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EDITORIAL

A Bugs Battle on Behalf of the Liver

No Tubo Digestivo Trava-se uma Batalha pelo Fígado

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Nonalcoholic fatty liver disease (NAFLD), the ectopic accumulation of fat in the liver, is the hepatic manifestation of the Western lifestyle-associated adiposopathy. Excessive caloric intake and defective energy expenditure overwhelm the adipose tissue, that becomes sick. The sick adipose tissue has impaired endocrine function releasing potentially nasty adipokines (such as leptin and TNF-α) in detrimental of health promoting ones such as adiponectin. It also cannot store fat appropriately, allowing fat to metastasize into distant organs such as the liver. A third element in the equation, intestinal microbiota, defines a potent axis, gut-adipose tissue-liver, in the pathogenesis of NAFLD.

Our gut houses ten to hundred trillion microbes, at least 10 times more the number of cells in our body.[1] These are not unwanted guests; they are lifelong residents that have a crucial role in maintaining whole-body homeostasis. Also, our gut microbiota is specific for each individual and is highly resilient, easily returning to the basal state after perturbation. Even though the microbiota is specific, because there is a huge overlap of metabolic functions and gene expression between different microorganisms, humans share a core functional microbiome.[2] Several factors can modulate the gut microbiota: host-related factors such as genetics and host immune system; external factors such as diet and antibiotics; and microbiota-related factors, because microbes competitively or cooperatively interact with each other.[3] When gut microbiota is biased to a disease-promoting phenotype, called dysbiota, it can promote the development and progression of NAFLD. Several studies in animal models and human patients with NAFLD/NASH tried to identify a NAFLD-specific dysbiota.[4] However, this could not yet been defined. Those studies were small, often without histological diagnosis, and with different populations precluding pooled evaluation.

Studies with animal models of NAFLD/NASH thought us major concepts regarding the gut-adipose tissue-liver axis: not only the gut dysbiota has a crucial role in the development and progression of NAFLD and NAFLD-associated carcinogenesis,[5] but also, NASH can be a transmissible disease through the sharing of the gut microbiota.[6,7] The gut microbiota/dysbiota can promote NAFLD/NASH through several mechanisms. It can increase the energy harvested from diet through fermentation of indigestible carbohydrates into different short-chain fatty acids, and promote obesity through modulation of adipose tissue and liver metabolism. Furthermore, dysbiota may increase intestinal permeability and endotoxemia, it can produce toxic products such as ethanol, impair choline metabolism and bile acids homeostasis.[4]

Although NAFLD is an extremely frequent condition, and growing to expectedly become the first cause of end-stage liver disease in the Western world,[8] we still lack an effective treatment. As such, enthusiasm and expectations in targeting the gut microbiota for the management of NAFLD are high. In fact, several pilot studies and two small

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Abbreviations: LPS, lipopolysaccharide; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; TGF, transforming growth factor; TLR, toll like receptor; TNF, tumor necrosis factor.

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randomized controlled studies addressed the effect of probiotics in the treatment of NAFLD/NASH.[9–16] Despite that, we still lack solid evidence on the role of probiotics in the management of NAFLD, which precludes its recommendation. Most studies are small, short-term, and heterogeneous in terms of design, population studied, probiotic/symbiotic used and end-points. Only two studies evaluated the effect on liver fibrosis, one noninvasively through the determination of hepatic transient elastography,[15] and the other one with liver biopsy,[12] both with divergent results. In fact, human studies have huge limitations in assessing a real impact of an intervention in the prognosis of NAFLD/NASH. First of all it is a very slowly progressing disease, which precludes the evaluation of potent endpoints such as survival or progression to end-stage liver disease. The best way to infer an impact in the prognosis is to evaluate the effect on liver fibrosis, however because fibrosis progression is also slow, it implies interventions of at least 1–2 years and invasive evaluation with liver biopsy, which may be unethical in an otherwise asymptomatic subject. In this scenario, and because there is a huge diversity of available probiotics and symbiotics (i.e. the combination of probiotics and prebiotics), animal studies seem crucial to move the field forward.

