

Revisión

# Is liver transaminases assessment an appropriate tool for the screening of non-alcoholic fatty liver disease in at risk obese children and adolescents?

G. Rodríguez<sup>1,2</sup>, S. Gallego<sup>3</sup>, C. Breidenassel<sup>4</sup>, L. A. Moreno<sup>5</sup> y F. Gottrand<sup>6</sup>

<sup>1</sup>“Growth, Exercise, Nutrition and Development” (GENUD) Research Group. Dpto de Pediatría, Radiología y Medicina Física, University of Zaragoza ; and Hospital Clínico Universitario ‘Lozano Blesa’. Zaragoza (Spain). <sup>2</sup>Instituto Aragonés de Ciencias de la Salud (Spain). <sup>3</sup>Servicio de Pediatría Hospital Ernest Lluch de Calatayud. Zaragoza (Spain). <sup>4</sup>Institut für Ernährungs- und Lebensmittelwissenschaften – Ernährungphysiologie. Rheinische Friedrich Wilhelms Universität (Germany). <sup>5</sup>“Growth, Exercise, Nutrition and Development” (GENUD) Research Group. EU. Ciencias de la Salud, University of Zaragoza (Spain). <sup>6</sup>Unité de Gastroentérologie, Hépatologie et Nutrition, Clinique de Pédiatrie, Hôpital Jeanne de Flandre. EA3925, IFR114, Faculté de médecine, université Lille2, Lille (France).

Abstract

Pediatric obesity has increased dramatically all over the world and nonalcoholic fatty liver disease (NAFLD) is one of the most frequent complications associated with excess adiposity. NAFLD causes serum transaminase elevation and liver disease, which could end up in fibrosis, cirrhosis and eventually hepatocellular carcinoma. NAFLD seems to be associated with the metabolic complications of obesity, mainly insulin resistance. The aim of the present article is to review the role of serum liver enzyme assessment as a suitable non invasive predictor of NAFLD in children. Although serum liver enzyme elevation does not accurately measure liver damage, it may be a valuable and non invasive test to screen NAFLD in children and adolescents and a marker to control NAFLD evolution. To detect NAFLD in obese children and adolescents, transaminases serum concentrations should be routinely determined in these patients. In this sense, it seems necessary to obtain transaminase reference standards for children and adolescents.

(Nutr Hosp. 2010;25:712-717)

DOI:10.3305/nh.2010.25.5.4758

Key words: *Transaminases. Metabolic syndrome. Obesity. Nonalcoholic fatty liver disease.*

## ¿ES ÚTIL LA VALORACIÓN DE LAS TRANSAMINASAS HEPÁTICAS PARA EL SCREENING DEL HÍGADO GRASO NO ALCOHÓLICO EN NIÑOS Y ADOLESCENTES OBESOS?

Resumen

La prevalencia de obesidad en niños ha aumentado considerablemente en todo el mundo y el hígado graso no alcohólico (HGNA) es una de las complicaciones más frecuentemente asociadas al exceso de adiposidad. El HGNA provoca elevación de las transaminasas hepáticas y una alteración hepática que pueden desencadenar fibrosis, cirrosis e incluso malignización. El HGNA aparece asociado a las complicaciones metabólicas de la obesidad, sobre todo a la resistencia a la insulina. El objetivo del presente artículo es revisar el rol que tienen las enzimas hepáticas como predictor no invasivo del HGNA en niños. Aunque la elevación de las enzimas hepáticas no valora con exactitud el daño hepático, pueden suponer un test no invasivo para el screening del HGNA en niños y adolescentes y un marcador de su evolución. Las transaminasas hepáticas deben ser determinadas de rutina en el diagnóstico del HGNA en niños y adolescentes. En este sentido, parece necesario encontrar unos estándares de referencia para los niveles de transaminasas en niños y adolescentes.

(Nutr Hosp. 2010;25:712-717)

DOI:10.3305/nh.2010.25.5.4758

Palabras clave: *Transaminasas. Síndrome metabólico. Obesidad. Hígado graso no alcohólico.*

**Correspondence:** Gerardo Rodríguez.  
Departamento de Pediatría, Radiología y Medicina Física.  
Facultad de Medicina, Universidad de Zaragoza.  
Domingo Miral, s/n.  
50009 Zaragoza  
E-mail: gereva@comz.org

Recibido: 23-II-2010.  
Revisado: 29-III-2010.  
Aceptado: 17-IV-2010.

