiate hepatopathic obese children at cut-off 3.2 µg/mL Resistin: sensitivity of 100% and specificity of 77.65% to differentiate hepatopathic obese children at cut-off 12.0 ng/mL RBP4: sensitivity of 100% and a specificity of 92.94% to differentiate hepatopathic obese children at cut-off 26 µg/mL
Koot et al (58) 119 severely obese children (47% prevalence of steatosis)	Mean adiponectin: 7.8±2.8 µg/mL at baseline Mean leptin: 33.9±6 32.4 at baseline	Adiponectin not associated with steatosis in univariate analysis (OR 0.96 [95% CI 0.84–1.09]; P=0.52) Leptin associated with steatosis in multivariate analysis (OR 1.04 [95% CI 1.01–1.09]; P=0.03)
Pacifico et al (59) 44 obese children with NAFLD 44 obese children without NAFLD	Leptin: mean 19.5 in NAFLD vs 20.8 µg/L in non-NAFLD Adiponectin: mean 9.0 in NAFLD vs 12.9 µg/L in non-NAFLD (P<0.05)	
Klein et al (60) 106 middle school students of varying body mass index	Mean adiponectin: 12.2±4.9 µg/mL Mean adiponectin: lower in males 10±4.2 vs females 12.8±4.1 µg/mL (P<0.05)	
Sanches et al (61) 79 obese adolescents (33 with NAFLD and 46 without NAFLD) Interdisciplinary (clinical, nutritional and psychological) therapy, exercise (60 min/day for 3 days/week) and physiotherapy for 1 year	Change in adiponectin at 1 year: 1.61±3.09 in non-NAFLD and 1.66±2.09 µg/mL in NAFLD Change in leptin at 1 year: mean −15.06 (P<0.05 vs baseline) in non-NAFLD and mean −16.17 µg/mL in NAFLD (P<0.05 vs baseline) Change in leptin/adiponectin ratio at 1 year: mean −3.36 (P<0.05 vs baseline) in non-NAFLD and mean −3.49 in NAFLD (P<0.05 vs baseline) Change in PAI-1 at 1 year: −3.58±5.81 (P<0.05 vs baseline) in non-NAFLD and −3.85±6.50 ng/mL in NAFLD (P<0.05 vs baseline)	

AT Aerobic training; ALT Alanine aminotransferase; n/a Not applicable; NAFLD Nonalcoholic fatty liver disease; PAI-1 Plasminogen activator inhibitor-1; RBP4 Retinol-binding protein-4; RT Resistance training; vs Versus

(divided into morbidly and nonmorbidly obese based on a BMI cut-off of 40 kg/m^2) (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 ≥40 kg/m^2). 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/m^2), with a slight rise approaching month 12 (mean 17.5 ng/mL) (77). Leptin significantly decreased during the first month in another small sample of obese patients undergoing intragastric balloon placement (median 67.1 ng/dL at baseline, 53.7 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 in the balloon group (P=0.05 at month 6 and P=0.04 at month 10 versus baseline) and 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).

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

TABLE 4
Intragastric balloon placement

Study and sample population	Association with nonalcohoilic fatty liver disease
Lee et al (70) 18 obese NASH patients Bioenterics* intragastric balloon + exercise + diet for 6 months (n=8) Sham balloon (500 mL of saline solution) + exercise + diet (n=10)	Balloon: reductions in mean body mass index (1.52 kg/m^2 vs 0.8 kg/m^2; P=0.0008) and median NAFLD activity scores (2 vs 4; P=0.0.03), with a trend toward improved steatosis scores (P=0.075) compared with sham No differences with respect to lobular inflammation, hepatocellular ballooning or fibrosis scores No significant changes in AST or ALT levels
Tai et al (71) 28 obese patients Bioenterics* intragastric balloon placement for 6 monhts	Improvement in body mass index (mean 32.4±3.7 kg/m^2 vs 28.5±3.7 kg/m^2; P<0.01), waist circumference (mean 101.9±8.9 cm vs 90.6±9.3 cm; P<0.01), glucose (median 93.5 mg/dL vs 91.0 mg/dL; P<0.01), AST level (median 33.0 IU/L vs 23.0 IU/L; P<0.01), ALT level (median 49.0 IU/L vs 22.0 IU/L; P<0.01), triglyceride (median 149.0 mg/dL vs 88.5 mg/dL; P<0.01), cholesterol (median 200.0 vs 186 mg/dL; P=0.13), high-density lipoprotein-cholesterol (median 45.0 vs 52.0 mg/dL; P=0.01), low-density lipoprotein-cholesterol (median 119.5 vs 114.0 mg/dL; P=0.03), the metabolic syndrome (64.3% vs 32.1%; P=0.01) at 6 months compared with baseline
Folini et al (72) 40 obese subjects Intragastric balloon or gastric banding for 6 months (n=24) Diet for 6 months (n=16)	Intragastric balloon or gastric banding: lower ALT (P=0.02), AST (P=0.03), total cholesterol (P=0.007), low-density lipoprotein-cholesterol (P=0.03) and fat-free mass (P=0.01) at the end of the study compared with diet
Nobili et al (73) 9 obese children Obalon† balloon for 3 months	Significant improvements in ALT, total and low-density lipoprotein cholesterol, insulin, HOMA-IR and uric acid levels No significant changes in ultrasonographic examination

