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Advances in paediatric nonalcoholic fatty liver disease: Role of lipidomics

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Abstract

Due its close relationship with obesity, nonalcoholic fatty liver disease (NAFLD) has become a major worldwide health issue even in childhood. The most accepted pathophysiological hypothesis is represented by the "multiple hits" theory, in which both hepatic intracellular lipid accumulation and insulin resistance mainly contribute to liver injury through several factors. Among these, lipotoxicity has gained particular attention. In this view, the pathogenic role of different lipid classes in NAFLD (*e.g.,* sphingolipids, fatty acids, ceramides, *etc.*) has been highlighted in recent lipidomics studies. Although there is some contrast between plasma and liver findings, lipidomic profile in the NAFLD context provides novel insights by expanding knowledge in the intricate field of NAFLD pathophysiology as well as by suggesting innovative therapeutic approaches in order to improve both NAFLD prevention and treatment strategies. Selective changes of distinct lipid species might be an attractive therapeutic target for treating NAFLD. Herein the most recent evidence in this attractive field has been summarized to provide a comprehensive overview of the lipidomic scenario in paediatric NAFLD.

Key Words: Fatty; Liver; Lipidomics; Children; Nonalcoholic fatty liver disease

Core Tip: Insightful data from lipidomics studies have recently expanded knowledge about nonalcoholic fatty liver disease pathophysiology. In fact, different lipids have been found to exert specific pathogenic roles in liver injury through several pathways

(in particular by impairing insulin signalling). Given the cardiometabolic burden of nonalcoholic fatty liver disease even in childhood, lipidomics findings might improve strategies for nonalcoholic fatty liver disease treatment by providing novel therapeutic targets.

INTRODUCTION

An increasing prevalence of nonalcoholic fatty liver disease (NAFLD) up to 25% has been found in adults, but alarming paediatric data have also been reported[1-3]. Due to the obesity epidemic, NAFLD has become the most prevalent liver chronic disease in childhood, affecting 3%-10% of the general paediatric population and up to 50% of obese children with a relevant cardiometabolic burden[2,4]. It includes different degree of hepatic steatosis ranging from simple fat accumulation to steatohepatitis and fibrosis, but its pathogenesis remains to be fully elucidated[1].

To date, "multiple hits" have been recognized in the NAFLD pathophysiology, with a pivotal role of the hepatic intracellular lipid accumulation and insulin resistance favouring liver damage through several factors such as lipotoxicity, oxidative stress, inflammation, genetics, gut axis, metabolic, and dietary factors[3,5]. Among these pathogenic factors, lipotoxicity has gained particular remarkable attention[6]. Lipid induced oxidative stress, inflammation, and cell death have been largely studied as major players of this process, and their interplay represent a critical step in both NAFLD development and progression[6-8]. Of note, it must be considered that the chronic inflammation closely related to lipotoxicity represents one of the most important features of several metabolic diseases such as obesity and type 2 diabetes, resulting in a dangerous "vicious circle" with dramatic clinical implications[6]. In fact, lipotoxicity affects a broad range of tissues such as adipose tissue, heart, brain, pancreatic islets, and skeletal muscle with a complex interrelation favouring the development of metabolic syndrome[6].

Although intrahepatic fat accumulation has been widely accepted as the hallmark of NAFLD, overwhelming evidence showed that both quantity and quality of accumulated hepatic lipids play a pathogenic role in NAFLD[7,9-12]. In particular, specific lipid classes such as sphingosine, diacylglycerols, and ceramides act as liver damaging agents through multiple pathways[6,7,9,13,14].

To date, the growing interest in lipidomic studies has provided meaningful data regarding lipid profiles involved in the pathogenesis of liver injury and its modulation as a potential therapeutic target[8,11,12-14]. In view of the clinical relevance of these findings in the NAFLD treatment scenario, evidence is currently available in adults and in children[15-19] (Tables 1 and 2).

We aimed to summarize the most recent findings regarding lipidomic studies in paediatric NAFLD by providing an overview of the different lipid class and their potential therapeutic implications.

LIPIDS IN NAFLD

Triacylglycerols (TAG) are the most representative lipidic class accumulated in the liver of NAFLD patients. Nevertheless, they would seem to be protective against lipotoxicity due to lipid overload. Lipotoxicity is mainly caused by increasing levels of saturated fatty acids (SFAs), free cholesterol, glycerophospholipids, sphingolipids, and deficient levels of phospholipids, u-3 polyunsaturated fatty acids (PUFAs), or PUFA-derived specialized proresolving mediators[20,21]. Monounsaturated fatty acids (MUFAs), lysophosphatidylcholine (LPC), and ceramide are also increased while phosphatidylcholine (PC) is reduced in nonalcoholic steatohepatitis (NASH)[22,23].

Table 1 Comparison between adult and paediatric lipidomic findings in nonalcoholic fatty liver disease

Lipid class	Changes in adult NAFLD patients	Changes in paediatric NAFLD patients
SFAs	Increased in liver[31] and in plasma[24]	Increased in liver and in plasma[32]
MUFAs	Increased in liver[35,36] and in plasma[25]	Increased in liver and in plasma[32]
PUFAs	Increased total PUFAs in liver[31] and n-6 LCPUFA in liver phospholipids[35]. Decreased LCPUFA of the n-6 and n-3 series in liver TAG, n-3 LCPUFA in phospholipids, total PUFA[35,36], n-3 PUFA[35], n-6 PUFA[32]	Increased in liver and in plasma[32]
PUFAs derived	Increased 5-HETE, 8-HETE, 11-HETE, and 15-HETE in NAFLD and NASH patients[27]. Increased 11,12-diHETrE, dhk PGD2, 20-COOH AA in NASH patients[26]	Increased EDPs, EEQs, EETs with progression of steatosis; reduced with progression of fibrosis[32]
TAG	Increased in liver[31] and in plasma[25]	Increased (TG[O]); TG (O-52:0), TG (O-52:1), TG (O-52:2), TG (O-52:3), TG (O-54:1), TG (O-54:2), TG (O-56:1) and TG (O-56:2) in serum[19]
DAG	Increased in liver[31] and in plasma[25]	No available data
FC	Increased in liver[31]	No available data
PC	Reduced in the liver[31], conflicting data for changes in serum [28,29]	Reduced serum alkyl/alkenyl-phosphatidylcholine (PC[O]) levels[19]
LPC	No statistically significant changes in plasma and serum[28,29]	No available data
PE	Decreased in liver[22] and increased in serum of NASH patients[28]	Increased PE in serum[19]
LPE	Decreased in serum of patients with NAFLD and NASH[28]	Increased LPE (20:3) and LPE (22:5); decreased [LPE(O)]) in serum[19]
PS	Reduced in liver[22], increased in plasma[29]	No available data
PI	Reduced in liver[22], increased in plasma[29]	No available data
PL	No change on liver[56]; decreased in plasma of NASH patients [25,57]	No available data
SM	Conflicting results in NAFLD and NASH patients[22,28,29,31,37,51]	Increased SM (d39:0), SM (d41:0) in serum[19]
CE	Increased in liver and in plasma[51,64,65]	Increased in serum[20]