## Introduction

The prevalence of childhood obesity has increased dramatically all over the world during the last decades. The rising incidence of obesity in today's environment is associated with many obesity-related health complications, including cardiovascular diseases, diabetes, hyperlipidemia, hypertension, and nonalcoholic fatty liver disease (NAFLD)<sup>1-3</sup>. In Europe the combined prevalence of childhood overweight and obesity is higher than 30% in countries such as Spain, Italy or Portugal; and about 20% in many other countries<sup>4,5</sup>. These trends are continuously increasing and, as it is well known, 'obesity epidemic' extends beyond Europe. However, a levelling-off of the obesity prevalence with reverse trends towards increasing childhood overweight have recently reported in the US, France or Sweden<sup>6-8</sup>. Despite these findings, overweight and obesity among children need to be considered as a global problem.

The increase in the prevalence of obesity is often related to a rise in obesity comorbidities, both during childhood and in adult age, because of its early onset and longer duration. Despite the fact that overweight has become a serious problem among children and teenagers, the data suggest low rates of diagnosis of overweight, obesity and related comorbidities. A high prevalence of obesity during childhood could trigger off NAFLD, that might end up in serious increases in liver related morbidity and mortality<sup>9</sup>.

One focus of interest in obesity research and clinical practice is the relation between obesity and the metabolic syndrome (MS), with the development of NAFLD as other consequence of this association. Obesity, especially central body fatness, is associated with dyslipidemia, hypertension, insulin resistance/hyperinsulinemia and impaired glucose tolerance/type 2 diabetes<sup>10,11</sup>. This cluster of metabolic abnormalities that already appears in obese children and adolescents increases the risk of cardiovascular diseases<sup>12</sup>. As well as obesity is associated with MS, fatness is also the major risk factor for NAFLD in children. There is a direct association between abdominal fat and liver fat content which is probably accounted for a general increase in visceral fat due to adiposity at this body region<sup>13</sup>. Studies from obese children populations report that about 35% of these children have NAFLD<sup>3,14-16</sup>. Parallel to the increasing prevalence of obesity, NAFLD could cause end stage liver disease already during childhood and adolescence while for other complications (dyslipidemia, hypertension, type 2 diabetes) the consequences are observed mostly during adulthood.

Serum liver enzymes are routinely utilised for the assessment of cytolysis in various conditions. They are also used as markers of NAFLD in children and adolescents but many unanswered questions remain: a) Are liver transaminases able to predict and/or to diagnose NAFLD appropriately? b) Are there adequate liver

enzyme cut-offs and population reference values for the screening of NAFLD? c) Are transaminases good markers of NAFLD evolution (to fibrosis/cirrhosis) d) Are transaminases a reliable tool for assessing NAFLD evolution under treatment (weight loss, drug therapy). The aim of this review is to address these questions and to try to provide the current evidence based status on these issues.

## NAFLD definitions

NAFLD covers a spectrum of liver diseases from steatosis to nonalcoholic steatohepatitis (NASH) and cirrhosis. According to the American Association for the Study of Liver Diseases, NAFLD is defined as fat accumulation in the liver exceeding 5% to 10% by weight, as determined from the percentage of fat-laden hepatocytes by light microscopy<sup>17</sup>. The accumulation of lipids, primarily in the form of triacylglycerols, has to be in individuals who do not consume significant amounts of alcohol and in whom other known causes of steatosis, such as certain drugs and toxins, have been excluded.

The spectrum of NAFLD includes steatosis alone (type 1), steatosis plus inflammation (type 2), steatosis plus hepatocyte injury or ballooning degeneration (type 3), and steatosis plus sinusoidal fibrosis, Mallory bodies, or both (type 4). NASH is considered to be the most severe form of NAFLD (types 3 and 4) and is associated with an array of adverse clinical outcomes, including cirrhosis, hepatocellular carcinoma, and advanced liver disease, which can lead to liver-related death<sup>18</sup>.

