

Clinical Advances in Pediatric Nonalcoholic Fatty Liver Disease

Jeffrey B. Schwimmer¹⁻³

The importance of nonalcoholic steatohepatitis (NASH) was first recognized formally at a National Institutes of Health Consensus Symposium in December 1998.⁽¹⁾ Since then, there has been a rapid growth of knowledge regarding nonalcoholic fatty liver disease (NAFLD) in children. The greatest advances have been made in the realms of awareness, diagnosis, and understanding of associated comorbidities. Challenges that lie ahead are centered on clinical management—the ongoing search for valid noninvasive diagnostic tools and effective therapy. This review addresses key clinical questions for which progress has been made.

Liver Chemistry in Pediatric NAFLD

WHAT IS THE UPPER OF LIMIT OF NORMAL FOR ALANINE AMINOTRANSFERASE IN CHILDREN?

Serum alanine aminotransferase (ALT) is the laboratory assay most used in the evaluation of NAFLD. Unfortunately, there are extreme differences in the cutoffs used by clinical laboratories for the upper limit of normal (ULN) for ALT in children. In the United States, the Screening

ALT for Elevation in Today's Youth (SAFETY) study used data from the National Health and Nutrition Examination Survey to determine biologically based thresholds for the ULN of ALT.⁽²⁾ The 95th percentile for ALT in children who had normal body mass index (BMI), glucose, and lipids, and the absence of infection or use of hepatotoxic medications was 26 U/L for boys and 22 U/L for girls. Park et al.⁽³⁾ used data from participants in the Korea National Health and Nutrition Examination Survey who were at low risk for liver disease to determine the ULN for ALT in Korean adolescents. The 97.5th percentile for ALT was 33 U/L for boys and 25 U/L for girls. The Canadian Laboratory Initiative in Pediatric Reference Intervals (CALIPER) determined that the normal range for liver chemistry varied not only by sex, but also by age.⁽⁴⁾ For children age 1-12 years, the ULN for ALT was 25 U/L for both boys and girls. For children age 13-18 years, the ULN for ALT was 24 U/L for boys and 22 U/L for girls. In aggregate, these studies make it clear that the ULN for ALT in children is approximately 25 U/L with small variations related to age and sex.

HOW WELL DOES ALT PERFORM IN THE DETECTION OF NAFLD IN CHILDREN?

In the SAFETY study, using the biology-based ALT cut points for detection of hepatic steatosis

Abbreviations: AASLD, American Association for the Study of Liver Diseases; ALT, alanine aminotransferase; BMI, body mass index; CALIPER, Canadian Laboratory Initiative in Pediatric Reference Intervals; MRI, magnetic resonance imaging; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; OSA, obstructive sleep apnea; PDFF, proton density fat fraction; QOL, quality of life; SAFETY, Screening ALT for Elevation in Today's Youth; SCALE, Study of Child and Adolescent Liver Epidemiology; SFF, signal fat fraction; ULN, upper limit of normal.

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determined by magnetic resonance imaging (MRI) yielded a sensitivity of 80% in boys and 92% in girls, and a specificity of 79% in boys and 85% in girls.⁽²⁾ The diagnostic accuracy of ALT was also tested in children with suspected NAFLD identified in primary care by screening according to pediatric guidelines.⁽²⁾ For the diagnosis of NAFLD in overweight and obese children age ≥ 10 , screening ALT of ≥ 80 U/L had a sensitivity of 57% and a specificity of 71%. As a comparison, using two times the biology-based ALT thresholds (ALT ≥ 50 for boys and ≥ 44 for girls) had a sensitivity of 88% and a specificity of 26%. Finally, Molleston and colleagues analyzed 483 children with NAFLD enrolled in the NASH CRN, of whom 3.5% (17/483) had normal ALT (boys, <26 U/L; girls, <23 U/L) and 15.3% (74/483) had mildly elevated ALT (boys, 26-50 U/L; girls, 23-44 U/L).⁽⁵⁾

Liver Imaging in Pediatric NAFLD

IS THERE A ROLE FOR ULTRASOUND IN THE MANAGEMENT OF NAFLD IN CHILDREN?

The presence of lipid droplets within hepatocytes can cause scatter and attenuation of ultrasound waves. Changes due to scattering and attenuation are used to infer hepatic steatosis, but currently available ultrasound-based techniques do not permit a direct quantifiable measurement of liver fat content. In aggregate, there are 492 children who have had a documented estimate of liver steatosis by ultrasonography compared with an estimate of grading of steatosis by liver histology or liver fat fraction by MRI.⁽⁶⁾ The overall positive predictive value of ultrasound in the detec-

tion of hepatic steatosis was 47%-62%. In the one study with available data, the accuracy for grading the severity of steatosis was 32%.⁽⁷⁾ Thus, an evidence-based assessment concluded that ultrasound does not meet the standard clinical threshold required to be used to diagnose fatty liver, grade hepatic steatosis, monitor disease, or be used as an outcome measure in clinical trials.⁽⁶⁾ However, it should also be noted that there are promising ultrasound-based technologies for the assessment of hepatic steatosis and fibrosis that are being developed.⁽⁸⁻¹⁰⁾ It will be important that these be properly evaluated in children with NAFLD.