In this issue of the Portuguese Journal of Gastroenterology, Cortez-Pinto et al. presented a preclinical study on the effect of a symbiotic preparation in the management of NASH.[17] They took advantage of the high fat choline deficient diet mouse model of NASH. This is a good animal model not only because it induces severe NASH with important fibrosis, but also because it does mimic human pathophysiology, since human NAFLD tends to associate with high fat content in the diet and the average consumption of choline in the general population is half the required dose.[18] The authors fed mice with a regular chow diet and compared to high fat choline deficient diet with or without supplementation with Synbiotic 2000® Forte. Synbiotic 2000® Forte is a mixture of four probiotic strains (*Pediococcus pentosaceus*, *Lactococcus raffinolectis*, *Latobacillus paracasei* subsp. paracasei and *Lactobacillus plantarum*) and four prebiotics (β-glucan, inulin, pectin and resistant starch) that was shown to decrease intestinal permeability and the risk of infection in critically ill children.[19]

Regarding the effect of the symbiotic preparation in the gut microbiota among mice fed the NASH-inducing diet, results were inconsistent, since most bacteria evaluated showed divergent results at week 6 or week 18 of treatment. The only consistent effect was a decrease in the abundance of Bacteroides in mice supplemented with the symbiotic. This is a relevant finding since in patients with NAFLD, NASH associates with higher abundance of Bacteroides.[20] The potential NASH-inducing effect of Bacteroides could be explained by an increase in toxic bile acid deoxycholic acid, which not only is increased in NASH, but it is a direct toxic to hepatocytes.[21,22] Also, Bacteroides produce branched-chain fatty acids from aminoacids fermentation, which have diabetogenic potential.[23] Supplementation with symbiotic also induced a decrease in serum lipopolysaccharide (LPS) and Gram-negative bacteria abundance in the stool, but only after long-term treatment (18 weeks).

Evaluating the effect of an intervention in the microbiota is a difficult task, because there is high variability in microbiota composition in the basal state, even in highly reproducible and controlled conditions such as in inbred mice. Microbiota, in this study, was assessed by Fluorescence in situ hybridization with fourteen 16S rRNA-targeted oligonucleotide probes for representative species, genera or families from five phyla: Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, Actinobacteria. This is a biased approach that does not allow fully characterization of changes in the microbiome induced by the intervention.

The most important finding in this study was partial prevention to NASH-related liver fibrosis (assessed through Sirius red staining and immunohistochemistry for the hepatic stellate cells activation marker α-smooth muscle actin), in mice supplemented with symbiotic. This is highly relevant because liver fibrosis is the histological feature that better dictates the hepatic prognosis in NAFLD.[24,25] Interestingly the decrease in fibrosis was not accompanied by an improvement in liver metabolism/steatosis or liver injury, hepatocellular ballooning or inflammation. This is surprising, since fibrosis is believed to be the consequence of an impaired/excessive response to liver injury.[26] However, in the same NASH mouse model, medication with non-absorbable antibiotics also reduced LPS serum levels and hepatic fibrosis, with no effect in liver injury.[27] Furthermore, in a different mouse model of NASH, methionine–choline deficient diet, supplementation with the probiotic VSL#3 partially prevented fibrosis, while having no effect in inflammation or steatosis.[28] On the contrary, studies on high fat diet did accomplish decreased steatosis and liver inflammation with probiotic supplementation.[29–31] The difference may be explained by the different mechanisms of steatogenesis in those models: while high fat diet induces steatosis through an increased uptake of circulating fatty acids arising from the adipose tissue, choline deficient diets are steatogenic through a decrease in the outflow of lipids by inhibiting lipoprotein synthesis.[32] The lack of effect on inflammation is more difficult to explain, but probably this study would need more sensitive assays to unravel an effect. Probiotics have shown to decrease inflammation, pro-inflammatory cytokines levels and function as well as a switch to an M2 anti-inflammatory phenotype in Kupffer cells.[29,33,34]

The protection from hepatic fibrogenesis conferred by the symbiotic mixture, may translate a decrease in LPS-induced activation of hepatic stellate cells. In fact, LPS signaling in hepatic stellate cells, through toll-like receptor (TLR)-4, sensitizes them to fibrogenic effect of transforming growth factor (TGF)-β, by down-regulation of the TGF-β pseudoreceptor Bambi.[35] However, the protection from liver fibrosis occurred previously to the decrease in serum LPS levels, which suggests LPS-independent mechanisms conferring protection against fibrosis.

In conclusion, gut dysbiosis seems to have a crucial role in the pathogenesis of obesity, metabolic syndrome and NAFLD/NASH. Since there is no effective available treatment for NAFLD/NASH, targeting the gut microbiota is an anticipated good candidate approach for the management of this disease. Despite the high hopes in this approach by the scientific community, lack of solid evidence precludes the implementation of probiotics in the clinical practice to treat NAFLD patients. The confusion in the field is extraordinary; there are many different formulations with different probiotics and/or prebiotics. There are little clues of how

long should treatment be applied, though the high resilience of our gut microbiota suggests that we might need life-long interventions. Human studies are difficult to implement in a slowly-progressive disease such as NAFLD, and it becomes essential to have strong preclinical studies guiding the design of future randomized controlled studies in humans. This study further increases the knowledge in the field and gives hepatologists extra hope in the probiotics approach in NAFLD, by showing a decrease in liver fibrosis, the strongest prediction of morbi-mortality in NAFLD.

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