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NOCTURNAL HYPOXIA IN OBESE-RELATED OBSTRUCTIVE SLEEP-APNEA AS A PUTATIVE TRIGGER OF OXIDATIVE STRESS IN PEDIATRIC NAFLD PROGRESSION.

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List of abbreviations: ALT, alanine aminotransferase; BS, bariatric surgery; BMI, body mass index; CPAP, continuous positive airway pressure; FFA, free fatty acids; GGT, γ -glutamyl transferase; HCC, hepatocellular carcinoma; Hct, blood hematocrit; HSCs, hepatic stellate cells; HIFs, hypoxia-inducible factors; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steato-hepatitis; NH, nocturnal hypoxia; OSA, obstructive sleep apnea; OSAS, obstructive sleep apnea syndrome; ROS, reactive oxygen species; WC, waist circumference.

Non-Alcoholic Fatty Liver Disease (NAFLD) is now considered as the hepatic component of metabolic syndrome. Its prevalence, paralleling the growing diffusion of obesity and type II diabetes, is increasing worldwide both in adults and children. NAFLD is estimated to affect up to 30% of the general population in Western countries, reaching an even higher prevalence among diabetic and obese individuals [1,2]. From 20 to 30% of NAFLD patients are expected to develop non-alcoholic steatohepatitis (NASH), characterized by steatosis, parenchymal damage, lobular/portal inflammation with or without perisinusoidal and/or portal fibrosis. NASH can eventually progress to more severe fibrosis and cirrhosis [3], with development of hepatocellular carcinoma (HCC) detected in both cirrhotic and non-cirrhotic adults [4] and sometimes also in children/young adults [5].

Notwithstanding the relevant clinical and social impact of NAFLD/NASH, the exact mechanisms responsible for NAFLD progression to more severe liver injury, in pediatric and adult individuals, remain elusive and we lack effective therapies and diagnostic tools to identify NAFLD patients at risk of progression. According to the hypothesis of multiple parallel hits, current literature indicates oxidative stress, inflammasome activation, lipotoxicity and changes in gut microbiota as most relevant mechanisms [6].

The involvement of oxidative stress, in particular, has been unequivocally documented in both pediatric and adult conditions of NAFLD, reproduced by experimental studies in rodents and suggested to favor NAFLD progression [7,8]. In experimental NAFLD oxidative stress can result from increased generation of reactive oxygen species (ROS) depending on several critical events, including impairment of mitochondria due to overload of free fatty acids (FFA), increased FFA metabolism in endoplasmic reticulum by cytochrome P450 isoforms 2E1 and 4A or in peroxisomes, lipotoxicity and endoplasmic reticulum stress, the presence of hypoxic conditions, as well as ROS generation through NADPH-oxidase isoforms associated to ligand-receptor interactions (growth factors, cytokines, chemokines, adipokines) or by activated inflammatory cells. Whatever the

source, ROS and oxidative stress are widely accepted to contribute to NAFLD progression by eliciting parenchymal death and by sustaining both inflammatory and fibrogenic responses [7,8].

On this background, we find the results reported by Sundaram et al. in this issue of *Journal of Hepatology* [9] intriguing. They provide clinically sound data suggesting that nocturnal hypoxia (NH), associated with obesity-related obstructive sleep apnea (OSA), may promote the progression of pediatric NAFLD by significantly contributing to hepatic oxidative stress. According to current guidelines OSA is defined as a “disorder of breathing during sleep characterized by prolonged partial upper airway obstruction and/or intermittent complete obstruction (obstructive apnea) that disrupts normal ventilation during sleep and normal sleep patterns” [10]. Patients with OSA syndrome (OSAS), in particular, can experience repeated episodes of NH alternating with normoxia (i.e., chronic intermittent hypoxia). This is accompanied by symptoms or signs that include habitual snoring (often with intermittent pauses, snorts, or gasps), disturbed sleep, and daytime neurobehavioral problems. Daytime sleepiness may occur, but is uncommon in young children. Pediatric OSA instead is also associated with neurocognitive impairment, behavioral problems, hypertension, cardiac dysfunction, and systemic inflammation [10]. Interestingly, in the present obesity epidemics, obese children represent a category of patients where those with OSA may reach a proportion of up to 36% [11].

Recent studies have reported a link not only between OSAS and cardiometabolic alterations, but also between OSAS and either direct (hepatic histology) or indirect (ALT or GGT levels) markers of liver damage in morbidly obese NAFLD adult and pediatric patients [reviewed in 12,13]. Two years ago the same group of Sundaram reported an alarming 60% OSA incidence in pediatric NAFLD. The severity/duration of hypoxemia was associated with biochemical and histologic measures of NAFLD severity [14]. The same year Nobili et al. confirmed similar incidence and the presence and severity of OSAS

associated with the presence of NASH significant fibrosis, and NAFLD activity score independently of body mass index, abdominal adiposity, metabolic syndrome, and insulin resistance. This relationship held also in non-obese children with NAFLD and the duration of hemoglobin desaturation correlated with increased intrahepatic leukocytes and activated macrophages/Kupffer cells and with circulating markers of hepatocyte apoptosis and fibrogenesis [15]. The association OSA-NASH severity finally was found to correlate with increased endotoxemia coupled with impaired gut barrier function, leading to increased TLR-4-mediated hepatic susceptibility to endotoxemia [16], in keeping with the concept that gut microbiota plays a role in the development of hepatic steatosis and its progression to NASH [17].