IS MRI READY FOR USE IN THE MANAGEMENT OF NAFLD IN CHILDREN?

MR methods are used increasingly to measure liver fat. Most studies have measured the signal fat fraction (SFF), the proportion of the MR signal that comes from fat. Because the fat signal is influenced by multiple factors, the SFF may not reliably reflect actual fat content. A newer method, proton density fat fraction (PDFF), is the SFF corrected for major confounding effects and thus more closely represents the actual fat content.⁽¹¹⁾ The MRI Rosetta Stone Project showed that liver PDFF is strongly correlated with histologic steatosis grade.⁽¹²⁾ This correlation was influenced by both sex and fibrosis stage. Moreover, there was not a singular cutoff for PDFF that separated children with and without NAFLD sufficient for diagnostic use. Therefore, at this time MRI is well suited for use in clinical research, but further studies are needed prior to its use in patient care for children with NAFLD.

ARTICLE INFORMATION:

From the ¹Division of Gastroenterology, Hepatology, and Nutrition, Department of Pediatrics, University of California, San Diego School of Medicine, La Jolla, CA; ²Department of Gastroenterology, Rady Children's Hospital San Diego, San Diego, CA; ³Liver Imaging Group, Department of Radiology, University of California, San Diego School of Medicine, San Diego, CA.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Jeffrey B. Schwimmer, M.D.
Division of Gastroenterology, Hepatology, and Nutrition
Department of Pediatrics
University of California, San Diego

3020 Children's Way, MC 5030
San Diego, CA 92123
E-mail: jschwimmer@ucsd.edu
fax: 858-560-6798

Liver Histology in Pediatric NAFLD

HOW SAFE IS LIVER BIOPSY AS A TEST FOR NAFLD IN CHILDREN?

The American Association for the Study of Liver Diseases (AASLD) position paper on liver biopsy estimates that the risk of clinically significant bleeding following liver biopsy is between 1/2500 and 1/10,000.⁽¹³⁾ There are over 2000 children with biopsy-proven NAFLD reported in the literature, and there have been no reports of any serious adverse events related to the liver biopsy procedure. However, most publications did not specifically detail adverse events. In a retrospective study, Harwood et al.⁽¹⁴⁾ demonstrated that liver biopsy in obese children was not associated with a rate of complications that was higher than that for nonobese children. In a prospective study, 255 children had an outpatient percutaneous liver biopsy for suspected NAFLD.⁽¹⁵⁾ There were no serious adverse events. In the immediate recovery period, 3% of children experienced mild pain or nausea. Once at home, an additional 3% of children noted minor self-limited discomfort.

WHAT IS THE ROLE OF LIVER BIOPSY AS A DIAGNOSTIC TEST FOR NAFLD IN CHILDREN?

The AASLD practice guideline on NAFLD states that “liver biopsy in children with suspected NAFLD should be performed in those where the diagnosis is unclear, where there is possibility of multiple diagnoses, or before starting therapy with potentially hepatotoxic medications. A liver biopsy to establish a diagnosis of NASH should be obtained prior to starting children on pharmacologic therapy for NASH.”⁽¹⁶⁾ The guideline does not address what makes the diagnosis clear in the absence of liver biopsy; certainly, elevated ALT alone does not. Algorithms such as the Fatty Liver Index have proven invalid in children.^(15,17) Liver ultrasound does not reliably determine hepatic steatosis in children. There is not a specific threshold for MRI fat fraction that accurately separates between all children with and without NAFLD. The value of liver biopsy in NAFLD evaluation was recently demonstrated in a prospective study of 347 overweight or obese children identified as having suspected NAFLD by way of screening in primary care and referral to a

pediatric gastroenterologist.⁽¹⁵⁾ The combination of histology, clinical, and laboratory features yielded a diagnosis of NAFLD in 55% (193/347) of those children. However, a total of 17 different liver diseases other than NAFLD were present in 24% of those who underwent liver biopsy. Finally, there are nearly 100 medications that can be considered as potentially hepatotoxic.⁽²⁾ These medications are most commonly used in dermatology, infectious disease, neurology, oncology, and rheumatology. The clinical challenge is to determine who needs how much of a workup. The greater potential for hepatotoxicity and the more advanced the disease is believed to be, the greater the need to be certain of the diagnosis and to properly grade and stage the disease.

