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Clinical features and metabolic complications for non-alcoholic fatty liver disease (NAFLD) in youth with obesity

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Pediatric obesity has become in the last forty years the most common metabolic disease in children and adolescents affecting about 25% of the pediatric population in the western world. As obesity worsens, a whole-body insulin resistance (IR) occurs. This phenomenon is more pronounced during adolescence, when youth experience a high degree of insulin resistance due the production of growth hormone. As IR progresses, the blunted control of insulin on adipose tissue lipolysis causes an increased flux of fatty acids with FFA deposition in ectopic tissues and organs such as the liver, leading to the development of NAFLD. In this brief review, we will discuss the clinical implications of IR and NAFLD in the context of pediatric obesity. We will review the pathogenesis and the link between these two entities, the major pathophysiologic underpinnings, including the role of genetics and metagenomics, how these two entities lead to the development of type 2 diabetes, and which are the therapeutic options for NAFLD in youth.

KEYWORDS

NAFLD, type 2 diabetes, youth, insulin resistance, genetics

1 Introduction

Nonalcoholic fatty liver (NAFL) is defined as the presence of biopsy-proven fat accumulation in more than 5% of hepatocytes without other causes of intrahepatic fat accumulation (i.e., excessive alcohol assumption, infectious diseases, autoimmunity, and metabolic diseases) (1). The gold standard for diagnosis is liver biopsy, as it allows a more accurate quantitative assessment of hepatocyte fat content. However, in clinical practice, less invasive methods, such as magnetic resonance imaging and liver ultrasound, are performed in pediatric age (1). Nonalcoholic fatty liver disease (NAFLD) refers to a variety of clinicopathological entities, ranging from simple hepatic steatosis (NAFL) to steatohepatitis (NASH), cirrhosis, and end-stage liver disease even at a young age (1). Children and adolescents with overweight and obesity present an increased risk for

developing NAFLD early on, with higher rates in male adolescents. Moreover, NAFLD is characterized by ethnic differences, with Hispanic youth showing higher rates than Non-Hispanic White (NHW) and Non-Hispanic Black (NHB), and the latter group having the lowest prevalence even when severe obesity is present (2).

Early autopsic reports (3) in 742 youth show that in the general pediatric population between age 2 and 19 years the prevalence of NAFLD is about 9.6%, and is the lowest (0.7%) between 2 and 4 years of age and the highest (17.3%) between 15 and 19 years of age (3). NAFLD prevalence increases with the increase of the degree of adiposity, being about 38% in youngsters with obesity (2–4). NAFLD prevalence changes among different ethnicities/races being about 40% among Hispanics, 13% in Non-Hispanic Black (NHB) and 30% in Non-Hispanic White (NHW) (4, 5). Data from Trico et al. show that in NHB individuals with diagnosis of NAFLD show the same degree of disease as NHW and H (2), and a more pronounced degree of insulin resistance as well as higher prevalence of prediabetes and type 2 diabetes (2). The reason for these ethnic/race differences is unknown and may be due to differences in metabolic pathways involved in the pathogenesis of NAFLD (such lipogenesis, adipose tissue lipolysis etc.) or to differences in adipose tissue distribution. Differences in the risk of developing NAFLD exist also between boys and girls, with girls showing a lower risk (3, 5), probably due to the estrogens, that seem to have a protective effect against intrahepatic fat accumulation (6, 7).

There are very few studies assessing the natural history of pediatric onset NAFLD and most of them are retrospective. Despite that, all show that early onset NAFLD has deleterious long-term effects. A landmark retrospective study by Feldstein et al. carried out reviewing the charts of 66 youth (average age 13.9±3.9 years) showed that youth with early onset NAFLD have standardized mortality ratio of 13.6 during the second decade of life compared to the general population of same age and gender (8). The long-term effects of NAFLD also affect insulin action worsening insulin resistance occurring in youth with obesity (9).

2 Methods

We performed a literature search in the PubMed database including the terms: “NAFLD”, “children and adolescents”, “insulin-resistance”, “type 2 diabetes mellitus”. These terms were used in different combinations. Only English language papers were included. Articles were evaluated for scientific relevance and pertinence to the topic. Relevant references from selected papers were included.

