Adiponectin, Structure, Function and Pathophysiological Implications in Non-Alcoholic Fatty Liver Disease

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Abstract: Obesity is a major risk factor for the development of the metabolic syndrome, a cluster of diseases including insulin resistance, type 2 diabetes, dyslipidemia, hypertension, microalbuminuria, atherosclerosis, and non-alcoholic steatohepatitis. On the other hand, it is now generally accepted that adipose tissue acts as an endocrine organ producing a number of substances with an important role in the regulation of food intake, energy expenditure and a series of metabolic processes. Adiponectin is a recently discovered hormone produced exclusively by adipocytes. In fact, adiponectin is considered currently as a major factor in obesity-related insulin resistance and atherosclerosis. This new hormone differs from other adipocytokines in that its production and concentrations are actually decreased in insulin resistant subjects. The aim of this review is to summarize the current knowledge about the chemistry and physiology of adiponectin and to discuss its implications in the pathophysiology and potential treatment of insulin resistance and non-alcoholic fatty liver disease.

Keywords: Adiponectin, insulin resistance, obesity, non-alcoholic fatty liver disease.

INTRODUCTION

Obesity is considered worldwide as one of the most important chronic diseases due to its high prevalence and related comorbidities (metabolic syndrome, insulin resistance,non-alcoholic fatty liver disease, etc.). These diseases impact not only in morbidity, but also in mortality, as 300,000 annual deaths are attributed to obesity in the United States [1]. The prevalence of obesity in this country has increased dramatically in the last two decades, from 8% in 1988 to 22% in 2002 [2], however, in subjects with diabetes, the prevalence is as high as 54.8% [3].

The use of body mass index (BMI) for the classification of weight status is based on epidemiological associations with morbidity and mortality [4], but it is not a measure of body fat distribution, although it is correlated with body fat percentage. In recent years there has been considerable interest about the consequences of central obesity, also referred to as abdominal, android, truncal, or upper-body obesity. Fat distribution, independent of absolute fat composition, has been related to a number of health risks. Measures of abdominal obesity (such as waist-to-hip ratio) are related to a number of metabolic variables, including cardiovascular risk factors and abnormal glucose metabolism. Furthermore, abdominal obesity is associated with an increased risk of developing diabetes, ischemic heart disease, and stroke. The risks associated with obesity itself are also exacerbated by the presence of increased levels of visceral fat and central obesity [5].

Obesity, or indirectly, BMI (associated with insulin resistance) is a major risk factor for developing non-alcoholic fatty liver disease [6,7]. The prevalence of non-alcoholic fatty liver disease ranges from 2.8 % to 25% in general population [8,9], but in high risk population (i.e. diabetics and obese people), this prevalence reaches 70-86% [10,11].

The risk factors associated with non-alcoholic fatty liver disease are female gender, waist circumference, hyperinsulinemia, hypertriglyceridemia and impaired glucose tolerance or type 2 diabetes [12,13].

Adiponectin is a recently described hormone mainly produced by the adipose tissue [14]. Experimental studies suggest that adiponectin plays a major role in the pathophysiology of insulin resistance and the metabolic syndrome [15,16]. Insulin resistance causes abnormalities on lipid storage and lipolysis in insulin-sensitive tissues, which may induce an increased flux of free fatty acids from adipose tissue to the liver and cause steatosis [17,18].

ADIPONECTIN STRUCTURE

Adiponectin consists of a carboxyl-terminal globular domain and an amino-terminal collagenous domain [22,23], the globular domain shares homology to those of collagens VIII and X. Adiponectin belongs to the family of complement 1q, known to form characteristics multimers [19-21]; gel filtration and velocity gradient studies revealed that serum adiponectin circulates as different molecular weighted species [24]. Pajvani *et al*. [25], demonstrated that adiponectin exists in two different complexes in serum, as a hexamer [low molecular weight (LMW), ~180 kDa] and a 12–18 subunits complex [high molecular weight (HMW), 400 kDa]. Experimental data indicate that disulfide bonds link all adiponectin monomers. This disulfide bond formation depends on cysteine-39 in the amino-terminal variable region as the necessary moiety. Single-nucleotide polymorphisms (G84R, G90S, Y111H, and I164T) modify this disulfide bond formation, and might alter adiponectin's ability to form multimers larger than a trimer, affecting its biological activity [24]. A recent controversial study by Tsao *et al*. [26], suggests that the majority of adiponectin in serum