20-COOH AA: 20-carboxy arachidonic acid; CE: Ceramides; DAG: Diacylglycerols; dhk PGD2: 13,14-dihydro-15-keto prostaglandin D2; diHETrE: Dihydroxy-eicosatrienoic acid; EDP: Epoxyeicosapentaenoic acid; EET: Epoxyeicosatrienoic acid; EEQ: Epoxyeicosatetraenoic acid; FC: Free cholesterol; HETE: Hydroxyeicosatetraenoic acid; LCPUFA: Long chain polyunsaturated fatty acid; LPC: Lysophosphatidylocholine; LPE: Lysophosphatidylethanolamine; LPE(O): Alkyl/alkenyl-lysophosphatidylethanolamine; MUFAs: Monounsaturated fatty acids; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis; PC: Phosphatidylcholine; PE: Phosphatidylethanolamine; PI: Phosphatidylinositol; PL: Plasmalogens; PS: Phosphatidylserine; PUFAs: Polyunsaturated fatty acids; SFAs: Saturated fatty acids; SM: Sphingomyelin; TAG: Triacylglycerols; TG(O): Alkyl-diacylglycerols.

Of interest, lipidomic studies conducted in both plasma and serum of NAFLD patients reported overlapping results with those found in the liver. In addition, increased levels of total SFA in phospholipids[24], metabolites of lipoxygenase, 5-hydroxyeicosatetraenoic acid (HETE), 8-HETE[25], 15-HETE, 5,6 dihydroxy- eicosatrienoic acid[26], palmitoleic acid in cholesteryl ester[27], PC and sphingomyelin[28], phosphatidylserine, and phosphatidylinositol[29] were found in plasma and serum of NAFLD patients. On the other hand, decreased levels of eicosanoic acid (C20: 0), cis-11-octadecenoic acid (C18: 1n-7), docosahexaenoic acid in phospholipids[24], 12,13-dihydroxy-9-octadecenoic acid[26], and lysophospatidylethanolamine[28] have been described.

Fatty acids
The increased share of free fatty acids reaching the liver in NAFLD has been related to three main mechanisms: lipolysis of adipose tissue, *de novo* lipogenesis, and diet[30].

Both hepatic[31] and plasma[24] findings from adults and children[32] with NAFLD showed an increased content of SFAs. Of concern, SFAs seem to be one of the major players involved in lipotoxicity. In fact, evidence linked inhibition (genetic or pharmacological) of the enzyme converting SFAs to MUFAs, namely stearoyl-CoA desaturase-

Table 2 Main findings of lipidomic studies in paediatric nonalcoholic fatty liver disease

Ref.	Study design and methods	Population (*n*)	Main findings
Wasilewska *et al*[20], 2018	Prospective study	80 children at median age 12 (7-17 yr)	Higher total serum CE concentration in NAFLD patients, compared to the controls and of certain CEs (C14:0, C16:0, C16:1, C18:0, C18:1, C22:0, C24:0). Total CE concentration was positively correlated with HOMA-IR and insulin levels
Draijer *et al* [8], 2020	Case-control study	21 children with obesity and steatosis and 21 with only obesity. Mean age of NAFLD patients: 14.8 yr; mean age of non-NAFLD patients 14.7 yr	Statistically significant alterations in 5 major lipid classes [TG(O), PE, PE(O), LPE(O), PC(O)] and 12 individual lipid species
Kalveram *et al* [32], 2021	Prospective study	40 children with biopsy-proven NAFLD. Mean age 14.2 ± 2.3 yr	Hepatic epoxyeicosanoids levels increased with higher degrees of steatosis. CYP epoxygenase activity increased, protein level, and activity of sEH decreased. In contrast, hepatic epoxyeicosanoids decreased with higher stages of fibrosis, with a decrease of CYP epoxygenase activity and protein expression

CE: Ceramides; CYP: Cytochrome P450; HOMA-IR: Homeostasis model assessment; NAFLD: Nonalcoholic fatty liver disease; LPE(O): Alkyl/alkenyl-lysophosphatidylethanolamine; PC(O): Alkyl/alkenyl-phosphatidylcholine; PE: Phosphatidylethanolamine; PE(O): Alkyl/alkenyl-phosphatidylethanolamine; sEH: Soluble epoxide hydrolase; TG(O): Alkyl-diacylglycerols.

1, to different processes such as hepatocyte apoptosis, lipotoxicity, and development of steatohepatitis[33]. Consequently, balancing between MUFAs and SFAs might represent a central player in the progression from isolated hepatic steatosis to progressive steatohepatitis and fibrosis[34].

MUFAs were also found to be increased in liver and plasma of both adult and paediatric patients with NAFLD[25,32,35-37]. This lipid class has been considered as less lipotoxic than SFAs because channelling free fatty acids into MUFAs allow their incorporation into triglycerides and storage in lipid droplets[34].

The long chain PUFAs such as eicosapentaenoic, docosahexanoic, and arachidonic acids were decreased in liver and plasma of patients with NAFLD[29,31,35,36]. This reduction could be due to impairments in dietary intake or the biosynthesis process. A pivotal pathogenic role in NAFLD progression (from simple steatosis to NASH) has been attributed to decreased activity of fatty acid desaturase 1, an enzyme involved in the PUFAs metabolism[6].

