The role of leptin and adiponectin in chronic liver diseases

E. Tsochatzis, G.V. Papatheodoridis

SUMMARY

Adipose tissue is currently considered to be a metabolically active organ that secretes hormones which regulate energy balance. Leptin and adiponectin are its main metabolic products and have been implicated in a wide spectrum of human diseases including liver diseases. These two hormones have been initially studied in non-alcoholic fatty liver disease, which is considered to be part of the metabolic syndrome. Leptin seems to have fibrogenic potential and serum leptin levels have been found to be higher in patients with non-alcoholic steatohepatitis (NASH) than in controls. On the other hand, serum adiponectin levels have been found to be inversely related to the presence of NASH. As steatosis is a common histopathological finding of chronic hepatitis C, serum leptin and adiponectin levels were measured in such patients and found to be significantly higher and lower compared to healthy controls, respectively. However, it is not yet clear whether they are just markers of liver steatosis and fibrosis or whether they have a direct pathogenic or protective role. Therefore, the associations between leptin-adiponectin and liver steatosis and/or fibrosis should be evaluated further in prospectively designed studies including larger cohorts of NASH and chronic hepatitis C patients with detailed assessment of metabolic and several potential confounding factors. Moreover, their measurement in other liver diseases, which are considered to be infrequently associated with steatosis, such as chronic hepatitis B, would clarify if their action is mediated by or is independent of steatosis.

INTRODUCTION

Adipose tissue had a major role in man’s evolution, as it served as an energy bank where excess calories could be stored as fat and used in periods of famine and starvation. Storage or mobilization of lipids, the predominant function of adipose tissue, depends on several self-produced cytokines or adipokines, which exert autocrine, paracrine and endocrine effects, regulate both lipogenesis and lipolysis and even participate in the inflammatory response. As a sedentary lifestyle with a positive caloric balance has increased in developed countries over the last years, the current epidemic of obesity and metabolic syndrome have emerged and shed light on the actions of adipokines. The hepatic manifestation of the metabolic syndrome is called non-alcoholic fatty liver disease (NAFLD), but fatty infiltration of the liver might affect the course of other liver diseases as well. This review focuses on the two most important adipose cytokines, leptin and adiponectin, aiming to clarify their role in liver diseases.

Leptin and adiponectin

Adipose tissue, once considered to be solely an energy storage organ, has emerged as an endocrine organ over the last decade, since its metabolic products, called adipokines, exert local, peripheral and central effects. Tumor necrosis factor α (TNFα), resistin, interleukin-6, plasminogen activator inhibitor 1, angiotensinogen, adipin, metallothionein and acylation-stimulating protein are included among the adipose tissue products, but leptin and adiponectin are the best-studied and probably the most important adipokines.

Leptin (from the Greek word leptos meaning thin) is a 16-kilodalton protein that circulates in the serum in free and bound form. It was discovered in 1994 as the product of the ob gene by positional cloning, using the leptin-deficient ob/ob mouse model of obesity. Classic experiments suggested that ob/ob mice lack a hormone that limits food intake, while db/db mice express a defec-
tive leptin receptor that makes them resistant to the actions of this hormone.\textsuperscript{4} Leptin levels increase with increasing fat mass,\textsuperscript{7,8} are higher in women than men\textsuperscript{7} and follow a circadian and ultradian variation.\textsuperscript{10} Leptin receptors are found in many areas of the brain as well as in peripheral tissues including the lung, kidneys, liver, pancreas, adrenals, ovaries, hematopoietic stem cells and skeletal muscle. Initially, leptin was considered to be solely an anorexigenic hormone that decreases food intake and increases energy expenditure. Despite these effects, it cannot be used therapeutically as an antiobesity agent, as most obese persons have increased leptin levels, indicating that obesity is associated with a leptin-resistant state in most cases.\textsuperscript{4}

In addition to the above activities, studies in animal models have recently shown that leptin has a key role in preventing lipid accumulation in nonadipose sites, referred to as lipotoxicity.\textsuperscript{11} In the liver, leptin achieves its antilipogenic effect by lowering the expression of sterol regulatory element binding protein 1 (SREBP-1).\textsuperscript{12} Leptin is currently considered to be an indicator of adequate energy supplies to support the general physiological functions,\textsuperscript{13} but also to have actions on the immune system, reproduction, development, haemopoiesis and angiogenesis. In particular, leptin is involved in wound repair and fibrogenesis,\textsuperscript{14-16} and seems to have an immunomodulatory role, since it is necessary for maturation and activation of macrophages and lymphocytes.\textsuperscript{17,18}