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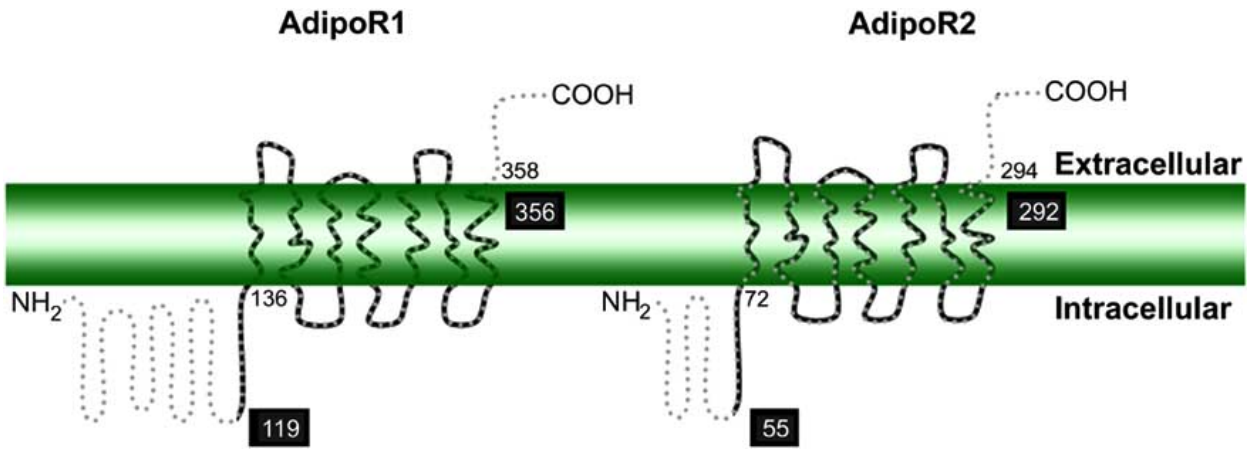

Fig. (1). Proposed structure of adiponectin receptors with their conserved region (dark numbers) and trasmembrane domain (white numbers).

circulates as the HMW form (>80%), and the rest in the hexameric (<10%) and trimeric (<10%) forms. Other important issue about structure of adiponectin is related with the post-translational modifications, particularly hydroxy-lysyl glycosylation of four lysines residues in the collagenous domain (K68, K71, K80, and K104) critical for its insulin-sensitizing activity with respect to inhibition of hepatic glucose production [27].

Wong *et al*. [21], described a family of proteins with high homology to adiponectin, designated as CTRP1-7, which exhibit similar structural and biological properties, however, the physiological importance of these proteins is uncertain.

ADIPONECTIN RECEPTORS

There are two distinct adiponectin receptors (AdipoR1 and AdipoR2). Human and mouse AdipoR1 share 96.8% identity and human and mouse AdipoR2 share 95.2% identity. AdipoR1 is located at chromosome 1p36.13-q41, whereas AdipoR2 is located at chromosome 12p13.31 [28, 29]. AdipoR1 encodes a protein of 375 amino acids with a predicted molecular mass of 42.4 kDa, whereas AdipoR2 encodes a protein of 311 amino acids with a predicted molecular mass or 35.4 kDa. AdipoR1 and AdipoR2 are structurally related, sharing 66.7% identity [30]. They use AMP-kinase as a second messenger but do not seem to be coupled with G protein. AdipoR2 also activates peroxisome proliferator-activated receptor-alpha (PPARα) and p38 mitogen-activated protein kinase (p38-MAPK). AdipoR1 is most abundantly expressed in skeletal muscle, whereas AdipoR2 is mainly expressed in the liver [31].

