The physiological and pathophysiological role of adiponectin and adiponectin receptors in the peripheral tissues and CNS

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Abstract Adiponectin is an abundantly expressed adipokine in adipose tissue and has direct insulin sensitizing activity. A decrease in the circulating levels of adiponectin by interactions between genetic factors and environmental factors causing obesity has been shown to contribute to the development of insulin resistance, type 2 diabetes, metabolic syndrome and atherosclerosis. In addition to its insulin sensitizing actions, adiponectin has central actions in the regulation of energy homeostasis. Adiponectin enhances AMP-activated protein kinase activity in the arcuate hypothalamus via its receptor AdipoR1 to stimulate food intake and decreases energy expenditure. We propose a hypothesis on the physiological role of adiponectin: a starvation gene in the course of evolution by promoting fat storage on facing the loss of adiposity. © 2007 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

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1. Introduction

Obesity-linked insulin resistance is a key feature of type 2 diabetes, metabolic syndrome and cardiovascular diseases [32,29,11]. White adipose tissue (WAT) has been recognized as an important endocrine organ that secretes a number of biologically active ‘adipokines’ [10,42,34,24]. Dysregulation of these adipokines has been shown to affect insulin sensitivity through modulation of insulin signaling pathway such as phosphorylation of the insulin receptor substrate (IRS) proteins (e.g. IRS1 and IRS2) [10] and the molecules involved in glucose and lipid metabolism in the peripheral tissues such as liver and skeletal muscle [33]. Of these adipokines, adiponectin has recently attracted much attention because of its antidiabetic and antiatherogenic effects [39,3,4,8,12,30,13]. Indeed, a decrease in the circulating levels of adiponectin by interactions between genetic factors and environmental factors causing obesity has been shown to contribute to the development of insulin resistance, type 2 diabetes, the metabolic syndrome and atherosclerosis (Fig. 1) [12,13].

In addition to these peripheral actions, adipokines have also been reported to have central actions in the regulation of energy homeostasis. Leptin binds is known to bind to the leptin receptor (LRb) in the hypothalamus and activates JAK2 (Janus kinase 2)-STAT3 (signal transducer and activator of transcription 3) and phosphatidylinositol-3 kinase (PI3K) pathway to increase metabolic rate and sympathetic tone and suppress feeding, thereby decreasing body weight [7]. Moreover, leptin has been reported to inhibit AMP-activated protein kinase (AMPK) activity in the hypothalamus and suppress food intake [28]. Recently, like leptin, adiponectin has been demonstrated to play an important role in the central nervous system (CNS). Adiponectin was shown to be present in the CSF of rodents [31,22] and human [23,19,6] and to enter the CSF from the circulation [31,22]. Moreover, the adiponectin receptors adipoR1 and adipoR2 [40] were found to be expressed in the hypothalamus and brain endothelial cells [35,22].

In this Review, we outline the recent progress in research on the physiological and pathophysiological role of adiponectin and adiponectin receptors in the peripheral tissues and CNS. Since the length of this Review is limited, we recommend that readers also consult other recent reviews on adiponectin research [4,12,13,30].

2. Cloning, function, and regulation of adiponectin receptors

In order to understand the molecular mechanism of adiponectin action, we isolated cDNA for human adiponectin receptors [40]. The cDNA encoded a protein designated adiponectin receptor 1 (AdipoR1) [40]. This protein is structurally conserved from yeast to humans (especially in the 7 transmembrane domains). Interestingly, the yeast homologue (YOL002c) plays a key role in metabolic pathways that regulate lipid metabolism, such as fatty acid oxidation [14]. Moreover, we found a gene that was significantly homologous (67% amino acid identity) with AdipoR1, which was termed AdipoR2 [40]. AdipoR1 is ubiquitously expressed, including in skeletal muscle and liver, whereas AdipoR2 is most abundantly expressed in the liver. AdipoR1 and AdipoR2 appear to be integral membrane proteins; the N-terminus is internal and the C-terminus is external-opposite to the topology of

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all other reported G protein-coupled receptors (GPCRs) [40]. Expression of AdipoR1 and AdipoR2 or suppression of AdipoR1 and AdipoR2 expression supports our conclusion that AdipoR1 and AdipoR2 serve as receptors for globular and full-length adiponectin and mediate increased AMPK, PPARα and p38 MAP kinase activities as well as fatty-acid oxidation and glucose uptake by adiponectin.

