Sleep Apnea, Hypoxia Inducible Factor, and Fatty Liver: More Questions Than Answers?

Nonalcoholic fatty liver disease (NAFLD) is the most prevalent chronic liver disorder (1). NAFLD affects ~25% of the adult population (2), but its prevalence exceeds 70% in obese patients (3). NAFLD is a heterogeneous condition, which includes a spectrum of disease progression ranging from steatosis without inflammation to nonalcoholic steatohepatitis (NASH), a progressive fibrotic disease (1). Although the majority of patients with NAFLD manifest liver steatosis without progression to end-stage liver disease, up to 30% of patients with NAFLD develop NASH (2). Hepatic fibrosis in NASH leads to poor outcomes, including cirrhosis and liver-related mortality (1). To this date, there is no approved therapy for NASH. Moreover, the underlying mechanisms mediating the progression from steatosis to NASH and liver fibrosis remain unknown.

Liver hypoxia has been identified as a potential stimulus of fibrogenesis and progression of NAFLD to NASH. Hypoxia activates hypoxia inducible factors HIF-1 and HIF-2. These transcription factors, which are heterodimers of hypoxia-sensitive α subunits and a constitutively expressed β subunit, regulate cellular transcriptional responses to hypoxia (4). *In vitro*, hypoxia activates both HIF-1α and HIF-2α in hepatic stellate cells, inducing an increase in profibrotic mediators (5). Hepatocyte-specific HIF-1α knockout reduces liver fibrosis *in vivo* (6). The fibrogenic effect of hepatocyte HIF-1 may be related, at least in part, to upregulation of LOX (lysyl oxidase) and LOX-like proteins. LOX is an enzyme that catalyzes collagen fiber cross-linking, an important process in the development of liver fibrosis (6, 7). HIF-2α is implicated in hepatic inflammation and lipid accumulation and augments the expression of fibrogenic genes (8). Thus, HIF-1 and HIF-2 may mediate NAFLD severity and the progression to NASH as a result of tissue hypoxia in hepatic steatosis. In this context, conditions that expose patients to hypoxia could increase the risk for hepatic fibrosis and NASH.

Obstructive sleep apnea (OSA) is a disease characterized by recurrent episodes of upper airway collapse that lead to periods of intermittent hypoxia (IH) during sleep. An association between OSA and NASH has been demonstrated in several cross-sectional clinical studies (9). The severity of oxyhemoglobin desaturation during apneic episodes is correlated with the degree of liver fibrosis independently of body mass index in patients who underwent liver biopsies, the gold standard of NAFLD/NASH diagnosis (9). A causal link between NAFLD and the hypoxic stress of OSA is yet to be established. Clinical trials of continuous positive airway pressure (CPAP), the preferred treatment of OSA, did not examine liver pathology. These trials failed to show beneficial effects of CPAP on fibrogenic biomarkers and liver steatosis, which, at least in part, could be attributed to a short-term treatment and low adherence with CPAP (9, 10). Mechanistic evidence of a possible contribution of IH to NAFLD progression has been provided by animal models.

Our group has previously shown that chronic IH, mimicking the oxyhemoglobin desaturations in patients with OSA, exacerbates liver tissue hypoxia and affects hepatic lipid metabolism in mice. IH induces hyperlipidemia and increases the levels of SREBP-1 (sterol regulatory element binding protein 1) and SCD-1 (stearoyl CoA desaturase) in the liver, which are key regulators of lipogenesis (11). Mice exposed to chronic IH show increased lipid peroxidation and hepatic levels of proinflammatory cytokines (e.g., TNF-α and NF-κB) (12). IH also causes oxidative stress and exacerbates insulin resistance, a major risk factor for NAFLD in diet-induced obesity (13). In mice with diet-induced hepatic steatosis, chronic IH induced lobular inflammation and liver fibrosis (12). Partial HIF-1α deficiency was associated with a reduction of IH-induced hyperlipidemia and hepatic levels of SREBP-1 and SCD-1 (14). Thus, it is conceivable that severe liver hypoxia induced by OSA causes liver inflammation and fibrosis, contributing to the development of NASH via the activation of hepatic HIF-1 pathways.

This hypothesis was tested by Mesarwi and colleagues (pp. 390–402) and is reported in this issue of the *Journal* (15). The investigators examined the role of HIF-1α in IH-induced progression of NAFLD using two protocols: *1*) mice with hepatocyte-specific deletion of *Hif1-α* (*Hif1-α*$^{-/-}$*hep*) and wild type (*Hif1-α*$^{F/F}$) were fed a trans-fat diet for 26 weeks and exposed to IH or intermittent air in the last 6 weeks, and *2*) C57BL/6J mice were fed with the same diet for 26 weeks and treated with HIF-1α antisense oligonucleotides (ASO), scrambled ASO, or saline while exposed to IH. The authors show that the *Hif1-α*$^{-/-}$*hep* mice had a slower weight gain, decreased fasting glucose and homeostatic model assessment-insulin resistance (HOMA-IR), and improved glucose tolerance compared with the wild-type mice (Figure 1). *Hif1-α* knockout mice also showed a reduced expression of proinflammatory cytokines TNF-α and IL-1β and fibrotic genes *Loxl1* and *Col3a1*. IH exacerbated liver fibrosis and inflammation. However, no significant interaction between IH and mouse genotype was found. In the second experiment, HIF-1α knockdown with ASO showed a trend to decrease collagen content and liver fibrosis (Figure 1). Collectively, both protocols complement each other and demonstrate that both HIF-1α and IH can independently contribute to the progression of

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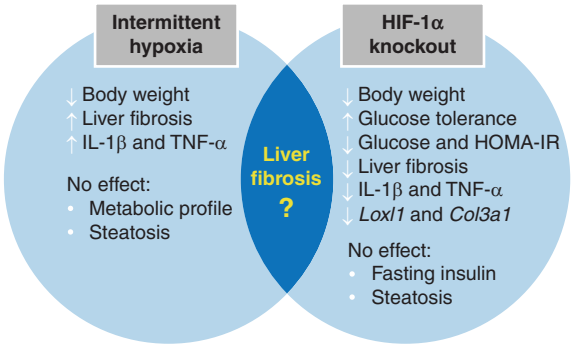

Figure 1. Summary of the main findings of Mesarwi and colleagues (15). Independent effects of intermittent hypoxia and HIF-1α knockout were observed on a model of nonalcoholic fatty liver disease, but there was no significant interaction. *Col3a1* = collagen type III α 1 chain; HIF-1α = hypoxia inducible factor 1-α; HOMA-IR = homeostatic model assessment-insulin resistance; LOX = lysyl oxidase.

NAFLD, without evidence of interaction or mechanistic link. The limitations of the study include the short duration of IH, which could account for the lack of IH-induced metabolic dysfunction, and the lack of intermittent air control in the ASO experiment.

Overall, Mesarwi and colleagues (15) provide additional evidence linking OSA to NAFLD and liver fibrosis. The investigators also confirm the pathological role of HIF-1α in NASH. However, the current report does not reveal a causal relationship between IH-induced NASH and HIF-1α. Future studies targeting other molecular pathways involved in liver fibrosis in IH are warranted, such as the activation of HIF-2α in the liver. The examination of downstream pathways of HIF-1α in the liver may provide insights into molecular targets for drug development in NAFLD and NASH. ■

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