AdipoR1 and AdipoR2 are seven transmembrane domain receptors, with an internal N-terminal domain and an external C-terminal domain (Fig. **1**). Scatchard plot analysis revealed that there are two binding sites for globular adiponectin: high-affinity binding sites (dissociation constant (K_d) ≈ 0.06 μg/ml^{-1}, equivalent to 1.14 nM of the globular adiponectin trimer) and intermediate-affinity binding sites (K_d ≈ 0.80 μg/ml^{-1}, equivalent to 14.4 nM of the globular adiponectin trimer). In contrast, there are intermediate (K_d value ≈ 6.7 μg/ml^{-1}, equivalent to 49.1 nM of the full-length adiponectin hexamer) and low-affinity binding sites for full-length adiponectin (K_d value approximately 329.3 μg/ml^{-1}, equivalent to 2,415 nM of the full-length adiponectin hexamer) [30].

MODE OF ACTION OF ADIPONECTIN

AdipoR1 has a high-affinity for globular adiponectin, and a low-affinity for full-length adiponectin. In contrast, AdipoR2 has an intermediate affinity for both globular and full-length adiponectin, the latter one being apparently responsible for its effects on the liver [30].

In vitro studies in hepatocytes, rhabdomyocytes, and adipocytes have shown that activation of the AMPK is necessary for the signaling effects of adiponectin [15,32,33]. Activation of AMPK might be a common mechanism by which adipocytokines increase insulin sensitivity [34,35]. Interestingly, an AMPK-mediated signaling pathway has been implicated in the mechanism of action of metformin and thiazolidinediones [36-38].

Adiponectin stimulates fatty-acid oxidation, glucose uptake and lactate production in rhabdomyocytes. In the liver, it stimulates fatty acid oxidation and reduces gluconeogenesis, which can account for the acute glucose-lowering effect of adiponectin *in vivo* [15].

The effect on hepatic insulin sensitivity could be partly explained by the upregulation of PPARα-target genes, like CD36, acyl-coenzyme A oxidase, and uncoupling protein 2, indicating that adiponectin could activate PPAR-α [39]. Actually, adiponectin itself has been described as a PPAR-γ target gene [40].

The insulin-sensitizing effects of adiponectin are accompanied by anti-inflammatory properties. Full-length adiponectin inhibits tumoral necrosis factor alpha (TNFα)-induced expression of several adhesion molecules on the surface of endothelial cells such as vascular cell adhesion molecule-1, E-selectin, and intercellular adhesion molecule-1 [41]. Full-length adiponectin also suppresses TNFα-induced inflammatory changes in endothelial cells by blocking nuclear factor-κB activation without affecting TNFα-mediated activation of c-Jun N-terminal kinase, p38, and protein kinase B (Akt) [42]. Additional anti-inflammatory effects of adiponectin include suppression of leukocyte colony formation, reduction of phagocytic activity, and reduction of TNFα secretion from macrophages [23,43]. Kumada *et al*. [44], demonstrated

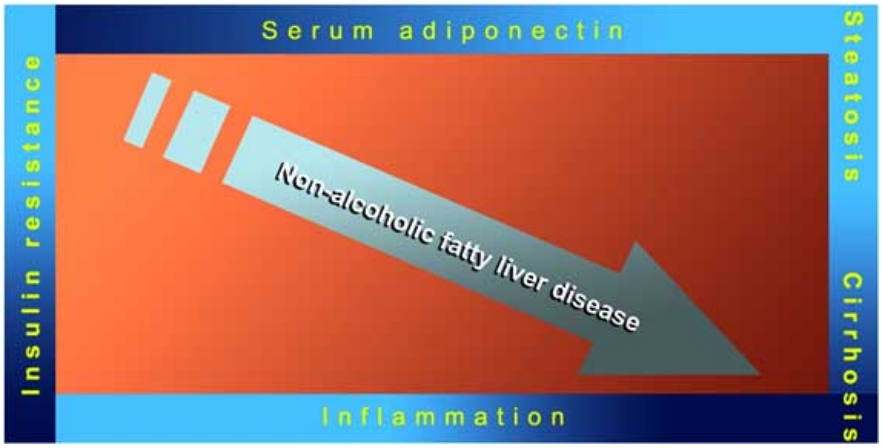

Fig. (2). Relation between phenomena associated with non-alcoholic fatty liver disease. Insulin resistance, a hallmark of the disease even without overweight or obesity, together with a chronic low-grade inflammatory state (local and systemic), are inversely associated with low serum adiponectin levels.

that adiponectin rapidly upregulates IL-10 and selectively increases the expression of tissue inhibitor of metalloproteinases-1 in both mRNA and protein levels, whereas the mRNA, protein levels, and activities of MMP-9 were not changed in human monocyte-derived macrophages, indicating that adiponectin may modulate the inflammatory response through IL-10. Finally, adiponectin might participate in angiogenesis through its ability to stimulate the AMPK-dependent signaling, which could lead to angiogenic factor synthesis in skeletal muscle [15, 30, 32, 45-49] and hypoxia-induced angiogenesis [50] and antiapoptotic cellular responses in endothelial cells [51].