Recently, a two-hybrid study revealed that the C-terminal extracellular domain of AdipoR1 interacted with adiponectin, whereas the N-terminal cytoplasmic domain of AdipoR1 interacted with APPL (adapter protein containing pleckstrin homology domain, phosphotyrosine-binding domain, and leucine zipper motif) [26]. Moreover, interaction of APPL with AdipoR1 in mammalian cells was stimulated by adiponectin binding, and this interaction appeared to play an important role in adiponectin-mediated AMPK activation and downstream effects [26]. The expression levels of both AdipoR1 and AdipoR2 were significantly decreased in insulin-resistant ob/ob mice, probably in part because of obesity-linked hyperinsulinaemia [36]. Moreover, adiponectin-induced activation of AMPK was significantly decreased, for example, in the skeletal muscle of ob/ob mice, suggesting that adiponectin resistance is present in ob/ob mice [36]. Thus, obesity decreases not only plasma adiponectin levels but also AdipoR1/R2 expression, thereby causing adiponectin resistance and leading to insulin resistance, which in turn aggravates hyperinsulinaemia, forming a ‘vicious cycle’ [36].

3. Expression of adiponectin receptors in the liver ameliorated diabetes

Consistent with the data in ob/ob mice, expression levels of AdipoR1 or AdipoR2 were decreased to approximately 65% or 55%, respectively, in the liver of db/db mice as compared with wild-type mice. To determine the role of decreased expression levels of AdipoRs in the development of the insulin resistance and diabetes observed in obese mice, we studied the effects of adenovirus-mediated restoration of AdipoR1 expression in db/db mice. In fact, adenovirus-mediated overexpression of AdipoR1 or AdipoR2 in the liver of db/db mice significantly improved insulin resistance and diabetes in db/db mice [41].

3.1. AdipoR1 increases AMPK activation by adiponectin in liver

Expression of AdipoR1 resulted in significantly increased activation of AMPK in the liver by adiponectin, whereas expression of AdipoR2 did not. Activation of AMPK in the liver has been reported to reduce the expression of genes encoding hepatic gluconeogenic enzymes such as glucose-6-phosphatase (G6pc) and phosphoenolpyruvate carboxykinase 1 (Pck1) [23] as well as genes encoding molecules involved in lipogenesis such as sterol regulatory element binding protein 1c (Srebf1) [37]. In fact, expression of AdipoR1 significantly decreased the expressions of G6pc, Pck1 and Srebf1 in the liver of db/db mice, which may be among mechanisms by which restoration of AdipoR1 in the liver reduced endogenous glucose production (EGP), apparently increased glucose infusion rate (GIR) and improved diabetes [41]. In contrast, expression of AdipoR2 had little effects on the expression levels of G6pc, Pck1 or Srebf1. These results suggested that AdipoR1 may be more involved in the activation of AMPK by adiponectin than AdipoR2 in liver in vivo [41].

3.2. AdipoR2 increases PPARα target genes in liver

Expression of AdipoR2 significantly increased the expression of genes encoding molecules involved in glucose uptake such as glucokinase (Gck) [27], unlike the molecules involved in gluconeogenesis, which appeared to be one possible mechanism by which AdipoR2 expression in the liver apparently increased GIR and improved diabetes. On the other hand, expression of AdipoR1 had little effect on the expression levels of Gck. Expression of AdipoR2 in liver of db/db mice increased PPARα (Ppara) itself [41] and its target genes [16] such as Acox1 (acyl-CoA oxidase) and Ucp2 (uncoupling protein 2), whereas expression of AdipoR1 in liver of db/db mice had little effects on PPARα itself and its target genes such as Acox1 and Ucp2. These observations suggested that AdipoR2 may be more involved in activation of the PPARα pathways than AdipoR1. Adenovirus-mediated expression of AdipoR1 or AdipoR2 in the liver of db/db mice significantly increased fatty-acid oxidation, and tended to decrease hepatic triglyceride content, which may be one mechanism by which expression of AdipoR1 or AdipoR2 in the liver improved insulin resistance and diabetes [41] (Fig. 2). The present data suggest that down-regulation of AdipoR1 and AdipoR2 in obesity plays causal roles, at least
in part, in the development of insulin resistance and diabetes.