Through the activity of hepatic cytochrome P450 enzymes, PUFAs derived from monoepoxides are collectively termed epoxyeicosanoids[38-40]. Those deriving from arachidonic acid (epoxyeicosatrienoic acid), eicosapentaenoic acid (epoxyeicosatetraenoic acid), and docosahexaenoic acid (epoxyeicosapentaenoic acid) have anti-inflammatory, antisteatotic, and antifibrotic properties[41]. In a recent paediatric study [32], 40 youths with biopsy-proven NAFLD underwent lipidomic evaluations by analysing liver tissue and blood samples. Upregulated hepatic epoxyeicosatrienoic acid, epoxyeicosatetraenoic acid, and epoxyeicosapentaenoic acid levels were found in children with steatosis. This might be due to reduced activity and protein expression of soluble epoxide hydrolase, metabolizing epoxyeicosanoids to vicinal diols. On the contrary, at the stage of fibrosis the aforementioned epoxyeicosanoids were found to be decreased in liver and plasma because of a potential reduction of cytochrome P450 epoxygenase expression. Therefore, the cytochrome P450 epoxygenase/soluble epoxide hydrolase pathway seem to represent a potential innovative pharmacologic target for NAFLD treatment.

Proinflammatory molecules are also derived from PUFAs. Puri *et al*[25] found increased plasma levels of arachidonic acid (5-HETE, 8-HETE, 11-HETE, and 15-HETE) lipoxygenase metabolites in NAFLD and NASH patients compared to lean normal controls. Moreover, plasma arachidonic acid-derived metabolites 11,12-dihydroxy-eicosatrienoic acid, 13,14-dihydro-15-keto prostaglandin D2, and 20-carboxy arachidonic acid levels were found to be significantly increased by Loomba *et al*[26] in subjects with NASH than those with NAFLD.

With respect to the wide cardiometabolic burden of NAFLD, changes in FA metabolism have also been linked to its related comorbidities including obesity, diabetes, and cardiovascular risk[20,28,29].

Neutral lipids
The hallmark of NAFLD is the accumulation of lipid droplets in the hepatocytes containing TAG[7]. TAGs were found to be increased in both plasma and liver of

patients with NAFLD compared to healthy controls[25,31]. They represent a less toxic form of storing lipids. The inhibition of diacylglycerol acyltransferase 2, the enzyme catalysing the final step in the assembly of TAG, reduced steatosis but at the same time increased hepatic free fatty acids, lipid peroxidation, oxidative stress, necroinflammation, and fibrosis[42]. In mice defective for perilipin-5, a protein binding lipid droplet and regulating TAG storage, the reduction in the size of lipid droplets caused increased lipolysis and lipotoxicity[43].

Diacylglycerols (DAG) were also increased in plasma and liver of patients with NAFLD[25,31], and the ratio of TAG/diacylglycerols seemed to increase in the evolution from NAFLD to NASH[31].

In a paediatric study, Draijer *et al*[8] performed lipidomic analyses in plasma samples of 21 children with obesity and proton magnetic resonance spectroscopy-detected hepatic steatosis compared to the lipidome of 21 samples of nonsteatotic subjects with obesity. The authors found an overall significant increase in NAFLD patients of serum alkyl-diacylglycerols [TG(O)], in particular 8 TG(O) species (TG(O-52:0), (TG(O-52:1) TG(O-52:2), TG(O-54:1), TG(O-54:2), TG(O-52:3), TG(O-56:1) and TG(O-56:2)].

Finally, it should also be noted that the amount of hepatic free cholesterol increases with the progression to NASH, without an increase in cholesterol esters[31]. It is considered a cytotoxic lipid disrupting membrane integrity and inducing oxidative stress, mitochondrial dysfunction, and apoptosis[44].

Glycerophospholipids

Glycerophospholipids represent a significant lipidic fraction of the cell membrane. Reduced hepatic PC levels were observed in both NAFLD and NASH subjects[22,31]. However, conflicting data about alterations in serum were reported[6]. Low hepatic levels of PC influenced circulating very low-density lipoproteins, which are therefore reduced with consequent hepatic accumulation of TAG[45,46]. Low levels also increased de novo lipogenesis and formation of lipid droplets in hepatocytes by activation of sterol regulatory element-binding protein 1[47].

Liver phosphatidylethanolamine (PE) content was found to be decreased among subjects with NASH[22], while serum PE increased[28]. The enzyme phosphatidylethanolamine n-methyltransferase catalyses the reaction converting PE to PC. Studies reported that a loss-of-function polymorphism in the phosphatidylethanolamine n-methyltransferase gene predisposed to NAFLD susceptibility[48]. Lower hepatic PC/PE ratio was also reported in NAFLD individuals. Interestingly, a reduced PC/PE ratio in red blood cell membranes has been found to enhance predisposition to NAFLD[49]. As a consequence, loss of membrane integrity and higher permeability to proinflammatory factors were observed[50].

Paediatric data reported significantly increased PE serum levels and reduced specific etherphospholipid classes such as alkyl/alkenyl-phosphatidylethanolamine, alkyl/alkenyl-lysophosphatidylethanolamine [LPE(O)], and alkyl/alkenyl-phosphatidylcholine in subjects with NAFLD. When looking at individual lipid species, two LPE species such as LPE (20:3) and LPE (22:5) were found to be increased[19].

Phosphatidylserine and phosphatidylinositol were decreased in the liver[22] but increased in the plasma[29] of NAFLD patients. However, these results are conflicting in other studies[25,28,51].

LPC was increased in the liver of NASH patients[6], while no statistically significant changes in plasma and serum of LPC content were reported in patients with NAFLD or NASH[28,29]. LPC derived from PC by the action of lipoprotein associated phospholipase A2 at the intracellular level, whereas in the extracellular milieu by lecithincholesterol acyltransferase activity. In patients with NAFLD, phospholipase A2 levels were found to be decreased, while those of lecithincholesterol acyltransferase increased[52,53]. LPC downregulates genes involved in fatty acid oxidation, upregulates genes involved in cholesterol biosynthesis, and promotes apoptosis of hepatocytes[54]. Inhibitors of phospholipase A2 decreased palmitate-induced lipotoxicity and cell apoptosis[54,55].

In the liver of NAFLD patients no change in plasmalogen content was reported[56], while this class was decreased in the plasma of NASH patients[25,57]. Animal data demonstrated that a specific mechanism (involving peroxisome proliferator-activated receptor-alpha) sustained by endogenous hepatic plasmalogens may prevent liver steatosis and NASH[58].

Circulating plasmalogen levels, particularly 16:0 and 18:1, were found to be reduced in NAFLD individuals with the GG-genotype of patatin-like phospholipase domain-containing 3 (*PNPLA3*) compared to those with the C or CG allele[28]. The *PNPLA3* gene is highly expressed in hepatic stellate cells of the liver and adipose tissue and

encodes adiponutrin, a protein exerting both lipase and acyltransferase activity[59]. Adiponutrin variant p.I148M [rs738409 (G)] enhanced PUFA content of TAGs and diacylglycerols and negatively affected both PC synthesis and lipid droplet hydrolysis [60].