## NAFLD physiopathology and prevalence

Given the close relations between obesity, the MS, and the development of NAFLD, it is not surprising that many NAFLD patients have multiple components of the MS, even whether they are no overweight or obese. This fact has been recently shown also in obese children with NAFLD which are more likely (50% versus 15%) to have MS than obese children without NAFLD (Odds ratio 5.0 after multiple adjustment; 95% CI 2.6 to 9.7) (19). Insulin resistance is present as an important factor in these associations and, by itself, is a significant predictor of NAFLD and NASH in most adult patients<sup>18,20,21</sup>, even in 10–15% of them who are not overweight<sup>22</sup>. Many studies have shown an association of insulin resistance with NAFLD and NASH on the basis of impaired glucose tolerance or impaired fasting glucose<sup>18,22</sup>. Despite elevated insulin concentrations, adipose tissue fatty acid flux is not suppressed in NAFLD patients, which indicates the presence of peripheral insulin resistance<sup>23</sup>. However, the association between NAFLD and obesity in children seems to be independent of hyperinsulinemia in this age group<sup>19</sup>. Further studies are needed to clarify this controversial aspect in children and whether dif-

ferences exists about NAFLD physiopathology between adults and children.

In most NAFLD patients, it is thought that overnutrition or inappropriate diet lead to chronic elevated plasma concentrations of glucose, insulin and free fatty acids (FFA)<sup>24</sup>. Glucose uptake in the liver is not insulin dependent, and increased glucose concentrations in the blood directly lead to increased glucose uptake by the liver. Insulin-mediated stimulation of de novo lipogenesis leads to an increased conversion of glucose to fatty acids<sup>25</sup>. Together, the increased concentrations of both glucose and FFA in the blood contribute to excessive accumulation of neutral lipids in the liver and subsequent elevation of serum transaminases. It has been recently shown that fast-food-based hyperalimentation can induce a rapid and severe elevation of serum alanine transaminase in healthy subjects<sup>26</sup>. In summary, NAFLD can be considered as a multifactorial disease that may involve a complex interaction of genetic, diet and lifestyle factors, all of them combined to form the NAFLD phenotype, as discussed in several recent reviews<sup>1-3,23</sup>.

The original “two-hit hypothesis” of NASH suggests that the accumulation of lipids in the liver (hit one) is followed by a cascade of prooxidative, hepatotoxic events (hit two), which are caused by a mechanism still unknown<sup>27</sup>. Elevated concentrations of circulating tumour necrosis factor (TNF) have been documented in NAFLD patients with implications in fatty liver disease physiopathology<sup>28,29</sup>. Small intestinal bacterial overgrowth and deficiencies in various nutrients, including essential fatty acids, choline, 25-hydroxyvitamin D<sub>3</sub> and amino acids, may also be involved in the development and progression of this disorder; however, limited evidence exists to support these mechanisms<sup>20,21</sup>.

In order to determine the real prevalence of NAFLD it is necessary to tackle various questions. The most important issue is that liver biopsy is required to make an appropriate diagnosis of NAFLD, although it is not well accepted in children as a diagnostic tool for NAFLD population prevalence studies. In consequence, increased serum transaminase concentrations are used for fatty liver disease screening in the majority of studies, although their sensitivity and specificity in the diagnosis of NAFLD has not yet been verified in children. Ultrasonographic techniques can also be employed for this aim, although this method is unlikely to determine the NAFLD intensity or the differences between simple steatosis and steatohepatitis. Finally, other challenge to estimate NAFLD prevalence is the choice of an adequate and representative study population<sup>32</sup>.

Nonalcoholic fatty liver is commonly associated with long-term elevations in liver enzymes such as alanine transaminase (ALT) and  $\gamma$ -glutamyl transferase (GGT) as useful and non invasive surrogate markers of NAFLD or liver dysfunction<sup>33</sup>. The overall prevalence of suspected fatty liver disease as determined by ele-

vated transaminase population data is estimated about 3-9% among children and adolescents, depending on the population background<sup>32,34</sup>. When the sample is composed of overweight / obese children and adolescents, the prevalence of suspected fatty liver from elevated transaminases in these groups range from 10 to 25% depending on age, gender and ethnicity<sup>15,16,32,35</sup>. From a retrospective study conducted from autopsies performed in 742 children and adolescents age 2 to 19 years, fatty liver (defined as >5% of hepatocytes containing macrovesicular fat) was present in 13% of subjects with the highest rate in obese children (38%)<sup>36</sup>. Therefore, results from NAFLD prevalence considerably varies depending on the screening method and population characteristics. NASH must be suspected if there is two- to threefold increase in transaminases levels and ultrasound liver brightness<sup>32,33</sup>. However, formal diagnosis requires liver biopsy<sup>2</sup>.