Although there is great passion in the debate over the role of liver biopsy in the diagnosis of NAFLD and NASH, no other diagnostic modality has shown sufficient accuracy to be appropriate for clinical use in the place of biopsy. One can choose not to perform a biopsy—but if so, rather than declaring a diagnosis of NAFLD, a child should be considered to have suspected NAFLD. There will be some children who are misclassified if a diagnostic certainty is proclaimed. We have observed this leading to a delay in diagnosis of important alternative diagnoses, such as autoimmune hepatitis. The benefits of liver biopsy will continue to need to be balanced against the potential risks on a case-by-case basis.

HOW IS LIVER HISTOLOGY EVALUATED IN CHILDREN WITH NAFLD?

Histology is the most sensitive means of obtaining pertinent, comprehensive information to identify steatohepatitis and stage liver fibrosis, which have important prognostic implications. Moreover, histology provides complex and integrated information at a cellular level and can therefore refine a diagnosis. NAFLD is a heterogeneous disorder with at least three patterns in children: those with steatosis and relatively mild features overall, those with portal features, and those with typical adult features of NASH.⁽¹⁸⁾ In contrast to the zone 3 predominance of lesions in adults, children are more likely to have zone 1 steatosis, inflammation, and fibrosis and greater severity of steatosis. It is unclear whether these patterns represent subphenotypic variations or are different disease processes altogether.

A constellation of histopathologic lesions are required for the diagnosis of steatohepatitis. Because of differences in the pattern of liver injury between children and adults with NAFLD, the diagnosis of steatohepatitis remains somewhat controversial in children. The determination of steatohepatitis is best made by an experienced pathologist reviewing the histology in aggregate. Scoring systems have been developed for clinical trials, but not for the diagnosis of NASH. There is a need for a consensus as to what constitutes steatohepatitis in children as determined by knowing which features are most associated with the risk for progression of disease, development of cirrhosis, and liver-related morbidity and mortality.

Associated Conditions

WHAT IS THE RELATIONSHIP BETWEEN NAFLD AND OBESITY?

Obesity is a consistent risk factor for NAFLD in children. However, NAFLD and obesity are not interchangeable. Most obese children do not have NAFLD. In the Study of Child and Adolescent Liver Epidemiology (SCALE), only one-third of obese children had NAFLD.⁽¹⁹⁾ Even many severely obese adolescents do not have NAFLD. In the Teen LABS study of 242 severely obese adolescents (mean BMI, 50.5 kg/m²) undergoing weight loss surgery, NAFLD was suspected in 37%.⁽²⁰⁾ NAFLD was then diagnosed in 87 of the 148 patients that had a liver biopsy at the time of bariatric surgery, representing 59% of those biopsied and 36% of those who had weight loss surgery.⁽²¹⁾ When assessed from the other vantage point, most children with NAFLD are obese. In SCALE, 60% of children with NAFLD were obese. In reports of children diagnosed with NAFLD in pediatric hepatology centers, approximately 70%-90% were obese. However, the severity of obesity differs by region and center. In reports from Europe and Asia, the mean BMI of children with NAFLD was 25-27 kg/m² compared with 30-32 kg/m² in reports from North America.⁽²²⁾ Thus, there may be differences in the risk for NAFLD or the severity of NAFLD among children around the globe. For example, the rate of advanced fibrosis among children with NAFLD ranged from 5% to 10% in Europe, 8% 10% in Asia, and 8% 20% in the United States.^(5,15,23-29) However, in the Teen LABS study, advanced fibrosis was observed in only 1% of adolescents with NAFLD.⁽²¹⁾

IS NAFLD ASSOCIATED WITH CARDIOVASCULAR DISEASE IN CHILDREN?

NAFLD is also associated with numerous serious health conditions over and above the expected rates attributable to obesity. Adults with NAFLD have increased rates of coronary heart disease, congestive heart failure, atrial fibrillation, and cardiovascular mortality.^(30,31) These associations are independent of traditional risk factors and have led many to speculate that NAFLD is a contributor to cardiovascular pathology via a combination of hepatic insulin resistance, atherogenic dyslipidemia, and liver derived inflammation.

The association between NAFLD and cardiovascular disease is also present in childhood. In a case-control study of 300 overweight children, we established that NAFLD was associated with metabolic syndrome independent of BMI.⁽³²⁾ Children with NAFLD had higher rates of abnormal glucose, insulin, triglycerides, and high-density lipoprotein cholesterol than overweight children matched for age, sex, and BMI. Overall, dyslipidemia is present in approximately half of children with NAFLD. Moreover, hepatic steatosis has been linked to higher rates of atherogenic small dense low-density lipoprotein particles that promote endothelial dysfunction and a hypercoagulable state.⁽³³⁾

Pediatric NAFLD is also associated with hypertension. In a study of 494 children with NAFLD, the estimated prevalence of high blood pressure was 36%.⁽³⁴⁾ Children with high blood pressure had more severe steatosis than children with NAFLD who did not have high blood pressure. Over 1 year of follow-up, girls with NAFLD had two-fold greater odds of having persistent high blood pressure than boys with NAFLD.

