New insights in the pathophysiology of chronic intermittent hypoxia-induced NASH: the role of gut–liver axis impairment

Judith Aron-Wisnewsky,[1,2,3] Jean-Louis Pepin[4,5]

Non-alcoholic fatty liver disease (NAFLD) is an increasingly prevalent condition paralleling the growing epidemic of obesity and type-2 diabetes. This disease shows a continuous spectrum of severity ranging from simple steatosis to overt non-alcoholic steato-hepatitis (NASH) that can further progress towards cirrhosis and hepatocarcinoma.[1] Although the complete physiopathology is not fully understood, many factors such as insulin resistance, inflammation, oxidative stress and lipotoxicity are involved in the onset of NAFLD.[2 3] Obstructive sleep apnoea (OSA) is independently associated with the different components of the metabolic syndrome, particularly visceral obesity, hypertension, insulin resistance and abnormal lipid metabolism.[4] The repetitive occurrence of partial or complete pharyngeal collapse during OSA induces chronic intermittent hypoxia (CIH) that subsequently results in low-grade inflammation, sympathetic overactivity and oxidative stress. Since OSA and NAFLD share common intermediary mechanisms, the presence of OSA, especially in the context of severe CIH, has been suggested as contributing to the pathogenesis and exacerbation of the severity of NAFLD.

Animal studies have demonstrated that in obesity, short-term or long-term exposure to CIH mimicking OSA induces NAFLD.[5–7] Next, studies in human adults, using either non-invasive surrogate markers of liver injury or biopsies to diagnose NAFLD, have also demonstrated that CIH is involved in the development of NAFLD, independently of body mass index (BMI), and contributes to the deleterious progression into overt NASH.[8–11] In contrast, other studies have shown that the absence of OSA seems to protect morbidly obese patients from developing NAFLD.[12]

In response to the currently emergent worldwide epidemic of childhood obesity, NAFLD has started to be more systematically investigated in children than previously.[13 14] The prevalence of NAFLD reaches 10% in the paediatric population and up to 60% in obese children.[15] The literature on paediatric NAFLD is growing in terms of epidemiology, diagnostic procedures and research into the mechanistic pathways.[16] A better understanding of the pathophysiology of the disease will help to delineate new treatment strategies, which are currently limited both for adults and even more so for children. Recent studies have demonstrated that OSA is more frequent in children with NAFLD even in non-obese individuals. The dose–response relationship between the amount of oxygen desaturation during the night and the severity of liver injury that has been established in adults also seems to prevail in children.[17 18]

Several factors have been demonstrated as mediating the effects of OSA/CIH on the liver. OSA exacerbates metabolic abnormalities such as insulin resistance and overt type-2 diabetes, independently of BMI.[19–21] OSA also plays a role in aggravating dyslipidaemia, which is known to be involved in NAFLD, both in humans[22] and in murine models.[6] Hepatic lipogenesis, the first stage of NAFLD, is increased by CIH. Indeed, gene expression of lipogenic factors, such as sterol regulatory element-binding proteins-1c and acetyl-CoA carboxylase, is upregulated in murine models of CIH.[23] As a result, hepatic triglyceride content is increased by CIH both in lean and obese mice.[7] OSA generates oxidative stress throughout the whole body as well as in the liver.[6 7] The production of reactive oxygen species favours liver lipid peroxydation, which can be prevented by the use of antioxidants.[7 24] Finally, systemic inflammation is also enhanced OSA patients with NAFLD.[8 20] Gene expression of the inflammation transcription factor NF-κβ is observed in the liver of patients with NAFLD.[25] In children, local liver inflammation has been documented by an increase in intrahepatic leucocytes and activated macrophages/Kupffer cells, particularly in those with the highest levels of nocturnal oxygen desaturation.[17]