3 Pathogenesis of NAFLD

Hepatic fat accumulation represents the main feature of NAFLD (1). Triglyceride's accumulation in the hepatocyte results from the excessive flux of adipose tissue-derived free fatty acids (FFA), enhanced hepatic *de novo* lipogenesis, chylomicron remnants accumulation, and impaired hepatic beta-oxidation (10, 11). The resulting imbalance between FFA intrahepatic concentration and esterification with glycerol to form triglycerides promotes

intrahepatic fat deposition and IR. Not only quantitative fat deposition, but also qualitative lipid composition is a relevant determinant of liver injury progression and NAFLD-related metabolic comorbidities occurrence (12, 13). In fact, excess of omega-6 polyunsaturated fatty acids have been shown to be detrimental for the liver health (14, 15).

Along with nutritional factors, the role of genetic, microbial, metabolic, and other environmental factors has been investigated (16–19). Several common genetic variants have been associated with NAFLD in adults and youth. The I148M variant in the *PNPLA3* gene is the most important genetic determinant of NAFLD (20). It interacts with nutrients, insulin resistance, and visceral adiposity (21–24) to convey susceptibility to the disease. The *PNPLA3* gene encodes for the adiponutrin, a protein expressed in the adipose tissue and the liver with a strong lipolytic activity on phospholipids (20). The I148M substitution causes a loss-of-function with impaired lipase activity, hepatic fat accumulation, and macrovesicular steatosis (25, 26). This variant displays a geographical distribution heterogeneity, being less frequent in individuals of African descents (27). This finding might explain the lower prevalence/incidence of NAFLD in this ethnic group even in presence of severe obesity and IR (27, 28). Another relevant genetic variant is the E167K allele in the Transmembrane 6 superfamily member 2 (*TM6SF2*) gene (29). *TM6SF2* is a 7-domain transmembrane transporter involved in VLDL secretion from the liver (29). When the aminoacid change occurs, the VLDL secretion is impaired leading to hepatic fat accumulation and at the same time conferring a lower cardiovascular risk, given to the lower concentrations of pro-atherogenic lipoproteins (30, 31). The P446L variant in the *GCKR* gene is another recognized common genetic variant associated with NAFLD (32). The *GCKR* encodes for the GCKRP (glucokinase regulatory protein) that binds the glucokinase (GCK) in the nucleus of the hepatocytes. When the *GCKR* the P446L occurs, the mutated protein shows an impaired ability to bind the GK, the consequence being that more GK is available in the cytoplasm to convert the glucose in glucose-6-phosphate, its active form (33). This leads to an enhanced glycolysis and hepatic *de novo* lipogenesis (34). Other variants have been reported in *MBOAT7* (35, 36) and *HSD17B13* (37, 38) genes with minor effect size, but interestingly enough only the variant in the *MBOAT7* gene has been associated also with IR in youth (35).

Gut-liver axis is another important player in the pathogenesis of NAFLD and its progression. The liver may affect the composition of gut microbiota *via* the bile acids and the secretion of antimicrobial compounds in the intestine (39, 40). At the same time, the gut microbiota is involved in nutrient absorption and gut permeability. Therefore, gut dysbiosis might induce hepatic fat deposition and inflammatory insults to the liver. It has been shown that an altered gut microbiota composition and reduced microbiota variety in subjects with NAFLD compared to those without NAFLD. A relative prevalence of Gram-negative to Gram-positive bacteria has been described in NAFLD compared to healthy controls, with an increase of Proteobacteria in more severe NAFLD (41). Another study reported a reduction of Bacteroides in pediatric NAFLD compared to non-affected subjects (42). Moreover, lower microbiota α -diversity has been described in pediatric NAFLD (42–45). The microbiota composition is associated with higher expression of genes encoding inflammatory compounds thus leading to more severe

forms (43). In a recent shotgun metagenomic study of gut microbiota involving youth with obesity with and without NAFLD, the authors found a higher prevalence of genes coding for branched chain aminoacids and short chain fatty acids in the group with NAFLD (46).