Hepatic steatosis in children, independent of degree of obesity, is associated with significant overall cardiac dysfunction. Sert et al.⁽³⁵⁾ reported that obese adolescents with abnormal liver echotexture and ALT >40 U/L had greater left ventricular mass and impaired diastolic function compared with obese adolescents with normal liver echotexture and ALT <40 U/L. Similarly, in a study of 400 obese children, Alp et al.⁽³⁶⁾ demonstrated systolic and diastolic impairment in children with abnormal liver echotexture compared with children with normal liver echotexture. Singh et al.⁽³⁷⁾ reported that obese adolescents with elevated hepatic SFF had decreased left ventricular global longitudinal

strain (systolic dysfunction) and decreased early diastolic longitudinal strain (diastolic dysfunction) compared with obese adolescents with normal hepatic SFF. Finally, Pacifico et al.⁽³⁸⁾ showed that obese children with NASH had more severe left ventricular systolic and diastolic dysfunction compared with obese children with NAFLD but not NASH or obese children without NAFLD. Thus, in children, there is a greater severity of cardiovascular structural and functional abnormalities associated with the presence and severity of NAFLD.

IS OBSTRUCTIVE SLEEP APNEA DIRECTLY OR INDIRECTLY ASSOCIATED WITH NAFLD IN CHILDREN?

Obstructive sleep apnea (OSA) may be associated with NAFLD in children over and above its association with obesity. There have been two studies to date that have performed polysomnography in a combined total of 90 children with NAFLD.^(39,40) These studies reported a prevalence of OSA of approximately 60% in children with NAFLD. Moreover, OSA was associated with the diagnosis of NASH and the severity of fibrosis. The pediatric hepatology community will need to work closely with pediatric sleep specialists to develop a validated strategy to guide which children with NAFLD should undergo polysomnography and to learn whether the appropriate treatment of OSA in children will improve NAFLD.

WHAT ABOUT QUALITY OF LIFE IN CHILDREN WITH NAFLD?

Quality of life (QOL), a subjective measure of a disease's overall impact from an individual's perspective, is an important component for the complete understanding of disease burden. Children with NAFLD have lower QOL than healthy children. The decrements have been reported in both physical health and psychosocial health. Kistler et al.⁽⁴¹⁾ reported that QOL was impaired in 39% of children with NAFLD. There were four symptoms most associated with impaired QOL: fatigue, trouble concentrating, sadness, and nausea. In addition, case-control studies have shown that children with NAFLD have a greater degree of negative mood and more symptoms of anxiety than obese children without NAFLD.^(42,43)

Treatment of Pediatric NAFLD

WHAT IS THE TREATMENT FOR CHILDREN WITH NAFLD?

Treatment of NAFLD in children is a major gap. To date, there is not an available, proven, safe, and effective treatment for NAFLD in children. One complicating factor is that there is not a universal definition of what represents a treatment success.

WHAT TREATMENT IMPROVES ALT IN CHILDREN WITH NAFLD?

The most studied outcome measure in clinical trials has been ALT. Based on multiple studies, including the large randomized controlled trial TONIC, there is sufficient evidence to conclude that neither vitamin E nor metformin improve ALT in children with NAFLD.⁽⁴⁴⁾ In pilot studies, there was no improvement in ALT with docosahexaenoic acid, ursodeoxycholic acid, combination probiotic (VSL #3), or a low fructose diet.⁽⁴⁵⁻⁴⁸⁾ There are no trials that have shown a definitive therapeutic improvement in ALT, but preliminary studies have shown the potential for improvement with cysteamine bitartrate, *Lactobacillus rhamnosus* GG, diets low in fat or glycemic index, and weight loss.⁽⁴⁹⁻⁵³⁾ Although lifestyle interventions have demonstrated the potential to improve ALT, they have been limited by study design, including confirmation of diagnosis, low baseline ALT, and multimodal interventions without adequate controls. Thus, which diet or what exercise or how much weight loss is effective are unknowns.

WHAT TREATMENT RESULTS IN IMPROVED HISTOLOGICAL PATTERN?