OBESITY AND INSULIN RESISTANCE

Adiponectin is found abundantly in the circulation with concentrations between 2 and 20 mg/mL, accounting for up to 0.05% of total serum protein [52,53]. Serum adiponectin levels are lower in obese subjects as compared with lean subjects [53-55], although one study did not establish this difference [56]. Visceral adiposity has been shown to be an independent negative predictor of adiponectin serum levels [57] and both adipose adiponectin mRNA expression and plasma adiponectin levels are decreased in most experimental obesity models [39,58]. Longitudinal studies in primates suggest that adiponectin decreases with weight gain [59] and studies in patients undergoing bariatric surgery (weight loss >20% of body weight) show a significant increase in circulating adiponectin levels within 6–12 months after surgery [60-62]. Although Hoffstedt *et al*. [56] found no difference in serum adiponectin concentration between obese and nonobese subjects, they showed that subcutaneous adipose secretion rate of adiponectin was reduced in obese compared with nonobese subjects. TNFα is expressed at higher levels in adipose tissue from obese patients than healthy controls, and it has been shown to attenuate insulin sensitivity *in vivo* and *in vitro* [63-66]. Several authors hypothesize that the increased adipose tissue levels of TNFα in obesity downregulate adiponectin production [67].

Several studies support the hypothesis that adiponectin functions as an insulin sensitizer.

In one study [68], obese insulin-sensitive subjects had higher adiponectin levels than the obese insulin-resistant subjects, despite the fact that weight and BMI were equal in both groups. Similarly, adiponectin levels in nonobese insulin-sensitive subjects were elevated compared with those of the nonobese subjects who were classified as insulin resistant. Thus, adiponectin concentration proved to be an indicator of insulin sensitivity status.

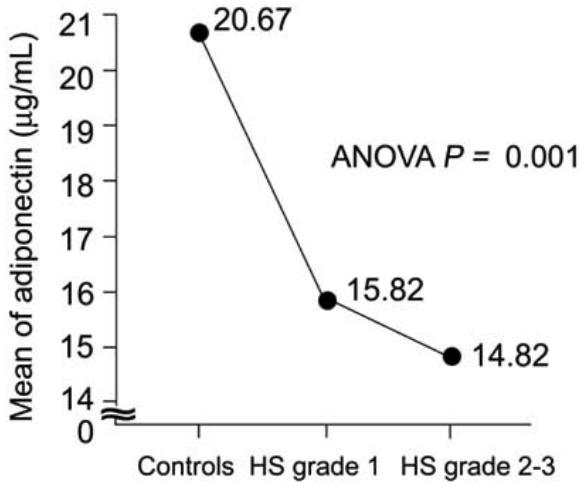

Fig. (3). Serum adiponectin concentrations according to HS severity [73].

There is a strong inverse statistical relation between adiponectin and diabetes. This association is supported by genetic studies where the identification of polymorphisms resulting in hypoadiponectinemia is associated with insulin resistance, and linkage analysis has identified the gene 3q27 encoding for adiponectin as a susceptibility locus for the metabolic syndrome and diabetes [69]. Furthermore, recent studies show that hypoadiponectinemia predicts the risk of the development of type 2 diabetes, even in the absence of any other indicators of insulin resistance [70,71].

