

EDITORIAL

Adiponectin in non-alcoholic steatohepatitis: An ideal culprit, but what are the proofs of its guilt?

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With the epidemics of obesity and type 2 diabetes mellitus, the adipose tissue attracts a great deal of attention, and is now not only considered as a passive energy depot but also as an active hormonal tissue producing adipo(cyto)kines acting locally and in distant organs. When facing excess lipid load, the adipose tissue gets inflamed and sources a pro-inflammatory systemic environment mechanistically implicated in obesity and diet-induced insulin resistance. This pro-inflammatory state is characterized, amongst others, by increased tumor necrosis factor (TNF) and leptin levels and in contrast a decrease in adiponectin release.^{1,2}

Despite advances in the understanding of the role of the inflamed adipose tissue in the pathogenesis of obesity-associated insulin resistance, the links between the metabolic syndrome and the development of non-alcoholic steatohepatitis (NASH), a fibrotic and inflammatory form of non-alcoholic fatty liver disease (NAFLD) frequently observed in this syndrome, remain partly obscure.

Adiponectin is proposed as an ideal culprit. Adiponectin regulates lipid metabolism and has insulin sensitizing, anti-inflammatory and antifibrogenic properties.³ Most of these metabolic effects are mediated through adiponectin receptor-dependent activation of activated protein kinase-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor- α (PPAR- α),⁴ two important pathways controlling energy partitioning and lipid metabolism. AMPK is a fuel-sensing enzyme that stimulates adenosine triphosphate (ATP)-generating catabolic pathways, such as glycolysis and lipid oxidation, and turns off energy-consuming processes, such as glycogen, lipid and protein synthesis, to restore energy balance. PPAR- α is a transcription factor controlling gene expression of key enzymes of the β -oxidation pathways. Thus, adiponectin favors lipid burning, glucose utilization and prevents hepatic fat accumulation. Whilst acting directly on inflammatory cells, adiponectin also counteracts TNF, a potent pro-inflammatory cytokine. These two adipocytokines have opposite biological effects and are reciprocally regulated, one repressing the expression of the other one. This is of particular interest in the context of NASH as TNF has been largely implicated as a mediator of insulin resistance and pathological progression towards steatohepatitis. Finally, studies in rodents demonstrated that adiponectin has a suppressive effect on liver fibrogenesis.²

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These promises for a key role of adiponectin in NAFLD/NASH were further sustained by countless observations of low serum adiponectin concentrations in obese^{5,6} and in NAFLD patients.⁷ An inverse correlation between serum adiponectin levels and the grade of hepatic inflammation was found in some studies,^{8,9} although not confirmed in others.¹⁰ Interestingly, both adiponectin and its receptors have been shown to be decreased in NASH livers compared with simple steatosis, even when patients were matched for body mass index, and to be associated with higher inflammation and fibrosis.^{11,12} Similarly, hepatic resistance to adiponectin has been described in animal models of steatohepatitis.¹³ Together, this suggested that alteration of hepatic adiponectin concentration or of hepatic adiponectin signaling may be of greater relevance than circulating levels of this adipocytokine. Furthermore, many studies underscored the potential role of hypoadiponectinemia in various cancers, including hepatocellular carcinoma (HCC).¹ This reinforces the possibility of an implication of low adiponectin signaling in the development and progression of NAFLD from simple steatosis to NASH and its complications.

To explore the potential role of decreased adiponectin in NAFLD/NASH pathogenesis, several research groups have used mice lacking adiponectin (adiponectin knockout [KO] mice) as a model. While it has been consistently reported that adiponectin-deficient mice have normal insulin sensitivity when fed a standard chow, the changes induced by dietary manipulations are less constant. Using two different strains of genetically engineered mice, Maeda's and Kubota's groups described that adiponectin KO mice become significantly insulin resistant when fed a high fat/high sucrose diet for 2 weeks,¹⁴ or a high fat diet for 6 weeks,¹⁵ but fail to develop hepatic steatosis. However, in a longer term high fat/high sucrose feeding experiment, adiponectin KO mice were not found to be more susceptible to insulin resistance than wild-type littermates.¹⁶ Also, unexpectedly given the antisteatosis and the anti-inflammatory properties of adiponectin, mice lacking adiponectin develop less severe steatohepatitis in response to a methionine and choline-deficient diet than wild-type controls.⁴

In this issue of the Journal, Asano and colleagues examined for the first time the consequences of a long-term exposure (24 and 48 weeks) of adiponectin KO and wild-type mice to a high fat diet on liver pathology.