There are no supplements or medications that have been shown to improve steatosis in children with NAFLD. In contrast, there is some evidence that steatosis may be improved by diet, exercise, or weight loss.⁽⁵⁴⁻⁵⁸⁾ To date, no therapy has been shown to improve inflammation (portal or lobular) or fibrosis. Both vitamin E and metformin had similar efficacy in improving ballooning compared with placebo over 2 years in the TONIC study; ballooning was improved in 38% (22/58) taking vitamin E, 39% (22/57) taking

metformin, and 17% (10/58) taking placebo.⁽⁴⁴⁾ The development of effective therapies for children with NAFLD and their demonstration of effectiveness in large, well-designed clinical trials is an urgent need.

WHAT ABOUT BARIATRIC SURGERY AS A TREATMENT FOR NAFLD IN CHILDREN?

Because of the lack of an established treatment for NAFLD in adolescents and the overlap with severe pediatric obesity, in 2015 the European Society for Paediatric Gastroenterology Hepatology and Nutrition released a detailed statement on bariatric surgery in adolescents, including those with NAFLD.⁽⁵⁹⁾ Although substantial weight loss can be achieved via weight loss surgery in adolescents,⁽⁶⁰⁾ there are currently no data on the direct effect of weight loss surgery on NAFLD in this population. Therefore, adolescents with NAFLD undergoing bariatric surgery should be cared for in centers with appropriate pediatric multidisciplinary expertise and a commitment to rigorously phenotype NAFLD histology at baseline and to follow outcomes prospectively as long as possible.⁽⁶¹⁾

Conclusion

NAFLD is the most common cause of chronic liver disease in children. National studies have provided stable estimates for the appropriate reference ranges for ALT in children. Clinical laboratories should be encouraged to use these data to improve the standardization of pediatric care. Noninvasive blood-based and imaging markers for NAFLD and NASH in children constitute an active research area, but they have yet to provide a tool validated for clinical use. Liver biopsy is safe in children with suspected NAFLD and has been shown to provide clinically meaningful data for children with chronic liver chemistry elevation. NAFLD has the potential to progress to cirrhosis and the need for liver transplantation in childhood and early adulthood.^(62,63) Beyond the risk for adverse hepatic outcomes, NAFLD is associated with dyslipidemia, hypertension, and early cardiac dysfunction in children. NAFLD is also associated with impaired QOL. Current treatment is individualized and focused on optimizing lifestyle, including nutrition, physical activity, and mental well-being. The

search for pharmacologic therapies for pediatric NAFLD is ongoing.

REFERENCES

- 1) Diseases NIDaDaK. Non-Alcoholic Steatohepatitis 1998. Available from: http://archives.niddk.nih.gov/conf1999/displaypage.aspx?page_name=conf1999/nashmtg.htm. Accessed May 27, 2015.
- 2) Schwimmer JB, Dunn W, Norman GJ, Pardee PE, Middleton MS, Kerkar N, et al. SAFETY study: alanine aminotransferase cutoff values are set too high for reliable detection of pediatric chronic liver disease. *Gastroenterology* 2010;138:1357-1364.
- 3) Park J, Kim S, Park M. Alanine aminotransferase and metabolic syndrome in adolescents: the Korean National Health and Nutrition Examination Survey Study. *Pediatr Obes* 2014;9:411-418.
- 4) Colantonio D, Kyriakopoulou L, Chan M, Daly C, Brinc D, Venner A, et al. Closing the gaps in pediatric laboratory reference intervals: a CALIPER database of 40 biochemical markers in a healthy and multiethnic population of children. *Clin Chem* 2012;58:854-868.
- 5) Molleston JP, Schwimmer JB, Yates KP, Murray KF, Cummings OW, Lavine JE, et al. Histological abnormalities in children with nonalcoholic fatty liver disease and normal or mildly elevated alanine aminotransferase levels. *J Pediatr* 2014; 164:707-713.
- 6) Awai HI, Newton KP, Sirlin CB, Behling C, Schwimmer JB. Evidence and recommendations for imaging liver fat in children, based on systematic review. *Clin Gastroenterol Hepatol* 2014;12: 765-773.
- 7) Shannon A, Alkhoury N, Carter-Kent C, Monti LD, Devito R, Lopez R, et al. Ultrasonographic quantitative estimation of hepatic steatosis in children with nonalcoholic fatty liver disease (NAFLD). *J Pediatr Gastroenterol Nutr* 2011;53:190-195.
- 8) Sasso M, Audiere S, Kemgang A, Gaouar F, Corpechot C, Chazouilleres O, et al. Liver steatosis assessed by controlled attenuation parameter (CAP) measured with the XL probe of the FibroScan: a pilot study assessing diagnostic accuracy. *Ultrason Med Biol* 2016;42:92-103.
- 9) de Lédinghen V, Wong GL, Vergniol J, Chan HL, Hiriart JB, Chan AW, et al. Controlled attenuation parameter (CAP) for the diagnosis of steatosis in nonalcoholic fatty liver disease. *J Gastroenterol Hepatol* 2015. doi: 10.1111/jgh.13219.
- 10) Cassinotto C, Lapuyade B, Ait-Ali A, Vergniol J, Gaye D, Foucher J, et al. Liver fibrosis: noninvasive assessment with acoustic radiation force impulse elastography— comparison with FibroScan M and XL probes and FibroTest in patients with chronic liver disease. *Radiology* 2013;269:283-292.
- 11) Tang A, Tan J, Sun M, Hamilton G, Bydder M, Gamst AC, et al. Nonalcoholic fatty liver disease: MR imaging of liver proton density fat fraction to assess hepatic steatosis. *Radiology* 2013;267:422-431.
- 12) Schwimmer JB, Middleton MS, Behling C, Newton KP, Awai HI, Paiz MN, et al. Magnetic resonance imaging and liver histology as biomarkers of hepatic steatosis in children with nonalcoholic fatty liver disease. *HEPATOLOGY* 2015;61:1887-1895.
- 13) Rockey DC, Caldwell SH, Goodman ZD, Nelson RC, Smith AD, American Association for the Study of Liver Diseases. Liver biopsy. *HEPATOLOGY* 2009;49:1017-1044.
- 14) Harwood J, Bishop P, Liu HL, Nowicki M. Safety of blind percutaneous liver biopsy in obese children: a retrospective analysis. *J Clin Gastroenterol* 2010;44:e253-e255.