An elegant paediatric study examining NAFLD genetic factors demonstrated that *PNPLA3* rs738409 (G) represented the strongest determinant of the presence of NAFLD as compared to healthy controls and conferred the highest risk of severity of steatosis. Interestingly, a specific steatosis pattern (including an increased percentage of portal inflammation) was reported in homozygous *PNPLA3* rs738409 (G) patients [61].

In addition to NAFLD, a significant association of these compounds has been found in a larger cardiometabolic-related context such as obesity and cardiovascular disease [51,57].

Sphingolipids

Sphingolipids are structural components of cellular membranes and signalling molecules in mammalian cells. Conflicting results were found about sphingomyelin (SM) trends in NAFLD and NASH patients[22,28,29,37,51]. Barr *et al*[62] found an increase in serum levels of certain sphingomyelin species, such as SM (36:3), (d18:2/16:0), (d18:2/14:0), (d18:1/18:0), (d18:1/16:0), (d18:1/12:0), and (d18:0/16:0) in NAFLD individuals compared to controls. Instead, reduced circulating levels of SM (d18:1/24:1), SM (d18:1/16:0), SM (d18:1/22:0), SM (d18:1/24:0), SM (d18:1/18:0), SM (d18:1/20:0), SM (d18:1/23:0), SM (d18:0/16:0), and SM (d18:0/20:4) were observed by Zhou *et al*[63] in NASH adult subjects compared to controls. Moreover, increased serum levels of two SM species such as SM(d39:0) and SM (d41:0) were found in the serum of NAFLD paediatric patients[19].

Higher ceramide levels were found in plasma and liver biopsies of NAFLD subjects [51,64,65]. These lipids decreased insulin sensitivity in skeletal muscle and hepatocytes [66] and enhanced several unfavourable biological processes such as oxidative stress, mitochondrial dysfunction, and cell apoptosis[66,67]. Moreover, they seem to regulate the synthesis of high-density lipoproteins. Animal data reported that myriocin, acting through ceramide biosynthesis inhibition–promoted insulin receptor and steatosis and enhanced apolipoprotein AI production rate, resulting in an increased high-density lipoprotein production rate[68].

In a prospective study[20] including 80 obese children, total ceramide concentration was significantly increased in the serum of obese and NAFLD patients than in the reference group. In addition, increased levels of distinct fatty acid ceramides, such as myristic, palmitic, palmitoleic, stearic, oleic, behenic, and lignoceric were observed in children with NAFLD compared to controls. Furthermore, a significant positive association of total ceramide levels with homeostasis model assessment and insulin levels was reported[20].

Taken together, these findings might pave the way for a wider risk assessment for these patients, as suggested by paediatric evidence indicating a significant association of distinct sphingolipids with NAFLD and with its cardiometabolic burden including obesity, cardiovascular disease, and metabolic derangements[20,69-71].

CONCLUSION

Lipidomic studies have added novelty by allowing an accurate characterization of lipidomic profile of both plasma and liver tissues in NAFLD[7,12,17]. Besides experimental data providing additional insights about the pathophysiology of NAFLD and its progression, there is a growing body of evidence from human studies[8,14,20]. In particular, a clear effect for specific ceramides in impairing insulin signalling pathways has been found[10,13,15].

Interestingly, different lipid classes have been demonstrated to exert pathogenic distinct roles in NAFLD and in other metabolic diseases such as obesity, metabolic syndrome, and type 2 diabetes[14,15]. Thus, manipulation of the expression of certain lipids (*e.g.,* selective lowering of specific ceramides) might represent a novel target for both prevention and treatment of these diseases. In fact, this attractive therapeutic approach might pave the way for novel strategies to counteract the increasing NAFLD-related cardiometabolic burden even in childhood.

Further research is needed to validate these findings and to provide a more comprehensive assessment of the exact pathogenic role of specific lipids in the NAFLD context.

REFERENCES

1   **Nobili V**, Alisi A, Valenti L, Miele L, Feldstein AE, Alkhouri N. NAFLD in children: new genes, new diagnostic modalities and new drugs. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 517-530 [PMID: 31278377 DOI: 10.1038/s41575-019-0169-z]

2   **Morandi A**, Di Sessa A, Zusi C, Umano GR, El Mazloum D, Fornari E, Miraglia Del Giudice E, Targher G, Maffeis C. Nonalcoholic Fatty Liver Disease and Estimated Insulin Resistance in Obese Youth: A Mendelian Randomization Analysis. *J Clin Endocrinol Metab* 2020; **105** [PMID: 32841326 DOI: 10.1210/clinem/dgaa583]

3   **Goldner D**, Lavine JE. Nonalcoholic Fatty Liver Disease in Children: Unique Considerations and Challenges. *Gastroenterology* 2020; **158**: 1967-1983.e1 [PMID: 32201176 DOI: 10.1053/j.gastro.2020.01.048]

4   **Di Bonito P**, Valerio G, Licenziati MR, Miraglia Del Giudice E, Baroni MG, Morandi A, Maffeis C, Campana G, Spreghini MR, Di Sessa A, Morino G, Crinò A, Chiesa C, Pacifico L, Manco M. High uric acid, reduced glomerular filtration rate and non-alcoholic fatty liver in young people with obesity. *J Endocrinol Invest* 2020; **43**: 461-468 [PMID: 31637675 DOI: 10.1007/s40618-019-01130-6]

5   **Di Sessa A**, Umano GR, Cirillo G, Marzuillo P, Arienzo MR, Pedullà M, Miraglia Del Giudice E. The rs72613567: TA Variant in the Hydroxysteroid 17-beta Dehydrogenase 13 Gene Reduces Liver Damage in Obese Children. *J Pediatr Gastroenterol Nutr* 2020; **70**: 371-374 [PMID: 31789772 DOI: 10.1097/MPG.0000000000002573]

6   **Marra F**, Svegliati-Baroni G. Lipotoxicity and the gut-liver axis in NASH pathogenesis. *J Hepatol* 2018; **68**: 280-295 [PMID: 29154964 DOI: 10.1016/j.jhep.2017.11.014]

7   **Kartsoli S**, Kostara CE, Tsimihodimos V, Bairaktari ET, Christodoulou DK. Lipidomics in non-alcoholic fatty liver disease. *World J Hepatol* 2020; **12**: 436-450 [PMID: 32952872 DOI: 10.4254/wjh.v12.i8.436]