4. AdipoR1 and AdipoR2 serve as the major adiponectin receptor in vivo

We generated AdipoR1 knockout mice, AdipoR2 knockout mice and AdipoR1/R2 double knockout mice [41]. AdipoR1 knockout mice showed significantly impaired glucose tolerance and insulin resistance. EGP was significantly increased and GIR was significantly decreased in AdipoR1 knockout mice as compared with the wild-type mice. These observations indicate increased hepatic glucose production and insulin resistance in liver of AdipoR1 knockout mice. Although glucose intolerance was not observed in AdipoR2 knockout mice, plasma insulin levels were found to be significantly higher in the AdipoR2 knockout mice than in the wild-type mice, suggesting the presence of insulin resistance in the AdipoR2 knockout mice. In contrast to AdipoR1 knockout mice, EGP was not significantly higher in AdipoR2 knockout mice. However, GIR was significantly decreased, and Rd tended to be decreased in AdipoR2 knockout mice. AdipoR1/R2 double knockout mice exhibited significantly impaired glucose tolerance and insulin resistance. There is a significant elevation of the insulin resistance index in the AdipoR1/R2 double knockout mice compared to the AdipoR1 knockout mice, and this can be attributed to the contribution of the AdipoR2 deficiency. These findings provided the first direct evidence that AdipoR1 and AdipoR2 do indeed play important physiological roles in the regulation of insulin sensitivity in vivo. Liver is a major target of adiponectin action [3]. We detected no appreciable adiponectin specific binding activity in the hepatocytes from AdipoR1/R2 double knockout mice, indicating undetectable levels of functional adiponectin receptors in hepatocytes from AdipoR1/R2 double knockout mice. Consistent with this, glucose lowering effect of adiponectin was completely abrogated in AdipoR1/R2 double knockout mice. Thus, AdipoR1 and AdipoR2 are the major adiponectin receptors in vivo, which mediate the major, if not the entire, part of adiponectin binding and adiponectin actions.

5. Adiponectin receptors are present in the hypothalamus, and adiponectin enters the CSF from the circulation

Since the end of the 19th century, several efforts have been made to determine the CNS role in the regulation of energy metabolism. In 1953, Gordon Kennedy proposed a “lipostat theory” that body fat content is maintained by factors secreted from adipose tissue, as a result of feedback signals arising from the fat depots that are sensed by the brain [15]. The hormone leptin circulates in proportion to body fat [2]. Leptin informs the CNS that adipose stores are expanding and prevents from accumulating excessive fat storage through coordinated regulation of feeding, metabolism, the autonomic nervous system and body energy balance. In contrast, no adipokine has been identified that promotes fat accumulation when energy balance is disrupted.

AdipoR1 and AdipoR2 [40], were found to be abundantly expressed in the hypothalamus, and their expression levels were comparable to those in the liver. In situ hybridization analysis revealed the expressions of AdipoR1 and AdipoR2 as well as the leptin receptor in the ARH. Immunohistochemical analysis revealed colocalization of AdipoR1 and the leptin receptor in the ARH of C57BL/6 mice. Adiponectin was detected in the CSF of C57BL/6 mice at approximately 1/4000th of its concentration in the serum; it was also detected in the CSF of adiponectin knockout mice [20,21] after intravenous (i.v.) injection of full-length adiponectin administered to raise the serum adiponectin levels in these mice to approximately 1/4000th of its concentration in the serum; it was also detected in the CSF of adiponectin knockout mice [20,21] after intravenous (i.v.) injection of full-length adiponectin administered to raise the serum adiponectin levels in these mice to approximately the same levels as in wild-type mice. These findings indicate that adiponectin does indeed enter the CSF from the circulation [22]. Adiponectin is known to exist in three forms, namely, trimers, hexamers, and HMW multimers, in the serum of wild-type mice. Interestingly, unlike in the serum, only trimers and hexamers, and not HMW multimers, were found in the CSF of the wild-type mice. In adiponectin knockout mice, after i.v. injection of full-length adiponectin, all three forms, i.e., trimers, hexamers, and HMW multimers, were found in the serum, while only trimers and hexamers, and not HMW multimers, were found in the CSF. These data indicate that the distribution of the multimeric forms of adiponectin in the CSF differs from that in the serum [22]. In wild-type mice,
while plasma levels of glucose and insulin and serum levels of leptin increased significantly after refeeding, serum and CSF adiponectin levels decreased significantly. In addition, the expression of AdipoR1 in the ARH decreased significantly after refeeding, whereas that of AdipoR2 remained unchanged.