- 15) Schwimmer JB, Newton KP, Awai HI, Choi LJ, Garcia MA, Ellis LL, et al. Paediatric gastroenterology evaluation of overweight and obese children referred from primary care for suspected non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2013;38:1267-1277.
- 16) Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *HEPATOLOGY* 2012;55:2005-2023.
- 17) Koot B, Van der Baan-Slootweg OH, Bohte A, nederveen AJ, Van Werven JR, Tammenga-Smeulders CL, et al. Accuracy of prediction scores and novel biomarkers for predicting nonalcoholic fatty liver disease in obese children. *Obesity* 2013;21:583-590.
- 18) Schwimmer JB, Behling C, Newbury R, Deutsch R, Nievergelt C, Schork NJ, et al. Histopathology of pediatric nonalcoholic fatty liver disease. *HEPATOLOGY* 2005;42:641-649.
- 19) Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. *Pediatrics* 2006;118:1388-1393.
- 20) Inge TH, Zeller M, Harmon C, Helmrath M, Bean J, Modi A, et al. Teen-longitudinal assessment of bariatric surgery (Teen-LABS): methodologic features of the first prospective multicenter study of adolescent bariatric surgery. *J Pediatr Surg* 2007;42:1969-1971.
- 21) Xanthakos S, Jenkins T, Kleiner D, Boyce T, Mourya R, Karns R, et al. High prevalence of nonalcoholic fatty liver disease in adolescents undergoing bariatric surgery. *Gastroenterology* 2015;149:623-634.
- 22) Pardee PE, Lavine JE, Schwimmer JB. Diagnosis and treatment of pediatric nonalcoholic steatohepatitis and the implications for bariatric surgery. *Semin Pediatr Surg* 2009;18:144-151.
- 23) Nobili V, Marcellini M, Devito R, Ciampalini P, Piemonte F, Comparcola D, et al. NAFLD in children: a prospective clinical-pathological study and effect of lifestyle advice. *HEPATOLOGY* 2006;44:458-465.
- 24) Patton HM, Lavine JE, Van Natta ML, Schwimmer JB, Kleiner D, Molleston J, et al. Clinical correlates of histopathology in pediatric nonalcoholic steatohepatitis. *Gastroenterology* 2008;135:1961-1971.
- 25) Ko JS, Yoon JM, Yang HR, Myung JK, Kim H, Kang GH, et al. Clinical and histological features of nonalcoholic fatty liver disease in children. *Dig Dis Sci* 2009;54:2225-2230.
- 26) Carter-Kent C, Brunt EM, Yerian L, Alkhoury N, Angulo P, Kohli R, et al. Relations of steatosis type, grade, and zonal distribution to histological features in pediatric nonalcoholic fatty liver disease. *J Pediatr Gastroenterol Nutr* 2011;52:190-197.
- 27) Takahashi Y, Inui A, Fujisawa T, Takikawa H, Fukusato T. Histopathological characteristics of non-alcoholic fatty liver disease in children: comparison with adult cases. *Hepatol Res* 2011;41:1066-1074.
- 28) Alkhoury N, Eng K, Lopez R, Nobili V. Non-high-density lipoprotein cholesterol (non-HDL-C) levels in children with nonalcoholic fatty liver disease (NAFLD). *Springerplus* 2014;3:407.
- 29) Mansoor S, Yerian L, Kohli R, Xanthakos S, Angulo P, Ling S, et al. The evaluation of hepatic fibrosis scores in children with nonalcoholic fatty liver disease. *Dig Dis Sci* 2015;60:1440-1447.
- 30) Mellinger JL, Pencina KM, Massaro JM, Hoffman U, Seshadri S, Fox CS, et al. Hepatic steatosis and cardiovascular disease outcomes: an analysis of the Framingham Heart Study. *J Hepatol* 2015;63:470-476.
- 31) Ballestri S, Lonardo A, Bonapace S, Byrne CD, Loria P, Targher G. Risk of cardiovascular, cardiac and arrhythmic complications in patients with non-alcoholic fatty liver disease. *World J Gastroenterol* 2014;20:1724-1745.