In wild-type mice, such regimen induces weight gain, hepatic steatosis and activation of fibrogenesis as assessed by upregulation of type I collagen gene expression. Despite this, perisinusoidal fibrosis, if any, remains discrete. In adiponectin KO mice, similar weight gain and hepatic steatosis were induced by the high fat diet whereas at the 48-week time point, perisinusoidal fibrosis appears slightly more sustained than in wild-type livers. Although at this

time type I collagen expression was similar in wild-type and adiponectin KO mice, the authors relate enhanced fibrosis to the cumulative consequences of a longer exposure to pro-inflammatory and profibrotic signals. Indeed, adiponectin-deficient mice display enhanced TNF and collagen I expression after 24 weeks exposure to high fat diet, while the expression of these genes was unchanged in wild-type mice at this earlier time point. The authors suggest that adiponectin may protect the liver from high fat diet-induced fibrosis by suppressing hepatic TNF expression.

The observation of increased fibrosis development in KO mice is of importance, because mice are quite refractory to fibrosis, and, specifically in the case of high fat diet regimen, fibrosis is generally inconspicuous. Together with a longer duration of exposure to a pro-inflammatory environment, insulin resistance as well as adiponectin deficiency itself may also participate to enhance fibrosis. Unfortunately, insulin sensitivity has not been evaluated by Asano *et al.* in their model. Recent *in vitro* experimental findings demonstrate that adiponectin has a suppressive effect on hepatic stellate cell (HSC) activation. This antifibrotic effect may be lost or diminished when adiponectinemia is low or absent, a situation that will promote fibrogenic transformation of HSC and fibrosis.

The effect of adiponectin on hepatic lipid metabolism is not clear. Histologically, steatosis appears more pronounced in KO livers but hepatic triglyceride content is similar to that of wild-type mice fed a high fat diet. This certainly calls for further investigations to resolve this apparent paradigm and to conclude whether adiponectin deficiency promotes steatosis or not. In particular, hepatic lipid metabolism machinery, and specifically enzymes and pathways regulated through adiponectin through AMPK and PPAR- α , and adipose tissue insulin sensitivity and free fatty acids release are worthy of further evaluation.

In this study, little information is provided about pro-inflammatory status, beside hepatic TNF gene expression which is upregulated earlier during the course of high fat feeding in adiponectin KO than in wild-type mice. At the later time point, however, hepatic TNF expression was similar in KO and wild-type mice. The timing of TNF upregulation, or rather the duration of exposure to increased TNF, may thus represent a factor contributing to increased susceptibility of adiponectin KO mice to a high fat diet. This early onset of inflammation could also provide an explanation for the insulin resistance observed in studies with short-term high fat feeding^{14,15} but not with long-term feeding protocols.¹⁶ Interestingly, the insulin resistance induced by a 3-day high fat diet in non-obese patients was more pronounced in patients with low serum adiponectin.¹⁷ All together, it appears that adiponectin may rather serve as counter regulatory mechanism upon acute changes in diet, nutritional load or inflammatory status, but may not be essential or be overpowered by other mechanisms in chronic long-term situations.

Additionally, Asano *et al.* report that one out of eight adiponectin KO mice fed the high fat diet for 48 weeks developed a liver adenoma and hyperplastic nodules. This may be an incidental event. However, as both acceleration of liver tumor formation in adiponectin KO mice fed a carcinogenic diet¹⁸ and increased prevalence of HCC on human NASH livers¹ have been reported, an association between low adiponectin and increased risk of HCC development remains plausible. However, this possibility has to be evaluated in animal and human studies specifically and properly designed to address this question.

Confronted with the alleged biological effects of adiponectin on lipid metabolism, insulin sensitivity, inflammatory and fibrotic processes mostly derived from study of cell systems, *in vivo* experimental data obtained using adiponectin KO mice do not deliver convincing evidences for the importance of this adipocytokine as a protective or counter-regulatory adipocytokine in diet-induced insulin-resistance and NAFLD/NASH. The absence of adiponectin during development and the entire lifespan of these animals is most probably compensated for by activation of adaptive and defense mechanisms to cope with this situation. A model with time-specific knockdown of adiponectin expression might be more (patho)physiologically informative.

We believe that further evaluation is needed to firmly and convincingly demonstrate that hypoadiponectinemia predisposes to or promotes NAFLD/NASH. If confirmed, then restoration of normal adiponectin circulating levels or of adiponectin signaling in the liver should be aimed for for prevention and treatment of NASH.

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