In the current issue of this journal, Nobili *et al* decipher new mechanistic aspects underlying the link between OSA and NAFLD in children. Using liver biopsies, they studied a large cohort of 81 children who also underwent polysomnography and an assessment of in vivo intestinal permeability. They first confirmed that NAFLD in children is related to OSA severity. Most importantly, they found that OSA increased intestinal permeability resulting in increased systemic levels of lipopolysaccharides (LPS). Interestingly, in the liver, this resulted in an upregulation of toll-like receptors (TLR-4) in hepatocytes, Kupffer cells and hepatic stellate cells (HSC) (figure 1). This family of molecules expressed on cell surfaces detects lipoproteins and lipids from bacteria, protozoa and fungi. LPS are a component of the Gram negative bacterial membrane and bind naturally to LPS-binding protein (LBP) and CD14. LBP is a soluble acute-phase protein primarily produced by hepatocytes in response to microbial translocation. The LPS-LBP-CD14 complex further activates TLR-4 situated on immune cells, adipocytes, hepatocytes and Kupffer cells. These results demonstrate that OSA induces a disruption of the gut–liver axis, increasing susceptibility to endotoxaemia, and suggest that gut microbiota might be one of the culprits of OSA-related NASH. Kheirandish-Gozal *et al*[26] have recently demonstrated, in a large cohort of 219 children, that systemic LPS levels increased along with OSA severity. Importantly, increased LBP serum levels have been associated with cardiovascular risk and mortality.[27] This suggests that OSA-related NAFLD may also contribute to the development of cardiovascular disease in children. The role of gut microbiota in NAFLD pathogenesis has been suggested (see for review ref. 28). Bäckhed *et al*[29] demonstrated that the transfer of gut microbiota from diet induced obese mice into axenic mice (ie, mice without microbiota) led to the development of NAFLD. Subsequently, the link between NAFLD, microbiota and intestinal permeability was assessed. A study in rodents elegantly demonstrated that increased intestinal permeability led to elevated endotoxaemia

[1]Institute of Cardiometabolism and Nutrition (ICAN), Assistance Pitié-Salpêtrière Hospital, Paris, France; [2]Sorbonne Universités, Université Pierre et Marie Curie-Paris 6, Paris, France; [3]INSERM, UMR_S U1166, Paris, France; [4]Institut national de la santé et de la recherche médicale (INSERM), U 1042, HP2 Laboratory (Hypoxia: Pathophysiology), University of Grenoble-Alpes, Grenoble, France; [5]Thorax and Vessels Division, Grenoble University Hospital, Grenoble, France

Correspondence to Professor Jean-Louis Pepin, Institut national de la santé et de la recherche médicale (INSERM), U 1042, HP2 Laboratory (Hypoxia: Pathophysiology), University of Grenoble-Alpes, Grenoble F-38000, France; jpepin@chu-grenoble.fr