Adiponectin, which has been studied less well compared to leptin, circulates in human serum in various forms of dimmers, trimmers or complexes.\textsuperscript{19} Two adiponectin receptors have been cloned, AdipoR1 and AdipoR2, with AdipoR2 having the highest expression in the liver among all body organs.\textsuperscript{20} Peroxisome proliferator-activated receptor (PPAR) \(g\) plays an important role in the transcriptional activation of the adiponectin gene\textsuperscript{21} and PPARg ligands\textsuperscript{22} increase mRNA expression and plasma concentration of adiponectin. Plasma adiponectin concentration correlates inversely with body mass index (BMI), percentage of body fat, fasting insulin concentration and plasma triglycerides.\textsuperscript{23} Adiponectin possesses anti-inflammatory properties and exerts significant metabolic effects, since it modulates the endothelial cell inflammatory response\textsuperscript{24} through inhibition of nuclear factor (NF)-\(\kappa\)B activation and blockage of TNF-\(\alpha\) release, suppression of macrophage function\textsuperscript{25} and suppression of proliferation and migration of vascular smooth muscle cells,\textsuperscript{26} while it also reduces body fat and improves hepatic and peripheral insulin sensitivity.\textsuperscript{27-29}

As it has become evident that obesity is associated with deregulation of both leptin and adiponectin levels and actions, there has been an increasing interest in the role of these two hormones in the metabolic syndrome and consequently in liver diseases, mostly in NAFLD.

**Non-alcoholic fatty liver disease (NAFLD)**

NAFLD was first described by Ludwig et al\textsuperscript{30} and encompasses a wide range of liver injury, from simple steatosis to steatohepatitis (non-alcoholic steatohepatitis or NASH). Its histological features are similar to those found in alcoholic liver disease, but there is no history of alcohol abuse. NASH is strongly associated with obesity\textsuperscript{31} and presence of type 2 diabetes mellitus.\textsuperscript{32,33} Truncal obesity is a risk factor even in patients with normal BMI.\textsuperscript{34} Furthermore, the majority of patients with NASH have biochemical evidence of insulin resistance.\textsuperscript{35} The conditions described above are features of the metabolic syndrome, which is defined as the presence of abdominal obesity, insulin resistance with or without frank hyperglycemia, dyslipidemia and hypertension.\textsuperscript{36} While all patients with NASH do not meet the criteria for the metabolic syndrome, insulin resistance and hyperinsulinemia are almost universal in these patients.\textsuperscript{35,37}

Insulin resistance, which is defined as impaired ability to clear a glucose load at any given plasma insulin concentration\textsuperscript{38} and measured by the euglycemic hyperinsulinemic clamp or more frequently by easier to perform indices such as the Homeostasis Model Assessment (HOMAIR),\textsuperscript{39} has a key role in the development of NAFLD.\textsuperscript{37} The primary effect of insulin is to increase glucose uptake by cells through upregulation of the glucose transporters expression on the cells surface. Insulin also induces lipogenesis and inhibits lipolysis in the adipose tissue, while it increases the synthesis of fatty acids in the liver. Insulin resistance and the metabolic syndrome lead to defective insulin-mediated inhibition of lipolysis, mostly in visceral fat,\textsuperscript{40} while hyperinsulinemia results in increased hepatic synthesis of free fatty acids and decreased synthesis of apolipoprotein B-100, thus leading to triglyceride accumulation in the liver. Thus, insulin resistance results in both increased adipose tissue lipolysis and increased hepatic lipogenesis\textsuperscript{41} leading to lipid accumulation in the hepatocytes, mainly in the form of triglycerides, which is a prerequisite for the development of NAFLD. The molecular pathogenesis of insulin resistance is multifactorial,\textsuperscript{41} but it has been shown that leptin\textsuperscript{42} and TNF\(\alpha\)\textsuperscript{43} among several molecules are involved in the inhibition of insulin activities.