4 Mechanisms underlying the association between NAFLD and IR

NAFLD is commonly associated with other metabolic comorbidities including dyslipidemia, and type 2 diabetes mellitus (T2D), with insulin resistance (IR) being the main pathogenic link between these conditions (2, 8). A bidirectional effect between NAFLD and IR has been postulated. In fact, IR is a predictor of NAFLD development in youth, and patients with T2D display higher rates of NAFL, nonalcoholic steatohepatitis (NASH), and advanced fibrosis compared to non-diabetic subjects (47). It has been estimated that about 30% of children with NAFLD might present with prediabetes or T2D (47). Studies investigating IR have demonstrated that NAFL per se is a risk factor for IR independent of the effect of other ectopic fat depots (i.e., visceral adipose tissue and intramyocellular fat depots (48). A study focusing on youth with obesity and NAFLD reported also a decrease in beta-cell function paralleling with hepatic steatosis severity degree (49). The association between NAFLD and IR is mediated by intracellular compounds, namely diacylglycerols (DAG) and ceramides that affect insulin transcription. Early studies by the Shulman lab at Yale University have shown in mice and humans that DAG accumulation impact insulin action in the liver, muscle and adipose tissue, the consequence being higher fasting glucose availability in tissues and plasma (50, 51). In fact, adipose tissue insulin resistance will result in an increased adipose tissue lipolysis with increased glycerol flux to the liver, where it serves as substrate for the gluconeogenesis (accounting for about 40% of neo-gluconeogenesis) (51). IR in the liver will result in an increased glucose production and in the muscle in a reduce glucose uptake. Overall, the increased availability of FFA and glucose in the liver will provide more substrate for the Acetyl-CoA formation and for the hepatic *de novo* lipogenesis, a pathway enhanced in the context of IR (52). From a clinical standpoint the consequence is a higher risk of T2D over time.

5 Therapeutic options for NAFLD in youth

The discovery of GLP-1 has resulted in the development of GLP-1 analogues, initially used to treat T2D in adults and youth and then approved also for the treatment of obesity as they have a strong anorexigenic effect. However, despite evidence showing an effect of semaglutide (53) on NAFLD, this class of medications is not approved for NAFLD, but for obesity (54) and type 2 diabetes (55) in youth. Even though the GLP-1 analogues are indeed game changer in the therapy of obesity and its complications, lifestyle intervention represent the first line of treatment. Nutritional approaches are, in fact, effective in ameliorating NAFLD and IR. A study testing the effect of 8-week low sugar diet (56) and a study testing a low omega 6

to omega 3 PUFA ratio for 12 weeks (12) showed that these nutritional interventions reduced MRI assessed intrahepatic fat of about 30% and had a similar effect on IR. Western diet is characterized by excess of free sugars and imbalance of high omega 6 and low omega 3 PUFA. The increased hepatic uptake of omega 6 PUFA leads to oxidized linoleic acid metabolites production and finally fatty acids accumulation (57, 58). Therefore, modification of dietary fat intake play an important role in NAFLD treatment. In addition, increased intake of certain dietary sugar, in particular fructose, have been associated with increased risk of NAFLD. Fructose is highly lipogenic and is added in processed foods and beverages (59). Low sugar diets are a promising tool for NAFLD therapy. Finally, preclinical studies have suggested that gut microbiome modulation might represent a therapeutic option for NAFLD. Several clinical studies have reported that NAFLD is associated with less microbiome diversity and relative prevalence of Gram-negative bacteria compared to non-NAFLD subjects (60). More recently, systematic review and metanalysis have investigated the effectiveness of probiotics in the treatment of NAFLD. The available RCT are highly heterogeneous in terms of probiotics supplementation, outcome measures, and diagnostic tools for NAFLD. Therefore, even if a decrease in liver enzymes and NAFLD severity has been reported, there is no sufficient evidence to recommend the use of probiotics for NAFLD treatment (60).

6 Conclusions

NAFLD is an important complication of childhood obesity strongly linked to IR and T2D in youth. Given the long-term implications that it can have on the mortality and the morbidity of young individuals it is imperative to find new and more effective preventive and therapeutic strategies for this disease.