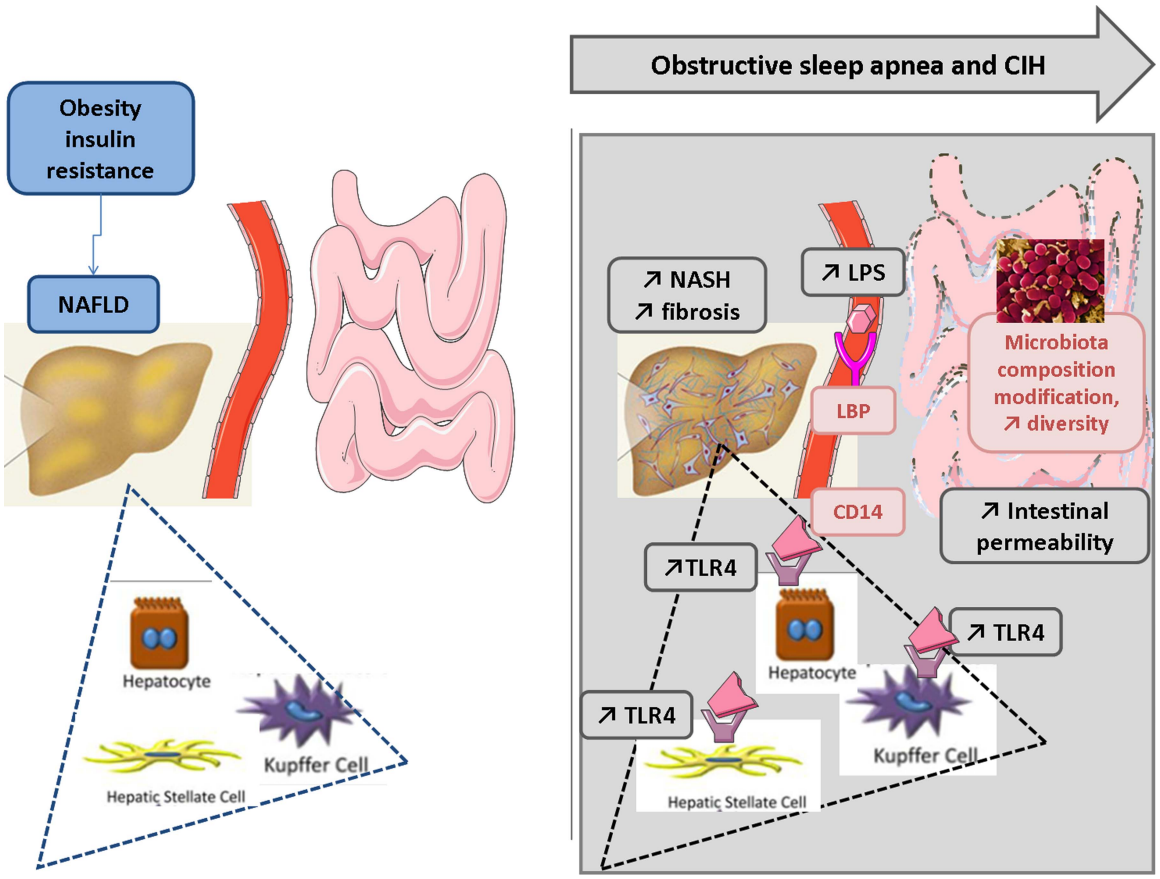

Figure 1 Mechanism of non-alcoholic fatty liver disease (NAFLD) exacerbation during obstructive sleep apnoea (OSA)-induced chronic intermittent hypoxia (CIH). OSA increases intestinal permeability, modifies microbiota composition and increases microbiota diversity,[34] which in turn lead to increased systemic lipopolysaccharide (LPS) levels along with increased prevalence and severity of non-alcoholic steato-hepatitis (NASH). In liver, there in an increase in toll-like receptors (TLR-4) on hepatocytes, hepatic stellate cells and Kupffer cells. The mechanism linking both phenomena could be gut dysbiosis where parts of gut microbiota leak into the systemic circulation, bind to LPS-binding protein, then onto its receptor CD14 and subsequently to TLR4 on hepatic cells. *The right panel shows what happens in normoxic conditions; the left panel (grey background) shows the effect of CIH*.

(systemic levels of LPS) and was associated with metabolic alterations such as insulin resistance, glucose intolerance, systemic and tissue inflammation as well as higher liver triglyceride levels.[30–32] In contrast, interventions that modify the microbiota, such as antibiotic and prebiotic use, induced a significant improvement in intestinal permeability, metabolic alterations and NAFLD.[32 33] Importantly, increased intestinal permeability is generally attributed to a high fat diet. In this current issue of *Thorax*, Nobili *et al* describe a new finding: the role of OSA/CIH in inducing intestinal permeability. This effect was apparently independent of differences in diet. Indeed, patients with or without NAFLD and with or without OSA displayed similar food intake profiles with regards to the proportion of saturated or unsaturated fat and carbohydrate in their diet as assessed by dietary records. This is in line with results by Moreno-Indias *et al* who used a mouse model of OSA to demonstrate that intermittent hypoxia exposure translated into distinct hypoxia/reoxygenation patterns in the faeces proximal to the bowel epithelium (<200 µm). This suggests increased intestinal permeability due to altered tight junction integrity. Intermittent hypoxia also altered the composition and diversity of the microbiota potentially leading to downstream metabolic dysregulation.[34]

Finally, Nobili *et al* looked deeper into the mechanistic ramifications. They show that OSA might exacerbate NAFLD features by inducing an upregulation of TLR-4 in HSC, thus explaining the increased severity of NAFLD they observed in children with OSA. This is in line with previous studies in mice which had shown that TLR-4 is essential for hepatic fat deposition.[35–37] Furthermore, it has been demonstrated that TLR-4 promotes fibrogenesis through activation of the inflammatory cascade in HSC.[38 39]

In summary, the literature provides a number of arguments implicating OSA and particularly CIH in NAFLD pathogenesis and exacerbation in both rodents and humans. Recent findings suggest that this can also occur in children. Nobili *et al* provide new evidence about the causal role of gut–liver axis disruption and the mechanistic pathway linking OSA and NAFLD, as shown in figure 1. They shed new light on the role of the microbiota in this disease, potentially opening novel therapeutic approaches to NAFLD.