According to the “two hits” hypothesis, the develop-
ment of NASH requires the presence of additional pathophysiological abnormalities. The second hit is currently believed to be increased oxidative stress within the hepatocytes, which is characterized by excessive production of reactive oxygen species (ROS). Free fatty acids that accumulate in the liver are metabolized through oxidation in mitochondria, peroxisomes and microsomes, while they can also re-esterify to triglycerides. PPARα has a regulatory role, as it controls the induction of genes involved in the fatty acids oxidation systems and its mediated signaling has been found to block liver injury in experimental NAFLD. Excess lipid accumulation can lead to metabolic overload and oxidative stress. Mitochondria and the cytochrome P-450 system are considered to be the major ROS generation sites in the liver. ROS promote progression from steatosis to steatohepatitis and fibrosis by three main mechanisms: lipid peroxidation, cytokine induction and Fas ligand induction.

In this complex setting, a significant role of leptin and adiponectin in the progression of liver fibrosis is beginning to emerge. Hepatic stellate cells (HSCs) have a central role in the development of liver fibrosis. When HSCs become activated by transformation to myofibroblast-like cells, they start to proliferate, migrate and produce transforming growth factor b1 (TGFb1) and various extracellular matrix proteins. Platelet-derived growth factor (PDGF)-BB and TGFb1 are considered to be the main cytokines that activate HSCs to produce fibrosis. Activated HSCs have been found to express leptin, which became of interest when leptin was shown to be a profibrogenic cytokine. In particular, Ikejima et al showed that leptin injections in rats receiving hepatotoxins resulted in greater expression of procollagen type I, increased expression of TGFb1 and of a smooth muscle actin, a marker of activated HSCs, and eventually increased production of fibrosis. In addition, the dramatic increase in serum TNFa levels after leptin injections in rats treated with carbon tetrachloride (CCl4) suggests that leptin may amplify inflammation and independently affect the development of fibrosis. Sinusoidal endothelial cells and Kupffer cells have been identified as the main targets of the profibrogenic action of leptin. On the other hand, rats that are resistant to the biologic actions of leptin have been found to exhibit a significantly reduced fibrogenic response to thioacetamide intoxication, while reduced fibrogenesis has been reported in leptin-deficient mice. According to these data, a direct effect of leptin on HSCs has been suggested and leptin is currently considered to be an essential mediator of hepatic fibrosis and to have effects that cannot be solely attributed to TNFa induction.

While leptin has a profibrogenic action, a protective action of adiponectin in liver injury is now emerging from several studies. Initial data suggested that adiponectin accumulates within the extracellular matrix of hepatocytes in CCl4 treated mice and that it may act as an anti-inflammatory hormone participating in the repair process of liver injury. Kamada et al also showed that treatment with adiponectin attenuated liver fibrosis in mouse models treated with CCl4 and that HSCs express both adiponectin receptors (AdipoR1 and AdipoR2). Thus, they speculated that serum circulating adiponectin binds to and may have a significant effect on HSCs, since adiponectin inhibited proliferation and migration of cultured HSCs and decreased TGFb1 gene expression. Masaki et al using a mouse model of endotoxin induced acute liver injury in KK-Ay obese mice found that adiponectin prevents hepatic injury by inhibiting the synthesis and/or release of TNFa. Finally, Xu et al also observed a protective effect of adiponectin in fatty liver disease in mice, as it alleviated steatosis, hepatomegaly and serum ALT abnormalities, and they concluded that this effect could not be solely explained by an antagonistic effect of adiponectin against TNFa.

Given all the above data, leptin and adiponectin levels were measured by several investigators initially in patients with NASH. In the most comprehensive study, leptin levels were found to be significantly higher in 47 patients with NASH than in 47 controls and to be correlated directly with the severity of hepatic steatosis but not with the degree of inflammation or fibrosis. Since leptin, c-peptide and age were the three factors independently associated with hepatic steatosis, it was suggested that such data support a pathogenic role of leptin in hepatic insulin resistance and/or failure of the antisteatotic action of leptin. In contrast to the previous findings, Chalasani et al failed to show a significant difference in serum leptin levels between 26 patients with NASH and 20 controls or an association between serum leptin levels and hepatic steatosis. However, in the latter study a type II error cannot be excluded, since serum leptin levels were relatively higher in NASH patients and there was a trend for a significant association with serum leptin levels and hepatic steatosis (P=0.06). Conflicting results have also been reported in other studies, which, however, did not evaluate the potential confounding effects of several important parameters, such as BMI, or used different criteria for the NASH diagnosis, like ultrasonography instead of liver histology.