**Inamed Health, USA; †Obalon Therapeutics, USA. ALT Alanine aminotransferase; AST Aspartate aminotransferase; HOMA-IR Homeostasis model assessment-insulin resistance; NAFLD Nonalcoholic fatty liver disease; NASH Nonalcoholic steatohepatitis; vs Versus*

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/m^2), 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 through the first six months of the study among morbidly obese individuals (BMI ≥40 kg/m^2), with a drop toward month 12 (mean 742.6 pg/mL versus mean 948.3 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 sham or balloon, 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 725.9±152.2 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

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 postbariatric 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, bariatric surgery led to decreased γ-GTP levels at follow-up, but had no effects on ALT and AST levels (88). Elsewhere, near normalization of ALT was observed in a sample of morbidly obese patients undergoing bariatric surgery, while mean serum γ-GTP levels decreased to levels below those of controls. No significant differences were observed in AST levels between controls and NAFLD patients, and between levels before and after bariatric surgery within the NAFLD subsample (91,92).

Roux-en-Y gastric bypass was associated with improved NAFLD parameters, and these effects were maintained for at least five years. Decreased AST levels following Roux-en-Y gastric bypass were observed in association with a decrease in NAFLD fibrosis score (1.142±1.261 at baseline versus 0.066±1.027 at 12 months; P=0.0394) in a large sample of 1236 obese patients (98). Bariatric surgery produced significant histopathological improvements in terms of steatosis (P<0.001), ballooning degeneration (P<0.001), Mallory-Denk hyaline bodies (P=0.005), glycogen nuclei (P=0.001), lobular inflammation (P<0.001), portal inflammation (P=0.005) and fibrosis (P<0.001) (88).

Adipokines

In general, serum adiponectin levels are lower (2.2±1.7 μg/mL in patients versus 4.1±2.5 μg/mL in control; P<0.001) and serum leptin levels are elevated (28.1±18.4 ng/mL in patients versus 4.7±3.9 ng/mL in control; P<0.001) in obese patients compared with nonobese controls (100). Bariatric surgery increases adiponectin levels and decreases leptin levels compared with presurgery levels. For example, mean serum leptin level was elevated, while mean serum adiponectin level was lower in a small sample of obese patients undergoing laparoscopic sleeve gastrectomy compared with nonobese controls. Leptin levels decreased, while adiponectin levels increased within one month following this intervention. These changes were maintained six months after the procedure (100). Roux-en-Y gastric bypass led to decreasing leptin levels in a small sample of morbidly obese patients (91). Adiponectin levels increased and leptin levels decreased in a time-dependent manner through month 12 in a sample of obese patients undergoing laparoscopic adjustable gastric banding (93). In another study, normalization of both adiponectin and leptin levels to values similar to those observed in controls were observed in morbidly obese patients following bariatric surgery (92).

Decreased adiponectin levels at baseline were observed in other samples of obese patients compared with nonobese controls. Following bariatric surgery, adiponectin levels increased in a time-dependent manner (90,94,96,97,101). Interestingly, no further increases were observed beyond four weeks in a sample of 108 morbidly obese NAFLD patients undergoing bariatric surgery. However, adiponectin levels continued to increase through six months in NASH patients (102).