Author contributions

EB and GU drafted the manuscript. NS conceptualized the review and reviewed the draft. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

- Chalasan N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American association for the study of liver diseases. *Hepatology* (2018) 67:328–57. doi: 10.1002/hep.29367
- Tricò D, Caprio S, Rosaria Umano G, Pierpont B, Nouws J, Galderisi A, et al. Metabolic features of nonalcoholic fatty liver (NAFL) in obese adolescents: Findings from a multiethnic cohort. *Hepatology* (2018) 68:1376–90. doi: 10.1002/hep.30035
- 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:e561–5. doi: 10.1542/peds.2004-1832
- D'Adamo E, Santoro N, Caprio S. Metabolic syndrome in pediatrics: old concepts revised, new concepts discussed. *Pediatr Clin North Am* (2011) 58:1241–55. doi: 10.1016/j.pcl.2011.07.005
- Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. *Pediatrics* (2006) 118:1388–93. doi: 10.1542/peds.2006-1212
- Vatner DF, Majumdar SK, Kumashiro N, Petersen MC, Rahimi Y, Gattu AK, et al. Insulin-independent regulation of hepatic triglyceride synthesis by fatty acids. *Proc Natl Acad Sci U.S.A.* (2015) 112:1143–8. doi: 10.1073/pnas.1423952112
- Goedeke L, Murt KN, Di Francesco A, Camporez JP, Nasiri AR, Wang Y, et al. Sex- and strain-specific effects of mitochondrial uncoupling on age-related metabolic diseases in high-fat diet-fed mice. *Aging Cell* (2022) 21:e13539. doi: 10.1111/acel.13539
- Feldstein AE, Charatcharoenwithaya P, Treerapratsuk 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) 58:1538–44. doi: 10.1136/gut.2008.171280
- Xanthakos SA, Lavine JE, Yates KP, Schwimmer JB, Molleston JP, Rosenthal P, et al. Progression of fatty liver disease in children receiving standard of care lifestyle advice. *Gastroenterology* (2020) 159:1731–1751.e10. doi: 10.1053/j.gastro.2020.07.034
- Lambert JE, Ramos-Roman MA, Browning JD, Parks EJ. Increased *de novo* lipogenesis is a distinct characteristic of individuals with nonalcoholic fatty liver disease. *Gastroenterology* (2014) 146:726–35. doi: 10.1053/j.gastro.2013.11.049
- Fletcher JA, Deja S, Satapati S, Fu X, Burgess SC, Browning JD. Impaired ketogenesis and increased acetyl-CoA oxidation promote hyperglycemia in human fatty liver. *JCI Insight* (2019) 5(11):e127737. doi: 10.1172/jci.insight.127737
- Van Name MA, Savoye M, Chick JM, Galuppo BT, Feldstein AE, Pierpont B, et al. A low omega-6 to omega-3 PUFA ratio (n-6:n-3 PUFA) diet to treat fatty liver disease in obese youth. *J Nutr* (2020) 150:2314–21. doi: 10.1093/jn/nxaa183
- Trico D, Galderisi A, Van Name MA, Caprio S, Samuels S, Li Z, et al. A low n-6 to n-3 polyunsaturated fatty acid ratio diet improves hyperinsulinemia by restoring insulin clearance in obese youth. *Diabetes Obes Metab* (2022) 24:1267–76. doi: 10.1111/dom.14695
- Puri P, Baillie RA, Wiest MM, Mirshahi F, Choudhury J, Cheung O, et al. A lipidomic analysis of nonalcoholic fatty liver disease. *Hepatology* (2007) 46:1081–90. doi: 10.1002/hep.21763
- Welsh JA, Sharma A, Cunningham SA, Vos MB. Consumption of added sugars and indicators of cardiovascular disease risk among US adolescents. *Circulation* (2011) 123:249–57. doi: 10.1161/CIRCULATIONAHA.110.972166
- Liu Y, Méric G, Havulinna AS, Teo SM, Åberg F, Ruuskanen M, et al. Early prediction of incident liver disease using conventional risk factors and gut-microbiome-augmented gradient boosting. *Cell Metab* (2022) 34:719–730.e4. doi: 10.1016/j.cmet.2022.03.002