Serum adiponectin levels have been found to be inversely related to the presence of NASH. In a relative-
ly large study\(^6\) including 80 patients with NASH, 29 with fatty liver and 82 controls, lower serum adiponectin levels were found to be associated with presence of NASH regardless of insulin resistance, higher grade of hepatic steatosis and of necroinflammatory activity. HOMA\(_{IR}\) was also independently associated with presence of fibrosis, while TNFa and soluble TNF receptor 2 levels were found to be increased in patients with NASH but not to correlate with the severity of fibrosis. Thus, the authors implied that determination of adiponectin and HOMA\(_{IR}\) might have diagnostic utility in differentiating between patients with fatty liver and patients with NASH. In a smaller study, hepatic expression of adiponectin mRNA and adipoR2 receptor were found to be significantly reduced in 13 patients with NASH compared with 9 patients with fatty liver.\(^6\) Since adiponectin was mainly expressed in the endothelial cells of portal vessels and liver sinusoids and adipoR2 was exclusively expressed in the hepatocytes, it was suggested that this hormone/receptor interaction, which may normally function in a paracrine way, could be impaired in NASH. Low serum adiponectin levels in NASH have also been reported in other small studies.\(^{64\text{-}66}\) In humans, a histological benefit from increased adiponectin hepatic expression has been suggested by the favorable effects of PPARg agonists, thiazolidinediones, in overweight patients with NASH.\(^{67,68}\) It should be noted that PPARg has been shown to play a significant role in the transcriptional activation of the adiponectin gene.\(^21\) These observations in combination with the protective role of adiponectin in animal models of liver injury support the need for further evaluation of adiponectin as a therapy for NASH.\(^{69}\)

**Chronic hepatitis C**

Steatosis is a common histopathological finding of chronic hepatitis C,\(^70\) reported to be present in 30% to 70% of patients.\(^71,72\) Presence or worsening of hepatic steatosis have been found to be associated with more advanced stages or more rapid progression of fibrosis.\(^73\text{-}76\) Moreover, steatosis has been observed to reduce the probability of response to combined antiviral treatment with interferon-alfa and ribavirin\(^77\text{-}80\) and therefore it was suggested to be a potential additional mechanism of resistance to therapy, independent of genotype, viral load, fibrosis, BMI and serum glucose.\(^78\) Finally, steatosis was recently reported to influence the development of hepatocellular carcinoma in chronic hepatitis C patients.\(^81\)

Several data suggest that there are host and virus related profiles of steatosis.\(^82\) In patients infected with genotype 3, there is a significant association between the presence or severity of steatosis and intrahepatic or serum viral load,\(^77,79,83\) but not between steatosis parameters and obesity or BMI.\(^84\) Moreover, steatosis improves in most patients with sustained virological response after therapy. All these data indicate that steatosis in chronic hepatitis C patients with genotype 3 develops due to a virus induced cytopathic effect.\(^85\)

In patients infected with genotype 1, presence of steatosis seems to be associated with presence of factors for NASH.\(^86,87\) In particular, presence of steatosis develops independently of serum HCV RNA levels and is associated with increased BMI and presence of visceral obesity.\(^88,89\) Moreover, weight reduction of obese patients has been shown to reduce liver steatosis and fibrosis despite virus persistence,\(^90\) while steatosis remains unchanged after an effective therapeutic course. All these indicate that steatosis in chronic hepatitis C patients with genotype 1 represents a host related reaction independent of the hepatitis C virus (HCV).