Bechmann et al (64) reported that obesity is negatively correlated with adiponectin levels, while adiponectin levels are inversely correlated with the NAFLD activity score. Low adiponectin levels (<13.5 mg/L in one study and <23 mg/L in another study) were a strong predictor of NAFLD severity elsewhere (95,103). Adiponectin levels were lower in NASH compared with simple steatosis. Adiponectin levels further decreased progressively with increasing steatosis severity and with more severe lobular inflammatory activity (95). Lower adiponectin levels were also found in patients with fibrosis compared with those without fibrosis (101).

Leptin levels increased in parallel with increasing steatosis severity and with the severity of lobular inflammatory activity. A trend for higher leptin levels in patients with more advanced fibrosis was also noted (95). Leptin levels did not correlate with fibrosis in another study (101).

Other adipokines measured in NAFLD patients include ghrelin, visfatin and resistin. Ghrelin levels were correlated with lobular inflammation but not with steatosis severity, and were an independent predictor of NASH (95,104). Ghrelin levels decreased significantly in a sample of obese patients who underwent laparoscopic sleeve gastrectomy and dieatry changes (127.5±96.9 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. Similarly, hepatic IL-6 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).

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 did not change (106).

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

Study; sample population	Changes following bariatric surgery
Vargas et al (88);	Mean (± SD) serum AST: 0.35±0.09 µkat/L during presurgery vs 0.36±0.17 µkat/L after surgery (P=0.862)
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)	Mean serum ALT: 0.49±0.20 µkat/L during presurgery vs 0.37±0.31 µkat/L after surgery (P=0.143)
	Mean serum γ-GTP: 40.2±17.4 during presurgery vs 19.2±12.8 IU/L after surgery (P<0.001)
Cazzo et al (89);	Mean (± SD) serum ALT: 30.7±17.1 mg/dL at baseline vs 20.7±7.2 mg/dL at month 12 (P<0.0001)
63 obese subjects undergoing Roux-en-Y gastric bypass surgery	Mean serum AST: 25.7±10.6 mg/dL at baseline vs 21.2±5.4 mg/dL at month 12 (P=0.0005)
Carazo et al (90);	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)
60 morbidly obese patients undergoing bariatric surgery	Mean plasma ALT: 31.0±3.1 IU/L before surgery vs 19.3±1.5 IU/L at month 12 (P=0.001)
	Mean plasma γ-GTP: 37.7±8.0 IU/L before surgery vs 17.5±2.1 IU/L at month 12 (P<0.001)
	Mean plasma adiponectin: 39.9±6.0 µg/mL before surgery vs 60.0±2.1 µg/mL at month 12 (P=0.002)
Tai et al (91);	Median serum AST: 27.0 IU/L before surgery vs 27.0 IU/L at month 12 (P=0.66)
21 morbidly obese patients undergoing Roux-en-Y gastric bypass	Median serum ALT: 34.0 IU/L before surgery vs 24.0 IU/L at month 12 (P<0.01)
	Median serum γ-GTP: 28.0 IU/L before surgery vs 12.0 IU/L at month 12 (P<0.01)
	Median serum leptin: 29.0 µg/L before surgery vs 4.1 µg/L at month 12 (P<0.01)
Felipo et al (92);	Mean (± SD) serum AST: 20±4.0 IU/L in controls, 23.5±15 IU/L in simple steatosis patients before surgery, 23±95 IU/L in simple steatosis patients after surgery, 24.5±16 IU/L in NASH patients before surgery and 20.5±6 IU/L in NASH patients after surgery
47 mordibly obese patients undergoing bariatric surgery (evaluated before and 18±5 months after surgery)	Mean serum ALT: 17±6.0 IU/L in controls, 31±17 IU/L in simple steatosis patients before surgery (P<0.05 vs control) , 23±17 IU/L in simple steatosis patients after surgery, 37±16 IU/L in NASH patients before surgery (P<0.05 vs control) and 20±12 IU/L in NASH patients after surgery
45 controls	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<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<0.05 vs before surgery)