PATHOPHYSIOLOGICAL IMPLICATIONS IN NON-ALCOHOLIC FATTY LIVER DISEASE

Xu *et al*. [72], demonstrated that adiponectin decreases the hepatic fat content in mice. They administered adiponectin to mice with fatty liver diseases and this resulted

in alleviation of both steatosis and hepatomegaly. These effects were at least in part attributed to enhanced hepatic fatty acid oxidation and decreased hepatic fatty acid synthesis. The increase in hepatic lipid oxidation by adiponectin might also play a role for the beneficial effect of adiponectin on hepatic glucose metabolism. In addition, our group showed that high adiponectin levels are protective against non-alcoholic fatty liver disease in humans (Fig. **3**) [73], but Bugianesi *et al*. [74] did not find a correlation with the necroinflammatory activity. Maeda *et al*. [75], using non-invasive methods to determine intramyocellular lipid content and hepatic lipid content, showed that serum adiponectin levels were inversely correlated with the hepatic lipid content, but it was not related to intramyocellular lipid content.

Vuppalanchi *et al*. [76], showed that patients with non-alcoholic steatohepatitis have a deregulated postprandial glucose and lipid homeostasis, and hypothesized that serum adiponectin levels in patients with non-alcoholic steato-hepatitis respond suboptimally to meals compared with the response in obese controls. There was a higher expression of AdipoR2 in liver tissue of patients with non-alcoholic steatohepatitis compared to that of simple steatotic and normal liver tissue. However, Kaser *et al*. [77], demonstrated that AdipoR2 staining was lower in biopsies of subjects with non-alcoholic steatohepatitis compared with simple steatosis. This upregulated expression and diminished density of AdipoR2 in non-alcoholic steatohepatitis might be explained by a post-transcriptional deregulation.

Experimental studies show that adiponectin is produced by quiescent hepatic stellate cell and that it induces caspase-mediated apoptosis in activated but not quiescent hepatic stellate cell apoptosis. This data indicates that adiponectin has a potential anti-fibrotic therapy in chronic liver disease, particularly nonalcoholic steatohepatitis [78]. Finally, recent data provide new insights about adiponectin and liver dysfunction, indicating that it could serve as an indicator of cholestasis [79] and pleiotropic effects that involve functional and hemodynamic variables in liver pathophysiology [80].

POTENTIAL ADIPONECTIN-ORIENTED THERAPIES

There are no reports concerning any form of synthetic AdipoR-agonist, however, there are several interesting studies showing that some pharmacological options impact favorably on adiponectinemia status and might, therefore, be of benefit in non-alcoholic fatty liver disease.

Given the fact that adiponectin is a PPARγ-target gene, thiazolidinediones impact on adiponectin levels has been evaluated. Yang *et al.* [81] showed in a small double-blinded, placebo-controlled, parallel-group comparative study of rosiglitazone and concurrent sulfonylurea therapy, a twofold-rise in adiponectin serum levels in type 2 diabetic patients treated with this combination for 6 months. Multivariate linear regression analysis showed that whether rosiglitazone was used was the single variable significantly related to the changes of plasma adiponectin. The amount of variance in changes of plasma adiponectin level explained by the treatment was approximately 24%**.** The results of this study expand the knowledge of the beneficial effects of exogenous PPARγ activation in type 2 diabetic patients and raise the possibility of an adiponectin-oriented insulin resistance and non-alcoholic fatty liver disease therapy.

The endocannabinoid system is a new pathway in obesity pathophysiology. Beyond its potential therapeutic benefit through modulation on food intake behavior, lipolysis, fatty acid synthesis and anti-fibrogenic activity [82], the synthetic CB_1-antagonist, rimonabant, has been shown to increase adiponectin mRNA expression in adipose tissue *in vitro* [83]. This has been confirmed by the recently published RIO-Lipids study [84], a large placebo-controlled, randomized, multicenter study comparing double-blinded therapy with either placebo or rimonabant at a dose of 5 mg or 20 mg daily for 12 months in addition to a hypocaloric diet in overweight or obese patients with dyslipidemia. The results of this study showed that rimonabant at a dose of 20 mg resulted in a 46.2 percent increase in plasma adiponectin levels, partly independent of weight loss alone.

CONCLUSION

Adiponectin has an important role in insulin-sensitivity through activation of fatty acid oxidation and reduction of hepatic glucose output. This effect, along with its anti-fibrotic action, makes it a therapeutic target in non-alcoholic fatty liver disease. Some pharmacological agents have been shown to induce adiponectin expression and should be evaluated in non-alcoholic steatohepatitis patients. Also, adiponectin-based therapies need to be explored in other liver diseases.

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