8   **Draijer LG**, Froon-Torenstra D, van Weeghel M, Vaz FM, Bohte AE, Holleboom AG, Benninga MA, Koot BGP. Lipidomics in Nonalcoholic Fatty Liver Disease: Exploring Serum Lipids as Biomarkers for Pediatric Nonalcoholic Fatty Liver Disease. *J Pediatr Gastroenterol Nutr* 2020; **71**: 433-439 [PMID: 32947564 DOI: 10.1097/MPG.0000000000002875]

9   **Samuel VT**, Shulman GI. Nonalcoholic Fatty Liver Disease, Insulin Resistance, and Ceramides. *N Engl J Med* 2019; **381**: 1866-1869 [PMID: 31693811 DOI: 10.1056/NEJMcibr1910023]

10   **Pei K**, Gui T, Kan D, Feng H, Jin Y, Yang Y, Zhang Q, Du Z, Gai Z, Wu J, Li Y. An Overview of Lipid Metabolism and Nonalcoholic Fatty Liver Disease. *Biomed Res Int* 2020; **2020**: 4020249 [PMID: 32733940 DOI: 10.1155/2020/4020249]

11   **Mann JP**, Feldstein AE, Nobili V. Update on lipid species and paediatric nonalcoholic fatty liver disease. *Curr Opin Clin Nutr Metab Care* 2017; **20**: 110-116 [PMID: 27906700 DOI: 10.1097/MCO.0000000000000346]

12   **Preuss C**, Jelenik T, Bódis K, Müssig K, Burkart V, Szendroedi J, Roden M, Markgraf DF. A New Targeted Lipidomics Approach Reveals Lipid Droplets in Liver, Muscle and Heart as a Repository for Diacylglycerol and Ceramide Species in Non-Alcoholic Fatty Liver. *Cells* 2019; **8** [PMID: 30909521 DOI: 10.3390/cells8030277]

13   **Park WJ**, Song JH, Kim GT, Park TS. Ceramide and Sphingosine 1-Phosphate in Liver Diseases. *Mol Cells* 2020; **43**: 419-430 [PMID: 32392908 DOI: 10.14348/molcells.2020.0054]

14   **Maceyka M**, Spiegel S. Sphingolipid metabolites in inflammatory disease. *Nature* 2014; **510**: 58-67 [PMID: 24899305 DOI: 10.1038/nature13475]

15   **Raichur S**. Ceramide Synthases Are Attractive Drug Targets for Treating Metabolic Diseases. *Front Endocrinol (Lausanne)* 2020; **11**: 483 [PMID: 32849276 DOI: 10.3389/fendo.2020.00483]

16   **Mucinski JM**, Manrique-Acevedo C, Kasumov T, Garrett TJ, Gaballah A, Parks EJ. Relationships between Very Low-Density Lipoproteins-Ceramides, -Diacylglycerols, and -Triacylglycerols in Insulin-Resistant Men. *Lipids* 2020; **55**: 387-393 [PMID: 32415687 DOI: 10.1002/lipd.12244]

17   **Apostolopoulou M**, Gordillo R, Koliaki C, Gancheva S, Jelenik T, De Filippo E, Herder C, Markgraf D, Jankowiak F, Esposito I, Schlensak M, Scherer PE, Roden M. Specific Hepatic Sphingolipids Relate to Insulin Resistance, Oxidative Stress, and Inflammation in Nonalcoholic Steatohepatitis. *Diabetes Care* 2018; **41**: 1235-1243 [PMID: 29602794 DOI: 10.2337/dc17-1318]

18   **Nikolova-Karakashian M**. Alcoholic and non-alcoholic fatty liver disease: Focus on ceramide. *Adv Biol Regul* 2018; **70**: 40-50 [PMID: 30455063 DOI: 10.1016/j.jbior.2018.11.004]

19   **Luukkonen PK**, Zhou Y, Sädevirta S, Leivonen M, Arola J, Orešič M, Hyötyläinen T, Yki-Järvinen H. Hepatic ceramides dissociate steatosis and insulin resistance in patients with non-alcoholic fatty liver disease. *J Hepatol* 2016; **64**: 1167-1175 [PMID: 26780287 DOI: 10.1016/j.jhep.2016.01.002]

20   **Wasilewska N**, Bobrus-Chociej A, Harasim-Symbor E, Tarasów E, Wojtkowska M, Chabowski A, Lebensztejn DM. Increased serum concentration of ceramides in obese children with nonalcoholic fatty liver disease. *Lipids Health Dis* 2018; **17**: 216 [PMID: 30208901 DOI: 10.1186/s12944-018-0855-9]

21   **Musso G**, Cassader M, Paschetta E, Gambino R. Bioactive Lipid Species and Metabolic Pathways in Progression and Resolution of Nonalcoholic Steatohepatitis. *Gastroenterology* 2018; **155**: 282-302.e8 [PMID: 29906416 DOI: 10.1053/j.gastro.2018.06.031]

22   **Chiappini F**, Coilly A, Kadar H, Gual P, Tran A, Desterke C, Samuel D, Duclos-Vallée JC, Touboul D, Bertrand-Michel J, Brunelle A, Guettier C, Le Naour F. Metabolism dysregulation induces a specific lipid signature of nonalcoholic steatohepatitis in patients. *Sci Rep* 2017; **7**: 46658 [PMID:

28436449 DOI: 10.1038/srep46658]

23   **Serhan CN**. Treating inflammation and infection in the 21st century: new hints from decoding resolution mediators and mechanisms. *FASEB J* 2017; **31**: 1273-1288 [PMID: 28087575 DOI: 10.1096/fj.201601222R]

24   **Zheng JS**, Xu A, Huang T, Yu X, Li D. Low docosahexaenoic acid content in plasma phospholipids is associated with increased non-alcoholic fatty liver disease in China. *Lipids* 2012; **47**: 549-556 [PMID: 22527845 DOI: 10.1007/s11745-012-3671-4]

25   **Puri P**, Wiest MM, Cheung O, Mirshahi F, Sargeant C, Min HK, Contos MJ, Sterling RK, Fuchs M, Zhou H, Watkins SM, Sanyal AJ. The plasma lipidomic signature of nonalcoholic steatohepatitis. *Hepatology* 2009; **50**: 1827-1838 [PMID: 19937697 DOI: 10.1002/hep.23229]