MS is very common in children affected by fatty liver disease, being observed in more than 50% of them<sup>9,19,37</sup>. Patients with MS show levels of transaminases significantly higher than children without MS<sup>38</sup>, and fibrosis and severe steatosis are frequently reported. Among comorbidities, dyslipidemia and hypertension are largely described in fatty liver disease, while insulin resistance and excessive body weight (either obesity or overweight) are almost universal findings<sup>29</sup>. Manco et al<sup>40</sup>, have shown significant histological findings in children with MS associated with clinical and biochemical variables. In this study, necroinflammation correlated positively with BMI z-score, insulin resistance, fasting glucose, total cholesterol, transaminase concentrations and white blood cell count. Fibrosis was associated with higher body weight and BMI z-score, cholesterol and triglyceride concentrations, insulin resistance, fasting insulin and insulin during the oral glucose tolerance test<sup>40,41</sup>. Abdominal rather than generalized obesity contributes to liver fibrosis in children with NAFLD and waist circumference is the main anthropometric predictor of this association<sup>42</sup>. In the presence of MS, NAFLD/NASH should be investigated by repeated measurement of liver enzymes and liver ultrasounds, and it is suggested that the role and timing of liver biopsy must be evaluated in future studies<sup>40</sup>.

### Screening and diagnosis of nafld in obese children and adolescents

Serum transaminases (alanine transaminase: ALT and aspartate transaminase: AST) play a key role in animal amino acid metabolism. Elevated levels of these enzymes are quite sensitive for liver injury indicating a high hepatocyte cell membrane permeability that let enzymes leak out into the blood stream. However, transaminases may also be elevated in other conditions. ALT is not commonly found outside the liver but AST elevation, which frequently indicates liver damage, may also be present in heart and skeletal mus-

cle injuries. For these reasons, serum ALT has been used as a surrogate marker for fatty liver disease in population studies. High serum ALT concentrations are associated with increased hepatic fat fraction and increased intra-abdominal visceral adipose tissue, even in statistical models excluding age as a contributing factor in serum ALT variability<sup>32</sup>. Therefore, subjects with elevated serum ALT and fatty liver have more severe steatosis and higher visceral adiposity than their counterparts and, also in children, these changes are likely the result of metabolic alterations derived from insulin resistance. At the beginning of insulin resistance setting up, affected individuals have mild steatosis and normal serum ALT. As insulin resistance progresses, individuals develop severe steatosis, elevated serum ALT, and visceral adiposity<sup>32,40</sup>. Persistent elevations of ALT and GGT over time even within the reference range have been related to MS and adverse cardiovascular risk profile in adolescents and young adults<sup>43</sup>.

There are no many publications about AST or ALT population reference values and normal individual aminotransferase level variations in children and adolescents<sup>32,44</sup>. Although there is no single standard cut-off point for abnormal high serum aminotransferase levels, the most commonly used criterion is a value of 40 U/L for both ALT and AST<sup>32,35,44</sup>. However, many other cut-off points ranging from 30 U/L to 45 U/L have been also used to define elevated serum ALT levels in children and adolescents<sup>32,45-47</sup>. Very recently, according to 95<sup>th</sup> percentile levels for ALT in healthy liver disease-free children, Schwimmer et al.<sup>48</sup> have proposed that 25.8 U/L in boys and 22.1 U/L in girls provided better sensitivity without less specificity to reliably detect chronic liver disease. Therefore, to assess appropriately the risk of NAFLD from aminotransferase measurement, it could be of interest to update and to standardize specific serum ALT/AST values depending on age, gender or race.

Even though most patients with NAFLD have elevated transaminases, it is known that many obese children show normal transaminases together with incipient evidence of fatty liver<sup>49</sup>. The levels of serum transaminases have been brought into question because population standards have been determined from samples that could include people with undiagnosed liver diseases such as NAFLD related to obesity<sup>32</sup>. In addition, liver biopsy, which is not well accepted for population studies in children, is required to evaluate the utility of serum transaminases level in NAFLD management<sup>49</sup>. Therefore, the reliability and cost analysis of serum transaminases in relation with NAFLD screening have not been appropriately determined to date and data from several risk populations are not available yet. Other non-invasive markers with high sensitivity and specificity for the screening of NAFLD/NASH were not available and serum transaminases assessment represented the best choice until current new useful tools have been reported<sup>50,51</sup>. The Enhanced Liver Fibrosis (ELF) panel of serum markers

(tissue inhibitor of matrix metalloproteinase 1, hyaluronic acid and aminoterminal peptide of pro-collagen III) is a test validated recently for staging liver fibrosis also in pediatric patients with NAFLD selecting those children that require biopsy<sup>50</sup>. Other non-invasive possibility to predict accurately liver fibrosis in children with NAFLD is FibroScan (transient elastography)<sup>51</sup>.