The association between steatosis and chronic hepatitis C, however, may be influenced by the presence of two risk factors for NAFLD, insulin resistance and/or diabetes mellitus, which are often observed in patients with chronic hepatitis C. In particular, insulin resistance is frequently present in early stages of chronic HCV infection and is an independent factor of the severity and progression rate of fibrosis.\(^93\) Furthermore, type 2 diabetes mellitus appears to be present significantly more frequently in chronic hepatitis C patients compared to the general population and to be associated with the severity and progression rate of fibrosis as well.\(^91\text{-}95\)

The mechanism for the high prevalence of diabetes mellitus in chronic hepatitis C is not clear, but several explanations have been proposed\(^96\) including either HCV induced hepatic steatosis, hepatic insulin resistance and eventually diabetes or alternatively an HCV core protein induced or immune-mediated extrahepatic diabetogenic effect.\(^94,95\) HCV core protein has been found to induce hepatic steatosis in either cell cultures\(^98\) or in transgenic mice\(^99\) with a more prominent effect observed in cells transfected with genotype 3 isolates-derived constructs.\(^100\) In addition, HCV inhibits the microsomal triglyceride transfer protein activity and therefore interferes with the hepatic assembly and secretion of apolipoprotein B-containing VLDL.\(^101\) An effect in lipid metabolism is further supported by co-localization.
of HCV core protein\textsuperscript{102} and HCV nonstructural protein 5A\textsuperscript{103} with cytoplasmic lipid structures in HCV transfected cells. There is evidence that interaction between HCV proteins and apolipoproteins may favor the secretion of viral proteins from the cell.\textsuperscript{104} Moreover, it has been shown that HCV RNA circulates in the serum in particles containing triglycerides, apoB and core protein.\textsuperscript{105} These particles enter hepatic cell lines in a competitive way with VLDL and LDL, which further supports the theory that HCV infects hepatocytes via the LDL receptor.\textsuperscript{106} This means that HCV virus achieves a reduced competition for the LDL receptor by inhibiting VLDL secretion by hepatocytes and thus lowering serum VLDL and LDL. Thus, steatosis could be an epiphenomenon of an HCV prosurvival effect.\textsuperscript{96} An intriguing finding that connects HCV virus and insulin resistance has also arisen. In a mouse model, insulin resistance was shown to be induced only by the expression of the HCV core protein, while signaling abnormalities in the insulin receptor IRS-1 pathway were found to be present before the development of steatosis.\textsuperscript{107} A defect in IRS-1 tyrosine phosphorylation was also found in liver biopsies from chronic hepatitis C patients but not from non-infected controls.\textsuperscript{108}

The associations among HCV infection, hepatic steatosis, insulin resistance and liver fibrogenesis have driven research into the evaluation of the role of leptin and adiponectin. Piche et al\textsuperscript{109} measured serum leptin levels in 77 chronic hepatitis C patients, of which 55 had biopsy proven steatosis, and in 20 healthy controls. The majority of their patients had minimal to moderate fibrosis and infection with a genotype 1. Serum leptin were found to be significantly and independently associated with the severity of liver fibrosis while a possible relation of leptin and adiponectin concentrations in chronic hepatitis C patients.\textsuperscript{110} Romero-Gomez et al\textsuperscript{110} studied 131 chronic hepatitis C patients, of which 63 had steatosis. In univariate analysis, leptin levels were significantly different in patients with than without steatosis. In the multivariate analysis, presence of steatosis was only associated with adiponectin levels and it was concluded that hypoadiponectinemia is at least partly responsible for steatosis in chronic hepatitis C. However, it should be noted that there were limitations in the latter study, such as the small number of patients with severe fibrosis and the lack of measurements of insulin resistance and visceral obesity, and therefore its conclusions should be seen with caution.

Conclusions - Future directions

Adipose cytokines are gaining increasing attention in the setting of chronic liver disease. Initially studied in NASH, they seem to have a role in chronic hepatitis C as well, while animal experiments justify their study in other forms of liver disease. However, it is not yet clear whether they are just markers of liver steatosis and fibrosis or whether they have a direct pathogenic or protective role. Therefore, the associations between leptin-adiponectin and liver steatosis and/or fibrosis progression should be evaluated further in prospectively designed studies including larger cohorts of NASH and chronic hepatitis C patients with detailed assessment of metabolic and several potential confounding factors. Moreover, their measurement in other liver diseases considered to be infrequently associated with steatosis, such as chronic hepatitis B, would clarify if their action is mediated by or is independent of steatosis. Finally, leptin and adiponectin levels should be measured in therapeutic trials in patients with NASH or HCV-related steatosis treated with agents like thiazolidinediones which often modify their levels.
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