26   **Loomba R**, Quehenberger O, Armando A, Dennis EA. Polyunsaturated fatty acid metabolites as novel lipidomic biomarkers for noninvasive diagnosis of nonalcoholic steatohepatitis. *J Lipid Res* 2015; **56**: 185-192 [PMID: 25404585 DOI: 10.1194/jlr.P055640]

27   **Walle P**, Takkunen M, Männistö V, Vaittinen M, Lankinen M, Kärjä V, Käkelä P, Ågren J, Tiainen M, Schwab U, Kuusisto J, Laakso M, Pihlajamäki J. Fatty acid metabolism is altered in non-alcoholic steatohepatitis independent of obesity. *Metabolism* 2016; **65**: 655-666 [PMID: 27085774 DOI: 10.1016/j.metabol.2016.01.011]

28   **Tiwari-Heckler S**, Gan-Schreier H, Stremmel W, Chamulitrat W, Pathil A. Circulating Phospholipid Patterns in NAFLD Patients Associated with a Combination of Metabolic Risk Factors. *Nutrients* 2018; **10** [PMID: 29883377 DOI: 10.3390/nu10050649]

29   **Ma DW**, Arendt BM, Hillyer LM, Fung SK, McGilvray I, Guindi M, Allard JP. Plasma phospholipids and fatty acid composition differ between liver biopsy-proven nonalcoholic fatty liver disease and healthy subjects. *Nutr Diabetes* 2016; **6**: e220 [PMID: 27428872 DOI: 10.1038/nutd.2016.27]

30   **Musso G**, Gambino R, De Michieli F, Cassader M, Rizzetto M, Durazzo M, Fagà E, Silli B, Pagano G. Dietary habits and their relations to insulin resistance and postprandial lipemia in nonalcoholic steatohepatitis. *Hepatology* 2003; **37**: 909-916 [PMID: 12668986 DOI: 10.1053/jhep.2003.50132]

31   **Puri P**, Baillie RA, Wiest MM, Mirshahi F, Choudhury J, Cheung O, Sargeant C, Contos MJ, Sanyal AJ. A lipidomic analysis of nonalcoholic fatty liver disease. *Hepatology* 2007; **46**: 1081-1090 [PMID: 17654743 DOI: 10.1002/hep.21763]

32   **Kalveram L**, Schunck WH, Rothe M, Rudolph B, Loddenkemper C, Holzhütter HG, Henning S, Bufler P, Schulz M, Meierhofer D, Zhang IW, Weylandt KH, Wiegand S, Hudert CA. Regulation of the cytochrome P450 epoxyeicosanoid pathway is associated with distinct histologic features in pediatric non-alcoholic fatty liver disease. *Prostaglandins Leukot Essent Fatty Acids* 2021; **164**: 102229 [PMID: 33388475 DOI: 10.1016/j.plefa.2020.102229]

33   **Alkhouri N**, Dixon LJ, Feldstein AE. Lipotoxicity in nonalcoholic fatty liver disease: not all lipids are created equal. *Expert Rev Gastroenterol Hepatol* 2009; **3**: 445-451 [PMID: 19673631 DOI: 10.1586/egh.09.32]

34   **Li ZZ**, Berk M, McIntyre TM, Feldstein AE. Hepatic lipid partitioning and liver damage in nonalcoholic fatty liver disease: role of stearoyl-CoA desaturase. *J Biol Chem* 2009; **284**: 5637-5644 [PMID: 19119140 DOI: 10.1074/jbc.M807616200]

35   **Araya J**, Rodrigo R, Videla LA, Thielemann L, Orellana M, Pettinelli P, Poniachik J. Increase in long-chain polyunsaturated fatty acid n - 6/n - 3 ratio in relation to hepatic steatosis in patients with non-alcoholic fatty liver disease. *Clin Sci (Lond)* 2004; **106**: 635-643 [PMID: 14720121 DOI: 10.1042/CS20030326]

36   **Allard JP**, Aghdassi E, Mohammed S, Raman M, Avand G, Arendt BM, Jalali P, Kandasamy T, Prayitno N, Sherman M, Guindi M, Ma DW, Heathcote JE. Nutritional assessment and hepatic fatty acid composition in non-alcoholic fatty liver disease (NAFLD): a cross-sectional study. *J Hepatol* 2008; **48**: 300-307 [PMID: 18086506 DOI: 10.1016/j.jhep.2007.09.009]

37   **Peng KY**, Watt MJ, Rensen S, Greve JW, Huynh K, Jayawardana KS, Meikle PJ, Meex RCR. Mitochondrial dysfunction-related lipid changes occur in nonalcoholic fatty liver disease progression. *J Lipid Res* 2018; **59**: 1977-1986 [PMID: 30042157 DOI: 10.1194/jlr.M085613]

38   **Konkel A**, Schunck WH. Role of cytochrome P450 enzymes in the bioactivation of polyunsaturated fatty acids. *Biochim Biophys Acta* 2011; **1814**: 210-222 [PMID: 20869469 DOI: 10.1016/j.bbapap.2010.09.009]

39   **Spector AA**, Kim HY. Cytochrome P450 epoxygenase pathway of polyunsaturated fatty acid metabolism. *Biochim Biophys Acta* 2015; **1851**: 356-365 [PMID: 25093613 DOI: 10.1016/j.bbalip.2014.07.020]

40   **Schunck WH**, Konkel A, Fischer R, Weylandt KH. Therapeutic potential of omega-3 fatty acid-derived epoxyeicosanoids in cardiovascular and inflammatory diseases. *Pharmacol Ther* 2018; **183**: 177-204 [PMID: 29080699 DOI: 10.1016/j.pharmthera.2017.10.016]

41   **Wells MA**, Vendrov KC, Edin ML, Ferslew BC, Zha W, Nguyen BK, Church RJ, Lih FB, DeGraff LM, Brouwer KL, Barritt AS 4th, Zeldin DC, Lee CR. Characterization of the Cytochrome P450 epoxyeicosanoid pathway in non-alcoholic steatohepatitis. *Prostaglandins Other Lipid Mediat* 2016; **125**: 19-29 [PMID: 27401401 DOI: 10.1016/j.prostaglandins.2016.07.002]

42   **Yamaguchi K**, Yang L, McCall S, Huang J, Yu XX, Pandey SK, Bhanot S, Monia BP, Li YX, Diehl AM. Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. *Hepatology* 2007; **45**: 1366-1374 [PMID: 17476695 DOI: 10.1002/hep.21655]