	Mean serum adiponectin: 11±4 i µg/mL n controls, 7±2 µg/mL in simple steatosis patients before surgery (P<0.05 vs control), 16±10 µg/mL in simple steatosis patients after surgery (P<0.001 vs before surgery), 6±2 µg/mL in NASH patients before surgery (P<0.05 vs control) and 12±7 µg/mL in NASH patients after surgery (P=0.008 vs before surgery)
	Mean serum leptin: 13±9 ng/mL in controls, 69±25 ng/mL in simple steatosis patients before surgery (P<0.001 vs control), 20±18 ng/mL in simple steatosis patients after surgery (P<0.001 vs before surgery), 54±22 ng/mL in NASH patients before surgery (P<0.001 vs control) and 24±11 ng/mL in NASH patients after surgery (P<0.001 vs before surgery)
Moschen et al (93);	Mean (± SD) serum AST: 30.1±2.6 IU/L at baseline, 26.8±2.4 IU/L at month 6 (P<0.05) and 23.9±1.2 IU/L at month 12 (P<0.05)
30 severely obese patients undergoing laparoscopic adjustable gastric banding	Mean serum ALT: 35.1±5.6 IU/L at baseline, 25.4±3.5 IU/L at month 6 (P<0.05) and 20.8±1.6 IU/L at month 12 (P<0.01)
	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 21.8±3.2 IU/L at month 12 (P<0.001)
	Mean serum adiponectin: 7.46±0.67 ng/mL at baseline, 8.65±0.84 ng/mL at month 6 (P<0.05) and 8.95±0.81 ng/mL at month 12 (P<0.01)
	Mean serum leptin: 27.4±1.38 µg/mL at baseline, 18.3±1.58 µg/mL at month 6 (P<0.01) and 15.15±1.50 µg/mL at month 12 (P<0.001)
	Mean serum resistin: 4.26±0.21 pg/mL at baseline, 5.18±0.26 pg/mL at month 6 (P<0.05) and 3.37±0.19 pg/mL at month 12 (P<0.01)
	Mean serum CRP: 0.86±0.08 at baseline, 0.63±0.06 at month 6 (P<0.01) and 0.42±0.05 mg/dL at month 12 (P<0.001)
	Mean serum TNF-α: 2.36±0.08 pg/mL at baseline, 1.77±0.06 pg/mL at month 6 (P<0.05) and 0.8±0.03 pg/mL at 12 (P<0.01)
Hosseinzadeh-Attar et al (94);	Mean (± SD) serum adiponectin: 36.5±11 ng/mL at baseline vs 41.3±11 ng/mL at week 6 (P<0.01)
35 severely obese patients undergoing gastric bypass	Mean serum visfatin: 5±3.5 ng/mL at baseline vs 3.4±3.2 ng/mL at week 6 (P<0.01)
Machado et al (95);	Mean (± SD) serum adiponectin: 22.9±9.8 ng/mL overall, 16.2±6.6 ng/mL in NASH and 24.1±9.9 ng/mL in no NASH (P=0.014 vs NASH)
82 morbidly obese individuals with biopsy-proven NAFLD (13.4% had NASH) undergoing bariatric surgery	Mean serum leptin: 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=0.291 vs NASH)
	Mean serum ghrelin: 19.9±11.5 pg/mL overall, 17.9±7.0 pg/mL in NASH and 20.2±12.3 pg/mL in no NASH (P=0.544 vs NASH)
Vilarrasa et al (96);	Median serum adiponectin: 11.5 µg/mL at baseline vs 23.4 µg/mL at month 12 (P<0.01)
65 morbidly obese subjects undergoing gastric bypass	Median serum IL-18: 229.8 pg/mL at baseline vs 168.9 pg/mL at month 12 (P<0.01)
	Median serum sTNFR1: 2.23 ng/mL at baseline vs 2.27 ng/mL at month 12
	Median serum sTNFR2: 5.07 pg/mL at baseline vs 4.32 pg/mL at month 12 (P<0.01)
	Median serum CRP: 7.9 mg/L at baseline vs 0.91 mg/L at month 12 (P<0.01)
Illán-Gómez et al (97);	Mean (± SD) blood adiponectin: 5.82±2.93 µg/mL at baseline vs 7.64±3.74 µg/mL at month 3 (P<0.001)
60 morbidly obese women undergoing gastric bypass	Mean blood IL-6: 3.84±1.67 pg/mL at baseline vs 3.36±1.53 pg/mL at month 3 (P<0.01)
30 lean controls	Mean blood hsCRP: 26.19±23.17 g/L at baseline vs 13.15±17.95 g/L at month 3 (P<0.001)

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 intrahepatocellular 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 NAFL-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 defined 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. Aditionally, 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 ouotcomes. Finally, we recommend continued collaboration between clinicians and laboratory, which will benefit patients.

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