Competing interests None declared.

Provenance and peer review Commissioned; internally peer reviewed.

CrossMark

To cite Aron-Wisnewsky J, Pepin J-L. *Thorax* 2015;**70**:713–715.

Published Online First 16 June 2015

Linked

► http://dx.doi.org/10.1136/thoraxjnl-2015-206782

Thorax 2015;**70**:713–715.
doi:10.1136/thoraxjnl-2015-207212

REFERENCES

1 Cohen JC, Horton JD, Hobbs HH. Human fatty liver disease: old questions and new insights. *Science* 2011;332:1519–23.
2 Day CP, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998;114:842–5.
3 Neuschwander-Tetri BA. Hepatic lipotoxicity and the pathogenesis of nonalcoholic steatohepatitis: the central role of nontriglyceride fatty acid metabolites. *Hepatol Baltim Md* 2010;52:774–88.
4 Lévy P, Bonsignore MR, Eckel J. Sleep, sleep-disordered breathing and metabolic consequences. *Eur Respir J* 2009;34:243–60.
5 Savransky V, Reinke C, Jun J, *et al*. Chronic intermittent hypoxia and acetaminophen induce synergistic liver injury in mice. *Exp Physiol* 2009;94:228–39.
6 Savransky V, Nanayakkara A, Vivero A, *et al*. Chronic intermittent hypoxia predisposes to liver injury. *Hepatol Baltim Md* 2007;45:1007–13.
7 Savransky V, Bevans S, Nanayakkara A, *et al*. Chronic intermittent hypoxia causes hepatitis in a mouse model of diet-induced fatty liver. *Am J Physiol Gastrointest Liver Physiol* 2007;293:G871–7.
8 Aron-Wisnewsky J, Minville C, Tordjman J, *et al*. Chronic intermittent hypoxia is a major trigger for non-alcoholic fatty liver disease in morbid obese. *J Hepatol* 2012;56:225–33.
9 Minville C, Hilleret MN, Tamisier R, *et al*. Nonalcoholic fatty liver disease, nocturnal hypoxia, and endothelial function in patients with sleep apnea. *Chest* 2014;145:525–33.
10 Musso G, Cassader M, Olivetti C, *et al*. Association of obstructive sleep apnoea with the presence and severity of non-alcoholic fatty liver disease. A systematic review and meta-analysis. *Obes Rev* 2013;14:417–31.
11 Polotsky VY, Patil SP, Savransky V, *et al*. Obstructive sleep apnea, insulin resistance, and steatohepatitis in severe obesity. *Am J Respir Crit Care Med* 2009;179:228–34.
12 Corey KE, Misdraji J, Zheng H, *et al*. The absence of obstructive sleep apnea may protect against non-alcoholic fatty liver in patients undergoing bariatric surgery. *PLoS ONE* 2013;8:e62504.
13 Schwimmer JB, Deutsch R, Kahen T, *et al*. Prevalence of fatty liver in children and adolescents. *Pediatrics* 2006;118:1388–93.
14 Molleston JP, Schwimmer JB, Yates KP, *et al*. Histological abnormalities in children with nonalcoholic fatty liver disease and normal or mildly elevated alanine aminotransferase levels. *J Pediatr* 2014;164:707–13.e3.
15 Della Corte C, Vajro P, Socha P, *et al*. Pediatric non-alcoholic fatty liver disease: recent advances. *Clin Res Hepatol Gastroenterol* 2014;38:419–22.
16 Patton HM, Sirlin C, Behling C, *et al*. Pediatric nonalcoholic fatty liver disease: a critical appraisal of current data and implications for future research. *J Pediatr Gastroenterol Nutr* 2006;43:413–27.
17 Nobili V, Cutrera R, Liccardo D, *et al*. Obstructive sleep apnea syndrome affects liver histology and inflammatory cell activation in pediatric nonalcoholic fatty liver disease, regardless of obesity/insulin resistance. *Am J Respir Crit Care Med* 2014;189:66–76.
18 Sundaram SS, Sokol RJ, Capocelli KE, *et al*. Obstructive sleep apnea and hypoxemia are associated with advanced liver histology in pediatric nonalcoholic fatty liver disease. *J Pediatr* 2014;164:699–706.e1.
19 Tasali E, Ip MSM. Obstructive sleep apnea and metabolic syndrome: alterations in glucose metabolism and inflammation. *Proc Am Thorac Soc* 2008;5:207–17.