In order to make a precise diagnosis of NAFLD, it is necessary to exclude other chronic liver diseases, including B and C hepatitis, autoimmune hepatitis, and drug-induced liver damage. On the other hand, the coexistence of various signs in an obese subject such as elevated aminotransferases, hepatomegaly or echogenic liver, together with insulin resistance or other MS feature, may suggest NAFLD development. Nevertheless, histological evaluation continues to be the only way of estimating precisely the degree of liver steatosis, necroinflammatory lesions and fibrosis found in NASH, distinguishing NASH from simple steatosis<sup>32</sup>.

### Treatment of NAFLD

To lose weight, a decrease in visceral fat, to improve insulin sensitivity and antioxidant therapy have been the approaches explored in NAFLD therapy. Several case series and uncontrolled trials have assessed the efficacy of weight loss programs in improving transaminases levels and the appearance of the liver by ultrasonography; however, there are very few published data in children showing the effect of diet and exercise on liver histology after weight loss<sup>32,52,53</sup>. Nobili et al<sup>52</sup> showed that a simple 12-month lifestyle educational program was able to improve both insulin resistance and liver disease in children with NAFLD monitored by serum levels of transaminases. Other study from the same group<sup>53</sup>, which is an extension of the aforementioned study and constitutes the first biopsy-proven trial after 24 months of NAFLD lifestyle intervention, has shown that diet and physical activity modification induce weight loss and a significant improvement in liver histology (grade of steatosis, lobular inflammation and hepatocyte ballooning).

With regard to drug therapy effects, recent studies in children with biopsy-proven fatty liver disease have shown different results<sup>41,42,53</sup>. Metformin is able to significantly improve NAFLD and ALT levels and no adverse effects have been found due to its use; in consequence, the NASH Clinical Research Network (CRN) is currently testing metformin NAFLD monotherapy in a randomised trial with children<sup>46</sup>. Therapy with antioxidants has also been tested in children with NASH. After biopsy verification in children and adolescents, alpha-tocopherol plus ascorbic acid association during 24 months does not seem to improve the efficacy of lifestyle intervention alone<sup>53</sup>. Ursodeoxycholic acid (UDCA) has also been studied as a potential therapy in adults and children with NAFLD. In obese children with increased serum

transaminases level and ultrasonographic images of bright liver, after lifestyle modification and/or UDCA treatment during 6 months, diet alone determined weight loss and resolved aminotranferase abnormalities and the appearance of steatosis but the addition of UDCA did not improve these results; therefore, UDCA administration seems not to be effective for the treatment of obesity-related NAFLD<sup>54</sup>.

In future trials in children and adolescents, NASH should be controlled with biopsy-proven randomised studies, following-up the patients using parameters such as liver histology, biomarkers of liver disease as well as non-invasive imaging techniques. Due to the close association of obesity and the MS across the entire spectrum of NAFLD, current recommendations for the treatment of fatty liver disease are aimed at weight loss and dietary modification. Weight reduction through surgical methods, even with quick weight-loss after surgery, has been successful in reducing disease progression<sup>55</sup>. Despite the fact that weight loss and dietary and lifestyle changes are recommended as primary treatment for fatty liver, no specific guidelines exist pertaining to diet. The inclusion of Mediterranean diet and specially n-3 fatty acids, high-MUFA (monounsaturated fatty acids) containing foods, fruits, vegetables, and low-GI (glycemic index) highfiber foods and reduced intakes of saturated fats, simple carbohydrates, and sweetened drinks may be universally recommended to NAFLD patients<sup>14</sup>.