43   **Wang C**, Zhao Y, Gao X, Li L, Yuan Y, Liu F, Zhang L, Wu J, Hu P, Zhang X, Gu Y, Xu Y, Wang Z, Li Z, Zhang H, Ye J. Perilipin 5 improves hepatic lipotoxicity by inhibiting lipolysis. *Hepatology* 2015; **61**: 870-882 [PMID: 25179419 DOI: 10.1002/hep.27409]

44   **Gan LT**, Van Rooyen DM, Koina ME, McCuskey RS, Teoh NC, Farrell GC. Hepatocyte free cholesterol lipotoxicity results from JNK1-mediated mitochondrial injury and is HMGB1 and TLR4-dependent. *J Hepatol* 2014; **61**: 1376-1384 [PMID: 25064435 DOI: 10.1016/j.jhep.2014.07.024]

45   **Waite KA**, Cabilio NR, Vance DE. Choline deficiency-induced liver damage is reversible in Pemt(-/-) mice. *J Nutr* 2002; **132**: 68-71 [PMID: 11773510 DOI: 10.1093/jn/132.1.68]

46   **Jacobs RL**, Zhao Y, Koonen DP, Sletten T, Su B, Lingrell S, Cao G, Peake DA, Kuo MS, Proctor SD, Kennedy BP, Dyck JR, Vance DE. Impaired de novo choline synthesis explains why phosphatidylethanolamine N-methyltransferase-deficient mice are protected from diet-induced obesity. *J Biol Chem* 2010; **285**: 22403-22413 [PMID: 20452975 DOI: 10.1074/jbc.M110.108514]

47   **Walker AK**, Jacobs RL, Watts JL, Rottiers V, Jiang K, Finnegan DM, Shioda T, Hansen M, Yang F, Niebergall LJ, Vance DE, Tzoneva M, Hart AC, Näär AM. A conserved SREBP-1/phosphatidylcholine feedback circuit regulates lipogenesis in metazoans. *Cell* 2011; **147**: 840-852 [PMID: 22035958 DOI: 10.1016/j.cell.2011.09.045]

48   **Song J**, da Costa KA, Fischer LM, Kohlmeier M, Kwock L, Wang S, Zeisel SH. Polymorphism of the PEMT gene and susceptibility to nonalcoholic fatty liver disease (NAFLD). *FASEB J* 2005; **19**: 1266-1271 [PMID: 16051693 DOI: 10.1096/fj.04-3580com]

49   **Arendt BM**, Ma DW, Simons B, Noureldin SA, Therapondos G, Guindi M, Sherman M, Allard JP. Nonalcoholic fatty liver disease is associated with lower hepatic and erythrocyte ratios of phosphatidylcholine to phosphatidylethanolamine. *Appl Physiol Nutr Metab* 2013; **38**: 334-340 [PMID: 23537027 DOI: 10.1139/apnm-2012-0261]

50   **Li Z**, Agellon LB, Allen TM, Umeda M, Jewell L, Mason A, Vance DE. The ratio of phosphatidylcholine to phosphatidylethanolamine influences membrane integrity and steatohepatitis. *Cell Metab* 2006; **3**: 321-331 [PMID: 16679290 DOI: 10.1016/j.cmet.2006.03.007]

51   **Anjani K**, Lhomme M, Sokolovska N, Poitou C, Aron-Wisnewsky J, Bouillot JL, Lesnik P, Bedossa P, Kontush A, Clement K, Dugail I, Tordjman J. Circulating phospholipid profiling identifies portal contribution to NASH signature in obesity. *J Hepatol* 2015; **62**: 905-912 [PMID: 25450212 DOI: 10.1016/j.jhep.2014.11.002]

52   **Liu Z**, Li H, Zheng Y, Gao Z, Cong L, Yang L, Zhou Y. Association of Lipoprotein-Associated Phospholipase A2 with the Prevalence of Nonalcoholic Fatty Liver Disease: A Result from the APAC Study. *Sci Rep* 2018; **8**: 10127 [PMID: 29973631 DOI: 10.1038/s41598-018-28494-8]

53   **Nass KJ**, van den Berg EH, Gruppen EG, Dullaart RPF. Plasma lecithin:cholesterol acyltransferase and phospholipid transfer protein activity independently associate with nonalcoholic fatty liver disease. *Eur J Clin Invest* 2018; **48**: e12988 [PMID: 29947103 DOI: 10.1111/eci.12988]

54   **Hollie NI**, Cash JG, Matlib MA, Wortman M, Basford JE, Abplanalp W, Hui DY. Micromolar changes in lysophosphatidylcholine concentration cause minor effects on mitochondrial permeability but major alterations in function. *Biochim Biophys Acta* 2014; **1841**: 888-895 [PMID: 24315825 DOI: 10.1016/j.bbalip.2013.11.013]

55   **Kakisaka K**, Cazanave SC, Fingas CD, Guicciardi ME, Bronk SF, Werneburg NW, Mott JL, Gores GJ. Mechanisms of lysophosphatidylcholine-induced hepatocyte lipoapoptosis. *Am J Physiol Gastrointest Liver Physiol* 2012; **302**: G77-G84 [PMID: 21995961 DOI: 10.1152/ajpgi.00301.2011]

56   **Vance JE**. Lipoproteins secreted by cultured rat hepatocytes contain the antioxidant 1-alk-1-enyl-2-acylglycerophosphoethanolamine. *Biochim Biophys Acta* 1990; **1045**: 128-134 [PMID: 2116174 DOI: 10.1016/0005-2760(90)90141-j]

57   **Weir JM**, Wong G, Barlow CK, Greeve MA, Kowalczyk A, Almasy L, Comuzzie AG, Mahaney MC, Jowett JB, Shaw J, Curran JE, Blangero J, Meikle PJ. Plasma lipid profiling in a large population-based cohort. *J Lipid Res* 2013; **54**: 2898-2908 [PMID: 23868910 DOI: 10.1194/jlr.P035808]

58   **Jang JE**, Park HS, Yoo HJ, Baek IJ, Yoon JE, Ko MS, Kim AR, Kim HS, Lee SE, Kim SW, Kim SJ, Leem J, Kang YM, Jung MK, Pack CG, Kim CJ, Sung CO, Lee IK, Park JY, Fernández-Checa JC, Koh EH, Lee KU. Protective role of endogenous plasmalogens against hepatic steatosis and steatohepatitis in mice. *Hepatology* 2017; **66**: 416-431 [PMID: 28073164 DOI: 10.1002/hep.29039]