- Demir M, Lang S, Hartmann P, Duan Y, Martin A, Miyamoto Y, et al. The fecal microbiome in non-alcoholic fatty liver disease. *J Hepatol* (2022) 76:788–99. doi: 10.1016/j.jhep.2021.11.029
- Yip TC, Vilar-Gomez E, Petta S, Yilmaz Y, Wong GL, Adams LA, et al. Geographical similarity and differences in the burden and genetic predisposition of NAFLD. *Hepatology* (2022) 9(5):957. doi: 10.1002/hep.32774
- Hsieh S, Leaderer BP, Feldstein AE, Santoro N, McKay LA, Caprio S, et al. Traffic-related air pollution associations with cytokeratin-18, a marker of hepatocellular apoptosis, in an overweight and obese paediatric population. *Pediatr Obes* (2018) 13:342–7. doi: 10.1111/jjpo.12228
- Romeo S, Kozlitzina J, Xing C, Pertsemliadis A, Cox D, Pennacchio LA, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* (2008) 40:1461–5. doi: 10.1038/ng.257
- Santoro N, Savoye M, Kim G, Marotto K, Shaw MM, Pierpont B, et al. Hepatic fat accumulation is modulated by the interaction between the rs738409 variant in the PNPLA3 gene and the dietary omega6/omega3 PUFA intake. *PLoS One* (2012) 7:e37827. doi: 10.1371/journal.pone.0037827
- Davis JN, Lê KA, Walker RW, Vikman S, Spruijt-Metz D, Weigensberg MJ, et al. Increased hepatic fat in overweight Hispanic youth influenced by interaction between genetic variation in PNPLA3 and high dietary carbohydrate and sugar consumption. *Am J Clin Nutr* (2010) 92:1522–7. doi: 10.3945/ajcn.2010.30185
- Marzuillo P, Grandone A, Perrone L, del Giudice EM. Weight loss allows the dissection of the interaction between abdominal fat and PNPLA3 (adiponutrin) in the liver damage of obese children. *J Hepatol* (2013) 59:1143–4. doi: 10.1016/j.jhep.2013.06.027
- Goran MI, Walker R, Le KA, Mahurkar S, Vikman S, Davis JN, et al. Effects of PNPLA3 on liver fat and metabolic profile in Hispanic children and adolescents. *Diabetes* (2010) 59:3127–30. doi: 10.2337/db10-0554
- BasuRay S, Wang Y, Smagris E, Cohen JC, Hobbs HH. Accumulation of PNPLA3 on lipid droplets is the basis of associated hepatic steatosis. *Proc Natl Acad Sci U.S.A.* (2019) 116:9521–6. doi: 10.1073/pnas.1901974116
- Wang Y, Kory N, BasuRay S, Cohen JC, Hobbs HH. PNPLA3, CGI-58, and inhibition of hepatic triglyceride hydrolysis in mice. *Hepatology* (2019) 69:2427–41. doi: 10.1002/hep.30583
- Santoro N, Kursawe R, D'Adamo E, Dykas DJ, Zhang CK, Bale AE, et al. A common variant in the patatin-like phospholipase 3 gene (PNPLA3) is associated with fatty liver disease in obese children and adolescents. *Hepatology* (2010) 52:1281–90. doi: 10.1002/hep.23832
- Santoro N, Feldstein AE, Enoksson E, Pierpont B, Kursawe R, Kim G, et al. The association between hepatic fat content and liver injury in obese children and adolescents: effects of ethnicity, insulin resistance, and common gene variants. *Diabetes Care* (2013) 36:1353–60. doi: 10.2337/dc12-1791
- Kozlitzina J, Smagris E, Stender S, Nordestgaard BG, Zhou HH, Tybjaerg-Hansen A, et al. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* (2014) 46:352–6. doi: 10.1038/ng.2901
- Holmen OL, Zhang H, Fan Y, Hovelson DH, Schmidt EM, Zhou W, et al. Systematic evaluation of coding variation identifies a candidate causal variant in TM6SF2 influencing total cholesterol and myocardial infarction risk. *Nat Genet* (2014) 46:345–51. doi: 10.1038/ng.2926
- Dongiovanni P, Petta S, Maglio C, Fracanzani AL, Pipitone R, Mozzi E, et al. Transmembrane 6 superfamily member 2 gene variant disentangles nonalcoholic steatohepatitis from cardiovascular disease. *Hepatology* (2015) 61:506–14. doi: 10.1002/hep.27490