### Final comment

The increase in obesity prevalence observed during the last decades has come along with a consequent rise of all obesity associated comorbidities. Nowadays, NAFLD is the most common form of liver disease in children and adolescents. Right now, liver biopsy is the most appropriate tool for NAFLD diagnosis and classification but, although biopsy is recommended when obesity-related liver disease is suspected, it is not well accepted as a population screening criterion in all obese children. Natural history of NAFLD in obese children, liver histopathology, later evolution during adulthood and response to different treatments have to be all clarified in the future. The role of liver enzyme measurements in the screening and management of NAFLD remains to be established. Reference transaminase cut-offs in children and adolescent populations should also be determined. Currently, there are still many important questions without clear solutions about the ability of liver transaminases to predict and/or to diagnose NAFLD appropriately and when liver biopsy has to be used for this aim.

### References

- Haynes P, Liangpunsakul S, Chalasani N. Nonalcoholic fatty liver disease in individuals with severe obesity. *Clin Liver Dis* 2004; 8: 535–547.

- Abrams GA, Kunde SS, Lazenby AJ, Clements RH. Portal fibrosis and hepatic steatosis in morbidly obese subjects: a spectrum of non-alcoholic fatty liver disease. *Hepatology* 2004; 40: 475–483.
- Chan DF, Li AM, Chu WC, Chan MH, Wong EM, Liu EK, Chan IH, Yin J, Lam CW, Fok TF, Nelson EA. Hepatic steatosis in obese Chinese children. *Int J Obes Relat Metab Disord* 2004; 28: 1257–1263.
- Lissau I. Overweight and obesity epidemic among children: Answer from European countries. *Int J Obes* 2004; 28 (Suppl): S10-S15.
- Moreno LA, Tomás C, González-Gross M, Bueno G, Pérez-González JM, Bueno M. Micro-environmental and socio-demographic determinants of childhood obesity. *Int J Obes Relat Metab Disord* 2004; 28 (Suppl 3): S16-S20.
- Ogden CL, Carroll MD, Flegal KM. High body mass index for age among US children and adolescents, 2003-2006. *JAMA* 2008; 299: 2401-2405.
- Romon M, Lommez A, Tafflet M, Basdevant A, Oppert JM, Bresson JL, Ducimetière P, Charles MA, Borys JM. Downward trends in the prevalence of childhood overweight in the setting of 12-year school- and community-based programmes. *Public Health Nutr* 2008 Dec 23:1-8. [Epub ahead of print]
- Ekblom OB, Bak EA, Ekblom BT. Trends in body mass in Swedish adolescents between 2001 and 2007. *Acta Paediatr* 2008 Dec 4. [Epub ahead of print]
- Riley MR, Bass NM, Rosenthal P, Merriman RB. Underdiagnosis of pediatric obesity and underscreening for fatty liver disease and metabolic syndrome by pediatricians and pediatric subspecialists. *J Pediatr* 2005; 147: 839-842.
- Brambilla P, Lissau I, Flodmark CE, Moreno LA, Widhalm K, Wabitsch M, Pietrobelli A. Metabolic risk-factor clustering estimation in children: to draw a line across pediatric metabolic syndrome. *Int J Obes* 2007; 31: 591–600.
- Cali AM, Zern TL, Taksali SE, de Oliveira AM, Dufour S, Otvos JD, Caprio S. Intrahepatic fat accumulation and alterations in lipoprotein composition in obese adolescents: a perfect proatherogenic state. *Diabetes Care* 2007; 30: 3093-3098.
- Tresaco B, Bueno G, Moreno LA, Garagorri JM, Bueno M. Insulin resistance and impaired glucose tolerance in obese children and adolescents. *J Physiol Biochem* 2003; 59: 217-223.
- Jakobsen M, Berentzen T, Sorensen I. Abdominal Obesity and Fatty Liver. *Epidemiol Rev* 2007; 9-11.
- Zivkovic AM, German JB, Sanyal AJ. Comparative review of diets for the metabolic syndrome: implications for nonalcoholic fatty liver disease. *Am J Clin Nutr* 2007; 86: 285-300.
- Tazawa Y, Noguchi H, Nishinomiya F, Takada G. Serum alanine aminotransferase activity in obese children. *Acta Paediatr* 1997; 86: 238–241.
- Strauss RS, Barlow SE, Dietz WH. Prevalence of abnormal serum aminotransferase values in overweight and obese adolescents. *J Pediatr* 2006; 136: 727–733.
- Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* 2003; 37: 1202–1219.
- Clark JM. The epidemiology of nonalcoholic fatty liver disease in adults. *J Clin Gastroenterol* 2006; 40 (suppl 1): S5–S10.
- Schwimmer JB, Pardee PE, Lavine JE, Blumkin AK, Cook S. Cardiovascular risk factors and the metabolic syndrome in pediatric non-alcoholic fatty liver disease. *Circulation* 2008; 118: 277-283.
- Iacobellis G, Moschetta A, Buzzetti R, Ribaldo MC, Baroni MG, Leonetti F. Aminotransferase activity in morbid and uncomplicated obesity: predictive role of fasting insulin. *Nutr Metab Cardiovasc Dis* 2007; 17: 442-447.
- Loria P, Lonardo A, Bellentani S, Day CP, Marchesini G, Carulli N. Non-alcoholic fatty liver disease (NAFLD) and cardiovascular disease: an open question. *Nutr Metab Cardiovasc Dis* 2007; 17: 684-698.
- Marchesini G, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, Forlani G, Melchionda N. Association of nonalcoholic fatty liver disease with insulin resistance. *Am J Med* 1999; 107: 450-455.

