Research Article

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Chronic intermittent hypoxia is a major trigger for non-alcoholic fatty liver disease in morbid obese

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See Editorial, pages 20–22

Background & Aims: Morbid obesity is frequently associated with low grade systemic inflammation, increased macrophage accumulation in adipose tissue (AT), obstructive sleep apnea (OSA), and nonalcoholic fatty liver disease (NAFLD). It has been suggested that chronic intermittent hypoxia (CIH) resulting from OSA could be an independent factor for early stage of NAFLD in addition to other well-recognized factors (dyslipidemia or insulin resistance). Moreover, macrophage accumulation in AT is associated with local hypoxia in fat tissue. We hypothesized that the association between CIH and morbid obesity could exert additional specific deleterious effects both in the liver and adipose tissues.

Methods: One hundred and one morbidly obese subjects were prospectively recruited and underwent bariatric surgery during which a liver needle biopsy as well as surgical subcutaneous and omental AT biopsies were obtained. Oxygen desaturation index (ODI) quantified the severity of nocturnal CIH.

Results: Histopathologic analysis of liver biopsies demonstrated that NAFLD lesions (ballooning of hepatocytes, lobular inflammation), NAFLD activity score (NAS), and fibrosis were significantly more severe in patients with the highest ODI tertile (p values $\leqslant$0.001 for all hepatic lesions). In multivariate analysis, after adjustment for age, obesity, and insulin resistance status, CIH remained independently associated with hepatic fibrosis, fibroinflammation, and NAS. By contrast, no association was found between CIH, macrophage accumulation, and adipocytes size in both subcutaneous and omental adipose tissue.

Conclusions: In morbidly obese patients, CIH was strongly associated with more severe liver injuries but did not worsen obesity induced macrophage accumulation in adipose tissue depots.

Keywords: NAFLD; Obesity; Intermittent chronic hypoxia; Adipose tissue.
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Abbreviations: NAFLD, nonalcoholic fatty liver disease; OSA, obstructive sleep apnea; CIH, chronic intermittent hypoxia; ODI, oxygen desaturation index; NAS, nonalcoholic fatty liver disease activity score; NASH, nonalcoholic steatohepatitis; AT, adipose tissue; BMI, body mass index; CPAP, continuous positive airway pressure; scWAT, subcutaneous white adipose tissue; oWAT, omental white adipose tissue; SaO_2, oxygen saturation; AHI, apnea/hypopnea index; PaO_2, partial pressure of oxygen in arterial blood; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase; HOMA-IR, homeostasis model assessment; SD, standard deviation; IL-6, interleukin-6.

Introduction

Obstructive sleep apnea (OSA) caused by repetitive partial or complete obstruction of the upper airway leads to chronic intermittent hypoxia (CIH) [1]. OSA is fivefold more prevalent in morbid obesity compared to leanness [2]. OSA results in chronic systemic inflammation [3–4] and is associated with insulin resistance and type 2 diabetes [5–6]. Studies in humans [7–9] or and in lean mice [10] have suggested that OSA also leads to liver injury and could be a trigger for early stage of nonalcoholic fatty liver disease (NAFLD) independently of obesity.

NAFLD is associated with insulin resistance, and its prevalence rises in parallel with worldwide increases in obesity and type 2 diabetes. NAFLD is associated with an increased risk of cardiovascular diseases and can progress to non-alcoholic steatohepatitis (NASH), liver cirrhosis, and cancer. The diagnosis of NAFLD relies on histopathologic findings and includes a wide spectrum of lesions (simple steatosis, steatohepatitis and fibrosis potentially leading to end stage cirrhosis) [11–12]. Nevertheless, the understanding of NASH pathogenesis remains debated. For a decade, the "two-hit" mechanism has been suggested to

explain the progression in liver alteration stages with the first step consisting in hepatocytes lipid accumulation mainly due to obesity and insulin resistance, in the absence of significant alcohol consumption or other liver disease [13]. Then a second hit was believed to play a role in the shift from steatosis to non-alcoholic steatohepatitis. Many possible factors such as oxidative stress and inflammatory mediators have been incriminated [14–15]. More recently, an alternative theory suggested a lipotoxic role of fatty acids in liver injury leading to NASH and occurring in parallel with triglycerides droplets accumulation (i.e.: steatosis) [16]. This emerging hypothesis might not account for all previously described contributory factors of liver injury. Among the culprits in NASH hepatocellular injury, the severity of CIH associated to OSA which is dramatically increased in obese compared to lean patients, and which induces oxidative stress and cytokines production could play a major role in the pathogenesis of NASH [17].