- 32) Schwimmer JB, Pardee PE, Lavine JE, Blumkin AK, Cook S. Cardiovascular risk factors and the metabolic syndrome in pediatric nonalcoholic fatty liver disease. *Circulation* 2008;118:277-283.
- 33) Siddiqui MS, Fuchs M, Idowu MO, Luketic VA, Boyett S, Sargeant C, et al. Severity of nonalcoholic fatty liver disease and progression to cirrhosis are associated with atherogenic lipoprotein profile. *Clin Gastroenterol Hepatol* 2015;13:1000-1008.
- 34) Schwimmer JB, Zepeda A, Newton KP, Xanthakos SA, Behling C, Hallinan EK, et al. Longitudinal assessment of high blood pressure in children with nonalcoholic fatty liver disease. *PLoS One* 2014;9:e112569.
- 35) Sert A, Pirgon O, Aypar E, Yilmaz H, Odabas D. Relationship between left ventricular mass and carotid intima media thickness in obese adolescents with non-alcoholic fatty liver disease. *J Pediatr Endocr Met* 2012;25:927-934.
- 36) Alp H, Ekliloglu B, Atabek M, Karaarslan S, Baysal T, Altin H, et al. Evaluation of epicardial adipose tissue, carotid intima-thickness and ventricular functions in obese children and adolescents. *J Pediatr Endocr Met* 2014;27:827-835.
- 37) Singh G, Vitola BE, Holland M, Sekarski T, Patterson B, Magkos F, et al. Alterations in ventricular structure and function in obese adolescents with nonalcoholic fatty liver disease. *J Pediatr* 2013;162:1160-1168.
- 38) Pacifico L, Di Martino M, De Merulis A, Bezzi M, Osborn JF, Catalano C, et al. Left ventricular dysfunction in obese children and adolescents with nonalcoholic fatty liver disease. *HEPATOLOGY* 2014;59:461-470.
- 39) Sundaram SS, Sokol RJ, Capocelli KE, Pan Z, Sullivan JS, Robbins K, et al. Obstructive sleep apnea and hypoxemia are associated with advanced liver histology in pediatric nonalcoholic fatty liver disease. *J Pediatr* 2014;164:699-706.
- 40) Nobili V, Cutrera R, Liccardo D, Pavone M, Devito R, Giorgio V, et al. Obstructive sleep apnea syndrome affects liver histology and inflammatory cell activation in pediatric nonalcoholic fatty liver disease, regardless of obesity/insulin resistance. *Am J Respir Crit Care Med* 2014;189:66-76.
- 41) Kistler KD, Molleston J, Unalp A, Abrams SH, Behling C, Schwimmer JB, et al. Symptoms and quality of life in obese children and adolescents with non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2010;31:396-406.
- 42) Mazzone L, Postorino V, De Peppo L, Della Corte C, Lofino G, Vassena L, et al. Paediatric non-alcoholic fatty liver disease: impact on patients and mothers' quality of life. *Hepat Mon* 2013;13:e7871.
- 43) Kerker N, D'Urso C, Van Nostrand K, Kochin I, Gault A, Suchy F, et al. Psychosocial outcomes for children with nonalcoholic fatty liver disease over time and compared with obese controls. *J Pediatr Gastroenterol Nutr* 2013;56:77-82.
- 44) Lavine JE, Schwimmer JB, Van Natta ML, Molleston JP, Murray KF, Rosenthal P, et al. Effect of vitamin E or metformin for treatment of nonalcoholic fatty liver disease in children and adolescents. The TONIC randomized controlled trial. *JAMA* 2011;305:1659-1668.
- 45) Nobili V, Bedogni G, Alisi A, Pietrobattista A, Rise P, Galli C, et al. Docosahexaenoic acid supplementation decreases liver fat content in children with non-alcoholic fatty liver disease: double-blind randomised controlled clinical trial. *Arch Dis Child* 2011;96:350-353.