59   **Sookoian S**, Pirola CJ. PNPLA3, the triacylglycerol synthesis/hydrolysis/storage dilemma, and nonalcoholic fatty liver disease. *World J Gastroenterol* 2012; **18**: 6018-6026 [PMID: 23155331 DOI: 10.3748/wjg.v18.i42.6018]

60   **Luukkonen PK**, Nick A, Hölttä-Vuori M, Thiele C, Isokuortti E, Lallukka-Brück S, Zhou Y, Hakkarainen A, Lundbom N, Peltonen M, Orho-Melander M, Orešič M, Hyötyläinen T, Hodson L, Ikonen E, Yki-Järvinen H. Human PNPLA3-I148M variant increases hepatic retention of polyunsaturated fatty acids. *JCI Insight* 2019; **4** [PMID: 31434800 DOI: 10.1172/jci.insight.127902]

61   **Hudert CA**, Selinski S, Rudolph B, Bläker H, Loddenkemper C, Thielhorn R, Berndt N, Golka K, Cadenas C, Reinders J, Henning S, Bufler P, Jansen PLM, Holzhütter HG, Meierhofer D, Hengstler JG, Wiegand S. Genetic determinants of steatosis and fibrosis progression in paediatric non-alcoholic fatty liver disease. *Liver Int* 2019; **39**: 540-556 [PMID: 30444569 DOI: 10.1111/liv.14006]

62   **Barr J**, Vázquez-Chantada M, Alonso C, Pérez-Cormenzana M, Mayo R, Galán A, Caballería J, Martín-Duce A, Tran A, Wagner C, Luka Z, Lu SC, Castro A, Le Marchand-Brustel Y, Martínez-Chantar ML, Veyrie N, Clément K, Tordjman J, Gual P, Mato JM. Liquid chromatography-mass spectrometry-based parallel metabolic profiling of human and mouse model serum reveals putative

biomarkers associated with the progression of nonalcoholic fatty liver disease. *J Proteome Res* 2010; **9**: 4501-4512 [PMID: 20684516 DOI: 10.1021/pr1002593]

63   **Zhou Y**, Orešič M, Leivonen M, Gopalacharyulu P, Hyysalo J, Arola J, Verrijken A, Francque S, Van Gaal L, Hyötyläinen T, Yki-Järvinen H. Noninvasive Detection of Nonalcoholic Steatohepatitis Using Clinical Markers and Circulating Levels of Lipids and Metabolites. *Clin Gastroenterol Hepatol* 2016; **14**: 1463-1472.e6 [PMID: 27317851 DOI: 10.1016/j.cgh.2016.05.046]

64   **Gorden DL**, Myers DS, Ivanova PT, Fahy E, Maurya MR, Gupta S, Min J, Spann NJ, McDonald JG, Kelly SL, Duan J, Sullards MC, Leiker TJ, Barkley RM, Quehenberger O, Armando AM, Milne SB, Mathews TP, Armstrong MD, Li C, Melvin WV, Clements RH, Washington MK, Mendonsa AM, Witztum JL, Guan Z, Glass CK, Murphy RC, Dennis EA, Merrill AH Jr, Russell DW, Subramaniam S, Brown HA. Biomarkers of NAFLD progression: a lipidomics approach to an epidemic. *J Lipid Res* 2015; **56**: 722-736 [PMID: 25598080 DOI: 10.1194/jlr.P056002]

65   **Meikle PJ**, Wong G, Barlow CK, Weir JM, Greeve MA, MacIntosh GL, Almasy L, Comuzzie AG, Mahaney MC, Kowalczyk A, Haviv I, Grantham N, Magliano DJ, Jowett JB, Zimmet P, Curran JE, Blangero J, Shaw J. Plasma lipid profiling shows similar associations with prediabetes and type 2 diabetes. *PLoS One* 2013; **8**: e74341 [PMID: 24086336 DOI: 10.1371/journal.pone.0074341]

66   **Holland WL**, Summers SA. Sphingolipids, insulin resistance, and metabolic disease: new insights from *in vivo* manipulation of sphingolipid metabolism. *Endocr Rev* 2008; **29**: 381-402 [PMID: 18451260 DOI: 10.1210/er.2007-0025]

67   **Marí M**, Colell A, Morales A, Caballero F, Moles A, Fernández A, Terrones O, Basañez G, Antonsson B, García-Ruiz C, Fernández-Checa JC. Mechanism of mitochondrial glutathione-dependent hepatocellular susceptibility to TNF despite NF-kappaB activation. *Gastroenterology* 2008; **134**: 1507-1520 [PMID: 18343380 DOI: 10.1053/j.gastro.2008.01.073]

68   **Kasumov T**, Li L, Li M, Gulshan K, Kirwan JP, Liu X, Previs S, Willard B, Smith JD, McCullough A. Ceramide as a mediator of non-alcoholic Fatty liver disease and associated atherosclerosis. *PLoS One* 2015; **10**: e0126910 [PMID: 25993337 DOI: 10.1371/journal.pone.0126910]

69   **Leal-Witt MJ**, Ramon-Krauel M, Samino S, Llobet M, Cuadras D, Jimenez-Chillaron JC, Yanes O, Lerin C. Untargeted metabolomics identifies a plasma sphingolipid-related signature associated with lifestyle intervention in prepubertal children with obesity. *Int J Obes (Lond)* 2018; **42**: 72-78 [PMID: 28947825 DOI: 10.1038/ijo.2017.201]

70   **Barbacini P**, Casas J, Torretta E, Capitanio D, Maccallini G, Hirschler V, Gelfi C. Regulation of Serum Sphingolipids in Andean Children Born and Living at High Altitude (3775 m). *Int J Mol Sci* 2019; **20** [PMID: 31212599 DOI: 10.3390/ijms20112835]

71   **León-Aguilar LF**, Croyal M, Ferchaud-Roucher V, Huang F, Marchat LA, Barraza-Villarreal A, Romieu I, Ramakrishnan U, Krempf M, Ouguerram K, Mercado-Camargo R, Bolaños-Jiménez F. Maternal obesity leads to long-term altered levels of plasma ceramides in the offspring as revealed by a longitudinal lipidomic study in children. *Int J Obes (Lond)* 2019; **43**: 1231-1243 [PMID: 30568270 DOI: 10.1038/s41366-018-0291-y]