The study by Sundaram and colleagues has addressed now NH as a potential source of oxidative stress in NAFLD [9]. Authors have studied 14 lean control adolescents and 36 adolescents (25 with OSA/NH) with biopsy proven NAFLD, polysomnographic characterization, liver histology scoring, and laboratory testing. Oxidative stress has been convincingly documented in this study by evaluating systemic (urine F2-isoprostanes) and hepatic (immunohistochemistry for 4-hydroxynonenal) reliable markers of “in vivo” lipid peroxidation [7,8].

This study is particularly welcome because of conflicting results reported in literature, correctly quoted in the Sundaram et al. paper [9], some reporting increased oxidative stress in adults with OSA as compared to healthy controls but others suggesting that oxidative stress in OSA patients was related to obesity rather than intermittent hypoxia. In their study, in addition to confirm the frequent association of OSA in pediatric NAFLD with deficiency of serum anti-oxidants, Authors call attention to a number of relevant points: i) NAFLD subjects with definite NASH (NAS histologic score > 5) had more severe sleep disordered breathing and significantly higher apnea/hypopnea index scores compared to those without definite NASH; ii) a correlation between the more severe fibrosis detected in

patients with OSA/hypoxia versus those without OSA/hypoxia; iii) a clear correlation between severity of the indexes of oxidative stress and the severity of the indexes used to evaluate OSA/NH; iv) the two populations of obese NAFLD adolescents did not differ for other relevant serum liver indexes and liver histology score (steatosis, ballooning degeneration, inflammation, NAS score). The suggestion is that obese NAFLD adolescents experiencing OSA/NH will then experience a surplus of hepatic oxidative stress that correlates with a more severe fibrosis than in non-OSA/NH NAFLD patients. This is reasonable since the repeated episodes of NH and normoxia (chronic intermittent hypoxia) in OSA/NH can easily lead to enhanced mitochondrial generation of ROS. Moreover, increased ROS generation in a hypoxic environment can sustain liver fibrogenesis through either ROS themselves or through responses operating through hypoxia-inducible factors (HIFs; i.e., known to be stabilized and then amplified by intracellular ROS) [7,8]. Although further studies designed to investigate specifically the role of HIFs are needed, both events have been suggested to modulate either the release of pro-inflammatory and pro-fibrogenic mediators by macrophages or activated hepatic stellate cells (HSCs) or by directly targeting HSCs. It should be noted, in particular, that the most relevant pro-fibrogenic phenotypical responses by activated HSCs are sustained by increased intracellular levels of ROS not able to induce cytotoxicity [reviewed in ref. 8]. Accordingly, this scenario closely resembles the described increase in liver injury and fibrosis reported in a rodent dietary model (high fat diet) of NAFLD/NASH when associated with chronic intermittent hypoxia [18].

However, like most investigations on this topic and as the Authors themselves acknowledge, this well-designed study is not exempt from some inevitable and evitable limitations. The major inevitable limitations are the poor generalizability (89% Hispanic subjects), the small sample size, the cross-sectional nature of the study not proving a causal link between OSA and liver damage, the possible bias towards a focus on more

severe liver disease due to enrollment of subjects who underwent liver biopsy for chronic elevation of aminotransferases, and the intrinsic ethics issues for the invasiveness of liver biopsy itself. Evidable limitations are the absence of a “healthy” obese control group and the lack of information about patients adiposity criteria other than BMI (e.g., the waist-to-height ratio, or WC cm > 95 %ile for age and gender [19]. For lean controls, a lesser disproportion of Hispanic subjects, polysomnography and liver ultrasounds data, and/or a specific validated OSA clinical score might have added weight to the Authors’ conclusions and possible insights into the bi-directionality of the events studied.

In any case, the study by Sundaram et al. [9] has merit and outlines a number of further relevant issues and perspectives. The reported significant relationships between i) blood hematocrit (Hct) and NAFLD fibrosis stage and ii) anti-oxidant blood values, and NAFLD and lipid peroxidation parameters, may suggest that their combined evaluation should help in deciding whether histological and polysomnographic evaluation are needed in order to early recognize subjects with more severe NAFLD and/or more severe OSA and hypoxia. Finally, since nocturnal Continuous Positive Airway Pressure (CPAP) is a treatment modality also for pediatric NAFLD patients with OSA/hypoxia, we definitively need trials designed to investigate whether CPAP treatment may significantly affect NAFLD progression in this age range. In fact at present the only randomized controlled trial on the possible efficacy of CPAP treatment was of relatively short duration, performed on adult patients with mild OSA/hypoxia and normal baseline transaminases, and apparently did not demonstrate any impact on steatosis, NASH or liver fibrosis, as evaluated by FibroMax score [20].

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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