23. Gaemers IC, Groen AK. New insights in the pathogenesis of non-alcoholic fatty liver disease. *Curr Opin Lipidol* 2006; 17: 268–273.
24. Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, Luketic VA, Shiffman ML, Clore JN. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* 2001; 120: 1183–1192.
25. Timlin MT, Parks EJ. Temporal pattern of de novo lipogenesis in the postprandial state in healthy men. *Am J Clin Nutr* 2005; 81: 35–42.
26. Kechagias S, Ernersson A, Dahlqvist O, Lundberg P, Lindström T, Nystrom FH; Fast Food Study Group. Fast-food-based hyper-alimentation can induce rapid and profound elevation of serum alanine aminotransferase in healthy subjects. *Gut* 2008 57: 649–654.
27. Day CP, James OF. Steatohepatitis: a tale of two “hits”? *Gastroenterology* 1998; 114: 842–845.
28. Hotamisligil GS. Adipose expression of TNF. *Science* 1993; 258: 87–91.
29. Wong VW, Wong GL, Tsang SW, Hui AY, Chan AW, Choi PC, So WY, Tse AM, Chan FK, Sung JJ, Chan HL. Genetic polymorphisms of adiponectin and tumor necrosis factor- $\alpha$  and nonalcoholic fatty liver disease in Chinese people. *J Gastroenterol Hepatol* 2008; 23: 914–921.
30. Ip E, Farrell GC, Robertson G, Hall P, Kirsch R, Leclercq I. Central role of PPAR-dependent hepatic lipid turnover in dietary steatohepatitis in mice. *Hepatology* 2003; 38: 123–132.
31. Targher G, Bertolini L, Scala L, Cigolini M, Zenari L, Falezza G, Arcaro G. Associations between serum 25-hydroxyvitamin D3 concentrations and liver histology in patients with non-alcoholic fatty liver disease. *Nutr Metab Cardiovasc Dis* 2007; 17: 517–524.
32. Patton HM, Sirlin C, Behling C, Middleton M, Schwimmer JB, Lavine JE. Pediatric nonalcoholic fatty liver disease: a critical appraisal of current data and implications for future research. *J Pediatr Gastroenterol Nutr* 2006; 43: 413–427.
33. Angelico F, Del Ben M, Conti R, Francioso S, Feole K, Maccioni D, Antonini TM, Alessandri C. Non-alcoholic fatty liver syndrome: a hepatic consequence of common metabolic diseases. *J Gastroenterol Hepatol* 2003; 18: 588–594.
34. Vos MB, McClain CJ. Nutrition and nonalcoholic fatty liver disease in children. *Curr Diab Rep* 2008; 8: 399–406.
35. Schwimmer JB, McGreal N, Deutsch R, Finegold MJ, Lavine JE. Influence of gender, race, and ethnicity on suspected fatty liver in obese adolescents. *Pediatrics* 2005; 115: e561–e565.
36. 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.
37. Kang H, Greenon JK, Omo JT, Chao C, Peterman D, Anderson L, Foess-Wood L, Sherbondy MA, Conjeevaram HS. Metabolic syndrome is associated with greater histologic severity, higher carbohydrate, and lower fat diet in patients with NAFLD. *Am J Gastroenterol* 2006; 101: 2247–2253.
38. Graham RC, Burke A, Stettler N. Ethnic and sex differences in the association between metabolic syndrome and suspected non-alcoholic fatty liver disease in a nationally representative sample of US adolescents. *JPGN* 2009; 49: 442–449.
39. Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, Rizzetto M. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 2003; 37: 917–923.
40. Manco M, Marcellini M, DeVito R, Comparcola D, Sartorelli MR, Nobili V. Metabolic syndrome and liver histology in paediatric non-alcoholic steatohepatitis. *Int J Obes (Lond)* 2008; 32: 381–387.