- 46) Alisi A, Bedogni G, Baviera G, Giorgio V, Porro E, Paris C, et al. Randomised clinical trial: the beneficial effects of VSL#3 in obese children with non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2014;39:1276-1285.
- 47) Mager D, Iñiguez I, Gilmour S, Yap J. The effect of a low fructose and low glycemic index/load (FRAGILE) dietary intervention on indices of liver function, cardiometabolic risk factors, and body composition in children and adolescents with nonalcoholic fatty liver disease (NAFLD). *JPEN J Parenter Enteral Nutr* 2015;39:73-84.
- 48) Vajro P, Franzese A, Valerio G, Iannucci MP, Aragione N. Lack of efficacy of ursodeoxycholic acid for the treatment of liver abnormalities in obese children. *J Pediatr* 2000;136:0739-43.
- 49) Vajro P, Mandato C, Licenziati MR, Franzese A, Vitale DF, Lenta S, et al. Effects of *Lactobacillus rhamnosus* strain GG in pediatric obesity-related liver disease. *J Pediatr Gastroenterol Nutr* 2011;52:740-743.
- 50) Ramon-Krauel M, Salsberg S, Ebbeling C, Voss S, Mulkern R, Apura M, et al. A low-glycemic-load versus low-fat diet in the treatment of fatty liver in obese children. *Child Obes* 2013;9:252-260.
- 51) Dohil R, Schmeltzer S, Cabrera BL, Wang T, Durelle J, Duke KB, et al. Enteric-coated cysteamine for the treatment of paediatric non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2011;33:1036-1044.
- 52) Vajro P, Fontanella A, Perna C, Orso G, Tedesco M, De Vincenzo A. Persistent hyperaminotransferemia resolving after weight reduction in obese children. *J Pediatr* 1994;125:239-241.
- 53) Suzuki A, Lindor K, St Staver J, Lymp J, Mendes F, Muto A, et al. Effect of changes on body weight and lifestyle in non-alcoholic fatty liver disease. *J Hepatol* 2005;43:1060-1066.
- 54) Lee S, Bacha F, Hannon T, Kuk JL, Boesch C, Arslanian S. Effects of aerobic versus resistance exercise without caloric restriction on abdominal fat, intrahepatic lipid, and insulin sensitivity in obese adolescent boys: a randomized, controlled trial. *Diabetes* 2012;61:2787-2795.
- 55) Lee S, Deldin AR, White D, Kim Y, Libman I, Rivera-Vega M, et al. Aerobic exercise but not resistance exercise reduces intrahepatic lipid content and visceral fat and improves insulin sensitivity in obese adolescent girls: a randomized controlled trial. *Am J Physiol Endocrinol Metab* 2013;305:E1222-E1229.
- 56) Van Der Heijden GJ, Wang ZJ, Chu Z, Toffolo G, Manesso E, Sauer PJ, et al. Strength exercise improves muscle mass and hepatic insulin sensitivity in obese youth. *Med Sci Sports Exerc* 2010;42:1973-1980.
- 57) van der Heijden GJ, Wang ZJ, Chu ZD, Sauer PJ, Haymond MW, Rodriguez LM, et al. A 12-week aerobic exercise program reduces hepatic fat accumulation and insulin resistance in obese, Hispanic adolescents. *Obesity* 2010;18:384-390.
- 58) Larson-Meyer DE, Newcomer BR, Heilbronn LK, Volaufova J, Smith SR, Alfonso AJ, et al. Effect of 6-month calorie restriction and exercise on serum and liver lipids and markers of liver function. *Obesity* 2008;16:1355-1362.
- 59) Nobili V, Vajro P, Dezsöfi A, Fischler B, Hadzic N, Jahnel J, et al. Indications and limitations of bariatric intervention in severely obese children and adolescents with and without nonalcoholic steatohepatitis: ESPGHAN Hepatology Committee Position Statement. *J Pediatr Gastroenterol Nutr* 2015;60:550-561.
- 60) Inge TH, Courcoulas AP, Jenkins TM, Michalsky MP, Helmrath MA, Brandt ML, et al. Weight loss and health status 3 years after bariatric surgery in adolescents. *N Engl J Med* 2016;374:113-123.
- 61) Xanthakos SA, Schwimmer JB. Paediatric gastroenterology. On a knife-edge—weight-loss surgery for NAFLD in adolescents. *Nat Rev Gastroenterol Hepatol* 2015;12:316-318.
- 62) Alkhoury N, Hanounch IA, Zein NN, Lopez R, Kelly D, Eghtesad B, et al. Liver transplantation for nonalcoholic steatohepatitis (NASH) in young patients. *Transplant Int* 2015. doi: 10.1111/tri.12694.
- 63) Feldstein AE, Charatcharoenwitthaya P, Treeprasertsuk S, Benson JT, Enders FB, Angulo P. The natural history of non-alcoholic fatty liver disease in children: a follow-up study for up to 20 years. *Gut* 2009;58:1538-1544.