20 Vgontzas AN, Papanicolaou DA, Bixler EO, *et al*. Sleep apnea and daytime sleepiness and fatigue: relation to visceral obesity, insulin resistance, and hypercytokinemia. *J Clin Endocrinol Metab* 2000;85:1151–8.
21 Drager LF, Lopes HF, Maki-Nunes C, *et al*. The impact of obstructive sleep apnea on metabolic and inflammatory markers in consecutive patients with metabolic syndrome. *PLoS ONE* 2010;5:e12065.
22 Trzepizur W, Le Vaillant M, Meslier N, *et al*. Independent association between nocturnal intermittent hypoxemia and metabolic dyslipidemia. *Chest* 2013;143:1584–9.
23 Li J, Thorne LN, Punjabi NM, *et al*. Intermittent hypoxia induces hyperlipidemia in lean mice. *Circ Res* 2005;97:698–706.
24 Jun J, Savransky V, Nanayakkara A, *et al*. Intermittent hypoxia has organ-specific effects on oxidative stress. *Am J Physiol Regul Integr Comp Physiol* 2008;295:R1274–81.
25 Paschetta E, Belci P, Alisi A, *et al*. OSAS-related inflammatory mechanisms of liver injury in nonalcoholic fatty liver disease. *Mediators Inflamm* 2015;2015:815721.
26 Kheirandish-Gozal L, Peris E, Wang Y, *et al*. Lipopolysaccharide-binding protein plasma levels in children: effects of obstructive sleep apnea and obesity. *J Clin Endocrinol Metab* 2014;99:656–63.
27 Lepper PM, Kleber ME, Grammer TB, *et al*. Lipopolysaccharide-binding protein (LBP) is associated with total and cardiovascular mortality in individuals with or without stable coronary artery disease–results from the Ludwigshafen Risk and Cardiovascular Health Study (LURIC). *Atherosclerosis* 2011;219:291–7.
28 Aron-Wisnewsky J, Gaborit B, Dutour A, *et al*. Gut microbiota and non-alcoholic fatty liver disease: new insights. *Clin Microbiol Infect* 2013;19:338–48.
29 Bäckhed F, Ding H, Wang T, *et al*. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci USA* 2004;101:15718–23.
30 Amar J, Chabo C, Waget A, *et al*. Intestinal mucosal adherence and translocation of commensal bacteria at the early onset of type 2 diabetes: molecular mechanisms and probiotic treatment. *EMBO Mol Med* 2011;3:559–72.
31 Cani PD, Amar J, Iglesias MA, *et al*. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 2007;56:1761–72.
32 Cani PD, Bibiloni R, Knauf C, *et al*. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes* 2008;57:1470–81.
33 Cani PD, Lecourt E, Dewulf EM, *et al*. Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal. *Am J Clin Nutr* 2009;90:1236–43.
34 Moreno-Indias I, Torres M, Montserrat JM, *et al*. Intermittent hypoxia alters gut microbiota diversity in a mouse model of sleep apnoea. *Eur Respir J* 2015;45:1055–65.
35 Ye D, Li FYL, Lam KSL, *et al*. Toll-like receptor-4 mediates obesity-induced non-alcoholic steatohepatitis through activation of X-box binding protein-1 in mice. *GUT* 2012;61:1058–67.
36 Poggi M, Bastelica D, Gual P, *et al*. C3H/HeJ mice carrying a toll-like receptor 4 mutation are protected against the development of insulin resistance in white adipose tissue in response to a high-fat diet. *Diabetologia* 2007;50:1267–76.
37 Saberi M, Woods NB, de Luca C, *et al*. Hematopoietic cell-specific deletion of toll-like receptor 4 ameliorates hepatic and adipose tissue insulin resistance in high-fat-fed mice. *Cell Metab* 2009;10:419–29.
38 Aoyama T, Paik YH, Seki E. Toll-like receptor signaling and liver fibrosis. *Gastroenterol Res Pract* 2010;2010:pii: 192543.
39 Paik YH, Schwabe RF, Bataller R, *et al*. Toll-like receptor 4 mediates inflammatory signaling by bacterial lipopolysaccharide in human hepatic stellate cells. *Hepatol Baltim Md* 2003;37:1043–55.