Obesity, a low-grade systemic inflammatory disease, favors *per se* the development of insulin-resistance, type 2 diabetes, OSA, and NAFLD [18–20]. Macrophages accumulate in adipose tissue (AT) [21–22] in proportion to body mass index (BMI). Macrophage accumulation and phenotype vary with AT anatomical sites [22–23] and we previously identified an association between omental AT macrophage accumulation and the severity of steatosis and liver fibro-inflammation [22,24]. Macrophage accumulation in obese AT also seems to relay with fat tissue local hypoxia [25]. Adipocytes exposure to hypoxia is promoting dysregulated production of adipocytokines which contributes to insulin resistance and metabolic syndrome. In consequence, CIH occurring during night in OSA may exacerbate AT inflammation which could trigger NASH.

We hypothesized that the association between CIH and obesity could exert additional deleterious inflammatory effects on both AT and the liver in morbid obesity. We addressed the following questions; (i) "Is CIH more severe in obese exhibiting NAFLD?" (ii) Could severe CIH in obese patients be associated with both more prevalent NASH and fibrosis and worse AT inflammation and macrophage accumulation?

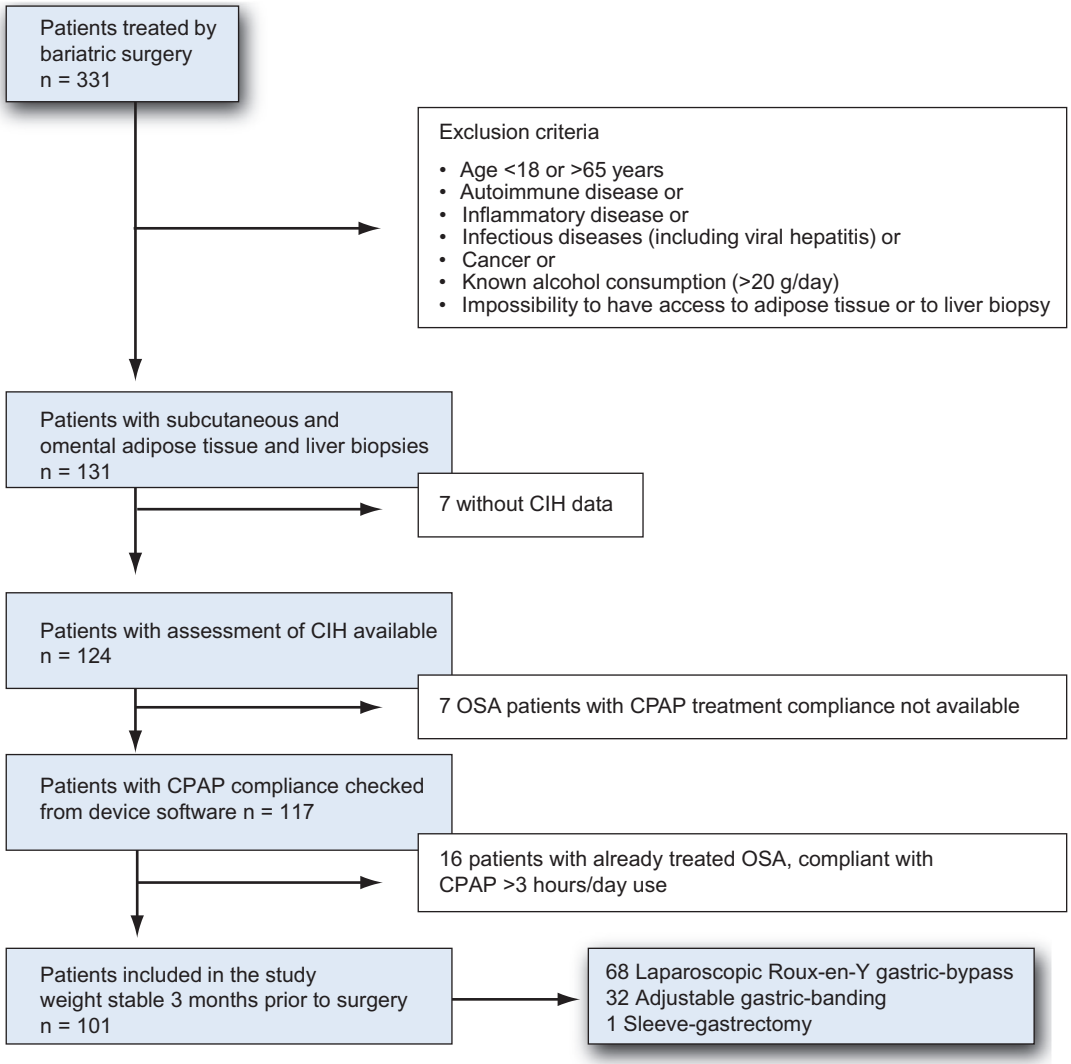

Fig. 1. Study flow chart.