41. Schwimmer JB, Middleton MS, Deutsch R, Lavine JE. A phase 2 clinical trial of metformin as a treatment for non-diabetic paediatric non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2005; 21: 871–879.
42. Manco M, Bedogni G, Marcellini M, Devito R, Ciampalini P, Sartorelli MR, Comparcola D, Piemonte F, Nobili V. Waist circumference correlates with liver fibrosis in children with non alcoholic steatohepatitis. *Gut* 2008 Apr 28. [Epub ahead of print].
43. Patel DA, Srinivasan SR, Xu JH, Chen W, Berenson GS. Persistent elevation of liver function enzymes within the reference range is associated with increased cardiovascular risk in young adults: the Bogalusa Heart Study. *Metabolism* 2007; 56: 792–798.
44. Siest G, Schiele F, Galteau MM, Panek E, Steinmetz J, Fagnani F, Gueguen R. Aspartate aminotransferase and alanine aminotransferase activities in plasma: statistical distributions, individual variations, and reference values. *Clin Chem* 1975; 21: 1077–1087.
45. Kinugasa A, Tsunamoto K, Furukawa N, Sawada T, Kusunoki T, Shimada N. Fatty liver and its fibrous changes found in simple obesity of children. *J Pediatr Gastroenterol Nutr* 1984; 3: 408–414.
46. Leung DH, Williams K, Fraley JK, Klish WJ. Age- and ethnic-specific elevation of ALT among obese children at risk for non-alcoholic steatohepatitis (NASH): implications for screening. *Clin Pediatr (Phila)* 2009; 48: 50–57.
47. Fraser A, Longnecker MP, Lawlor DA. Prevalence of elevated alanine aminotransferase among US adolescents and associated factors: NHANES 1999–2004. *Gastroenterology* 2007; 133: 1814–1820.
48. Schwimmer JB, Dun W, Norman GJ, Pardee PE, Middleton MS, Kerkar N, Sirlin CB. SAFETY Study: alanine aminotransferase cutoff values are set too high for reliable detection of pediatric chronic liver disease. *Gastroenterology* 2010; Jan 11 [Epub ahead of print].
49. Patton HM, Lavine JE, Van Natta ML, Schwimmer JB, Kleiner D, Molleston J; Nonalcoholic Steatohepatitis Clinical Research Network. Clinical correlates of histopathology in pediatric non-alcoholic steatohepatitis. *Gastroenterology* 2008; 135: 1961–1971.e2.
50. Nobili V, Parkes J, Bottazzo G, Marcellini M, Cross R, Newman D, Vizzutti F, Pinzani M, Rosenberg WM. Performance of ELF serum markers in predicting fibrosis stage in pediatric non-alcoholic fatty liver disease. *Gastroenterology* 2009; 136: 160–167.
51. Nobili V, Vizzutti F, Arena U, Abraldes JG, Marra F, Pietrobattista A, Fruhwirth R, Marcellini M, Pinzani M. Accuracy and reproducibility of transient elastography for the diagnosis of fibrosis in pediatric nonalcoholic steatohepatitis. *Hepatology* 2008; 48: 442–448.
52. Nobili V, Marcellini M, Devito R, Ciampalini P, Piemonte F, Comparcola D, Sartorelli MR, Angulo P. NAFLD in children: a prospective clinical-pathological study and effect of lifestyle advice. *Hepatology* 2006; 44: 458–465.
53. Nobili V, Manco M, Devito R, Di Ciommo V, Comparcola D, Sartorelli MR, Piemonte F, Marcellini M, Angulo P. Lifestyle intervention and antioxidant therapy in children with nonalcoholic fatty liver disease: a randomized, controlled trial. *Hepatology* 2008; 48: 119–128.
54. 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: 739–743.
55. Mummadi RR, Kasturi KS, Chennareddygar S, Sood GK. Effect of Bariatric Surgery on Nonalcoholic Fatty Liver Disease: Systematic Review and Meta-Analysis. *Clin Gastroenterol Hepatol* 2008 Aug 19. [Epub ahead of print]