- Santoro N, Zhang CK, Zhao H, Pakstis AJ, Kim G, Kursawe R, et al. Variant in the glucokinase regulatory protein (GCKR) gene is associated with fatty liver in obese children and adolescents. *Hepatology* (2012) 55:781–9. doi: 10.1002/hep.24806
- Beer NL, Tribble ND, McCulloch LJ, Roos C, Johnson PR, Orho-Melander M, et al. The P446L variant in GCKR associated with fasting plasma glucose and triglyceride levels exerts its effect through increased glucokinase activity in liver. *Hum Mol Genet* (2009) 18:4081–8. doi: 10.1093/hmg/ddp357
- Santoro N, Caprio S, Pierpont B, Van Name M, Savoye M, Parks EJ. Hepatic *De novo* lipogenesis in obese youth is modulated by a common variant in the GCKR gene. *J Clin Endocrinol Metab* (2015) 100:E1125–32. doi: 10.1210/jc.2015-1587
- Umano GR, Caprio S, Di Sessa A, Chalasan N, Dykas DJ, Pierpont B, et al. The rs626283 variant in the MBOAT7 gene is associated with insulin resistance and fatty liver in Caucasian obese youth. *Am J Gastroenterol* (2018) 113:376–83. doi: 10.1038/ajg.2018.1
- Teo K, Abeysekera KWM, Adams L, Aigner E, Anstee QM, Banales JM, et al. rs641738C>T near MBOAT7 is associated with liver fat, ALT and fibrosis in NAFLD: A meta-analysis. *J Hepatol* (2021) 74:20–30. doi: 10.1016/j.jhep.2020.08.027
- Abul-Husn NS, Cheng X, Li AH, Xin Y, Schurmann C, Stevis P, et al. A protein-truncating HSD17B13 variant and protection from chronic liver disease. *N Engl J Med* (2018) 378:1096–106. doi: 10.1056/NEJMoa1712191
- Di Sessa A, Umano GR, Cirillo G, Marzuillo P, Arienzo MR, Pedullà M, et al. The rs72613567: TA variant in the hydroxysteroid 17-beta dehydrogenase 13 gene reduces liver damage in obese children. *J Pediatr Gastroenterol Nutr* (2020) 70:371–4. doi: 10.1097/MPG.0000000000002573
- Tripathi A, Debelius J, Brenner DA, Karin M, Loomba R, Schnabl B, et al. The gut-liver axis and the intersection with the microbiome. *Nat Rev Gastroenterol Hepatol* (2018) 15:397–411. doi: 10.1038/s41575-018-0011-z
- Hrcir T, Hrcirova L, Kverka M, Hromadka R, Machova V, Trckova E, et al. Gut microbiota and NAFLD: Pathogenetic mechanisms, microbiota signatures, and therapeutic interventions. *Microorganisms* (2021) 9(5):957. doi: 10.3390/microorganisms9050957
- Loomba R, Seguritan V, Li W, Long T, Klitgord N, Bhatt A, et al. Gut microbiome-based metagenomic signature for non-invasive detection of advanced fibrosis in human nonalcoholic fatty liver disease. *Cell Metab* (2019) 30:607. doi: 10.1016/j.cmet.2019.08.002
- Del Chierico F, Nobili V, Vernocchi P, Russo A, De Stefanis C, Gnani D, et al. Gut microbiota profiling of pediatric nonalcoholic fatty liver disease and obese patients unveiled by an integrated meta-omics-based approach. *Hepatology* (2017) 65:451–64. doi: 10.1002/hep.28572
- Schwimmer JB, Johnson JS, Angeles JE, Behling C, Belt PH, Borecki I, et al. Microbiome signatures associated with steatohepatitis and moderate to severe fibrosis in children with nonalcoholic fatty liver disease. *Gastroenterology* (2019) 157:1109–22. doi: 10.1053/j.gastro.2019.06.028
- Stanislowski MA, Lozupone CA, Wagner BD, Eggesbø M, Sontag MK, Nusbacher NM, et al. Gut microbiota in adolescents and the association with fatty liver: the EPOCH study. *Pediatr Res* (2018) 84:219–27. doi: 10.1038/pr.2018.32
- Monga Kravetz A, Testerman T, Galuppo B, Graf J, Pierpont B, Siebel S, et al. Effect of gut microbiota and PNPLA3 rs738409 variant on nonalcoholic fatty liver disease (NAFLD) in obese youth. *J Clin Endocrinol Metab* (2020) 105:e3575–85. doi: 10.1210/clinem/dgaa382

