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Editorial

Nash, insulin resistance and iron

Non-alcoholic steatohepatitis (NASH) is part of the spectrum of non-alcoholic fatty liver disease (NAFLD), which can progress to hepatic cirrhosis and end-stage liver disease. Its pathogenesis is associated with insulin resistance (IR) and the metabolic syndrome. Hepatic steatosis has also been considered an early marker of IR. It is now accepted that NASH is a multistep process with a prominent role for IR, where oxidative stress and cytokines retain a central role (1).

The relation between NASH and iron metabolism/overload is still controversial. It may be of relevance for pathogenesis and progression to more severe forms of disease. A strong association between iron overload and IR/metabolic syndrome and therefore with hepatic steatosis and NASH has been proposed. In fact, Mendler et al. (2) defined a syndrome of 'IR-associated iron overload', in the presence of unexplained hepatic iron overload and at least one component of the IR syndrome. Besides, elevated serum ferritin levels have been frequently reported in patients with diabetes mellitus and IR (3–7). Also, Fargion et al. (8) studied a cohort of 40 patients with hyperferritinemia and found IR in 69%. Several hypotheses have been postulated to explain this association. Iron overload is believed to lead to IR, as suggested by the fact that iron depletion can improve insulin sensitivity (9–11). Also, a recent study showed a decrease in the development of diabetes mellitus in blood donors, which correlated with a reduction in iron deposits and an increase in insulin sensitivity (12). Besides, iron overload can interfere with insulin signaling through the induction of reactive oxygen species, the latter impairing insulin uptake through a direct effect on insulin receptor function, by inhibiting the translocation of GLUT4 to the plasma membrane (13, 14) and iron can also induce IR of glucose transport in adipocytes (15). Furthermore, hepatic extraction and metabolism of insulin decreases with increasing iron stores, leading to hyperinsulinism (16, 17). This relation IR/iron overload is also important in reverse, as insulin stimulates cellular iron uptake through increased transferrin receptor externalization (18, 19). It is also interesting to note that hepatic iron overload has been described in hereditary defects of insulin receptors (20).

Although iron may then be an important cofactor in the first hit, through IR, it is in the subsequent hits that iron may play its major role. Some authors believe that iron may be the substrate of oxidative stress (21). In steatotic livers, the saturation of β-oxidation by excess free fatty acids will ultimately lead to the generation of hydrogen peroxide, which in turn can be converted to highly reactive hydroxyl radicals in the presence of free iron (22, 23). There is strong evidence, from *in vitro* and *in vivo* studies, that iron overload enhances oxidative stress (24–29). Iron can also promote fibrosis through hepatocellular necrosis (the so-called sideronecrosis) and inflammation with activation of Kupffer cells which release profibrogenic mediators, as direct fibrogenic promoter acting as a paracrine activator of hepatic stellate cells, or as a cofactor in fibrogenesis in conjunction with other hepatotoxins (30).

The association of NASH with iron overload is still not clear, as opposite results have been presented. Some important studies suggest an increased prevalence of common mutations of the *HFE* gene associated with hereditary hemochromatosis in patients with NASH. George et al. (31), evaluated 51 patients with NASH, and found that 31% were either homozygous or heterozygous for the Cys282Tyr mutation, a prevalence significantly greater than in controls, although the prevalence of His63Asp was not higher in these patients. Of note, however, patients with NASH carrying the Cys282Tyr mutation also had significantly less steatosis. Bonkowsky et al. (32) also found an increased prevalence of total *HFE* mutations, in 23 NASH patients vs the control group (69% vs 40%, $P = 0.001$). However this was only true when Cys282Tyr and His63Asp mutations were considered together, the frequency of Cys282Tyr heterozygosity alone was not increased. More recently, Chitturi et al. (33) also found an increased frequency of Cys282Tyr heterozygosity among 93 patients with NASH compared with healthy blood donors (22% vs 9%, $P = 0.035$).

However, three other studies did not confirm an increased prevalence of *HFE* mutations in patients with NASH (34–36). This discrepancy of results may be related to a referral or ascertainment bias, as the former studies are from reference centers for hemochromatosis.

Another topic of interest is the relation between NAFLD and its progression to more aggressive forms with necroinflammation and fibrosis and iron overload. Most studies are consensual in demonstrating an association between hepatic steatosis and NASH with hyperferritinemia (2, 8, 31, 33, 34). Hyperferritinemia also correlates with the severity of histologic activity, in terms of necroinflammation and fibrosis (2, 31, 34, 37). However, most studies failed to demonstrate higher iron deposits in patients with NASH. George et al. (31) found that stainable hepatic iron stores, hepatic iron concentration and hepatic iron index were positively associated with fibrosis. Those results were not confirmed by subsequent studies, in which, elevation of transferrin saturation and hepatic iron stores did not associate with histologic severity of NASH (2, 34–39). In fact, most authors believe that hyperferritinemia cannot be considered a marker of iron overload in this setting, and it has been suggested to be a marker of the IR syndrome (40).

Kirsch et al.'s (41) work is very interesting, as it tries to bring more clues to a controversial topic. An animal study was conducted, evaluating the relation between iron and NASH, using the methionine choline deficiency (MDC) diet model, with supplementation of iron. The first 'unexpected' result was a decrease in hepatic steatosis in the iron-supplemented rats. However, the link between iron and hepatic steatosis has been proposed to be IR, and the MDC diet model do not have IR, and usually have a lower body weight, what might explain the negative correlation with steatosis. Iron has also been shown to induce crucial changes in lipid metabolism (42–44) (of note these animals also had lower triglyceride serum levels), which may favor these results, in a setting were IR does not play a role. Another 'unexpected' issue is the fact that iron supplementation was also associated to lower aminotransferase levels, but as aminotransferase levels correlated to the severity of steatosis, that might explain the difference.

Concerning hepatic inflammation, Kirsch et al. (41) found it to be increased in the iron supplemented group at 4 weeks although it was similar at 12 weeks. This may be explained by the fact that iron overload can induce oxidative stress in an acute setting, but maintained iron overload also elicits an up-regulation of antioxidant defenses, thus limiting the accumulation of oxidative damage, as proposed by Brown et al. (24). Perivenular fibrosis was also more severe in this group.

The authors also evaluated hepatic lipid peroxidation products, and found a decrease in both early and end products of lipid peroxidation (CD and free TBARS, respectively). Of note, they did not study markers of oxidative stress, rather markers of oxidative damage, and as pointed out by the authors, the iron supplemented rats had lesser degrees of steatosis resulting in less substrate for lipid peroxidation. When they analyzed the relation between early and end products of lipoperoxidation, an increase in TBARS:CD ratio, favoring end products, was found, what may translate an increment in oxidative damage. In fact, transition metal ions, especially iron, which frequently have unpaired electrons are excellent catalysts in the generation of very reactive species from the less reactive ones (30).

Iron appears to be an important cofactor in the pathogenesis and progression of some cases of NASH, although it is not universal, as most studies are not able to correlate it with more aggressive forms of NASH. The somewhat intriguing results of Kirsch et al., should encourage further studies on the effect of iron in lipid metabolism, in the setting of NAFLD, using alternative models of NAFLD, such as genetically obese rodents, or the high-fat diet model (45), in order to tackle this difficult issue.

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