46. Testerman T, Li Z, Galuppo B, Graf J, Santoro N. Insights from shotgun metagenomics into bacterial species and metabolic pathways associated with NAFLD in obese youth. *Hepatol Commun* (2022) 6:1962–74. doi: 10.1002/hep4.1944
47. Newton KP, Hou J, Crimmins NA, Lavine JE, Barlow SE, Xanthakos SA, et al. Prevalence of prediabetes and type 2 diabetes in children with nonalcoholic fatty liver disease. *JAMA Pediatr* (2016) 170:e161971. doi: 10.1001/jamapediatrics.2016.1971
48. D'Adamo E, Cali AM, Weiss R, Santoro N, Pierpont B, Northrup V, et al. Central role of fatty liver in the pathogenesis of insulin resistance in obese adolescents. *Diabetes Care* (2010) 33:1817–22. doi: 10.2337/dc10-0284
49. Cali AM, De Oliveira AM, Kim H, Chen S, Reyes-Mugica M, Escalera S, et al. Glucose dysregulation and hepatic steatosis in obese adolescents: is there a link? *Hepatology* (2009) 49:1896–903. doi: 10.1002/hep.22858
50. Samuel VT, Shulman GI. Nonalcoholic fatty liver disease as a nexus of metabolic and hepatic diseases. *Cell Metab* (2018) 27:22–41. doi: 10.1016/j.cmet.2017.08.002
51. Samuel VT, Shulman GI. The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux. *J Clin Invest* (2016) 126:12–22. doi: 10.1172/JCI77812
52. Perry RJ, Camporez JG, Kursawe R, Titchenell PM, Zhang D, Perry CJ, et al. Hepatic acetyl CoA links adipose tissue inflammation to hepatic insulin resistance and type 2 diabetes. *Cell* (2015) 160:745–58. doi: 10.1016/j.cell.2015.01.012
53. Newsome PN, Buchholtz K, Cusi K, Linder M, Okanoue T, Ratziu V, et al. A placebo-controlled trial of subcutaneous semaglutide in nonalcoholic steatohepatitis. *N Engl J Med* (2021) 384:1113–24. doi: 10.1056/NEJMoa2028395
54. Kelly AS, Auerbach P, Barrientos-Perez M, Gies I, Hale PM, Marcus C, et al. Controlled trial of liraglutide for adolescents with obesity. *N Engl J Med* (2020) 382:2117–28. doi: 10.1056/NEJMoa1916038
55. Tamborlane WV, Barrientos-Pérez M, Fainberg U, Frimer-Larsen H, Hafez M, Hale PM, et al. Liraglutide in children and adolescents with type 2 diabetes. *N Engl J Med* (2019) 381:637–46. doi: 10.1056/NEJMoa1903822
56. Schwimmer JB, Ugalde-Nicalo P, Welsh JA, Angeles JE, Cordero M, Harlow KE, et al. Effect of a low free sugar diet vs usual diet on nonalcoholic fatty liver disease in adolescent boys: A randomized clinical trial. *Jama* (2019) 321:256–65. doi: 10.1001/jama.2018.20579
57. Santoro N, Caprio S, Giannini C, Kim G, Kursawe R, Pierpont B, et al. Oxidized fatty acids: a potential pathogenic link between fatty liver and type 2 diabetes in obese adolescents? *Antioxid Redox Signal* (2014) 20(2):383–9. doi: 10.1089/ars.2013.5466
58. Trico D, Di Sessa A, Caprio S, Chalasani N, Liu W, Liang T, et al. Oxidized derivatives of linoleic acid in pediatric metabolic syndrome: is their pathogenic role modulated by the genetic background and the gut microbiota? *Antioxid Redox Signal* (2017) 30(2):241–50. doi: 10.1089/ars.2017.7049
59. Vos MB, Lavine JE. Dietary fructose in nonalcoholic fatty liver disease. *Hepatology* (2013) 57(6):2525–31. doi: 10.1002/hep.26299
60. Sharpton SR, Maraj B, Harding-Theobald E, Vittinghoff E, Terrault NA. Gut microbiome-targeted therapies in nonalcoholic fatty liver disease: a systematic review, meta-analysis, and meta-regression. *Am J Clin Nutr* (2019) 110(1):139–49. doi: 10.1093/ajcn/nqz042