6. Adiponectin increases AMPK activity in the ARH via AdipoR1 to stimulate food intake

In view of the increases in adiponectin concentrations in the serum and CSF and the increases in the AdipoR1 expression level in the ARH under fasting conditions, adiponectin signals may be involved in the stimulation of food intake. It has been suggested that adiponectin is an orexigenic hormone and that it may stimulate the phosphorylation of AMPK and acetyl-CoA carboxylase (ACC), downstream of AMPK, in the hypothalamus [9,5,38]. Phosphorylation of AMPK and ACC was suppressed after refeeding [1,28,38]. Administration of adiponectin increased the phosphorylation of AMPK and ACC. Next, we investigated whether the enhanced AMPK activation by adiponectin was mediated by the adiponectin receptors expressed in the ARH by injecting adeno-AdipoR1 siRNA or adeno-AdipoR2 siRNA into the ARH, using adeno-LacZ as a control. The phosphorylation of AMPK and ACC in the ARH was significantly suppressed in the adeno-AdipoR1 siRNA-treated mice as compared with the adeno-LacZ-treated mice under fasting conditions. After refeeding, phosphorylation of AMPK and ACC was also suppressed in the control group treated with adeno-LacZ, and this was reversed by the administration of adiponectin. However, in the animals in which AdipoR1 expression in the ARH was decreased by treatment with AdipoR1 siRNA, adiponectin failed to reverse the suppression of AMPK and ACC phosphorylation observed after refeeding. On the other hand, when AdipoR2 expression in the ARH was reduced by the administration of AdipoR2 siRNA, the suppression of AMPK and ACC phosphorylation after refeeding was still reversed by adiponectin. These findings suggest that adiponectin directly activates AMPK in the ARH via AdipoR1, but not AdipoR2. Since increased AMPK activity in the ARH has been shown to stimulate food intake [9,28,5], we then investigated the effect of adiponectin on food intake. Food intake after refeeding was significantly lower than after fasting, as the AMPK and ACC phosphorylation levels decreased. Adiponectin injection significantly increased food intake after refeeding, as the AMPK and ACC phosphorylation levels increased. In the mice in which AdipoR1 expression in the ARH was decreased by treatment with AdipoR1 siRNA, the stimulation of food intake by adiponectin injection was blunted. On the other hand, in the mice in which AdipoR2 expression in the ARH was decreased by treatment with AdipoR2 siRNA, no such blunting of the effect of adiponectin injection was observed. These findings suggest that adiponectin stimulates food intake via AdipoR1 in the ARH. Next, in order to investigate whether the stimulation of food intake induced by adiponectin is actually mediated by AMPK, dominant-negative AMPK (D/N-AMPK) was expressed in the ARH, and the amount of food intake was measured. In the control group treated with LacZ, the amount of food consumed after adiponectin injection was significantly higher than after saline injection. In contrast, in the group treated with D/N-AMPK, the stimulation of food intake by the adiponectin injection was blunted, suggesting that adiponectin has a central action of stimulating food intake by activating AMPK in the ARH. In addition to regulating food intake, AMPK in the hypothalamus is also thought to regulate energy expenditure [9,5,17]. Examination of the effect of adiponectin on energy expenditure revealed that oxygen consumption was significantly decreased by adiponectin. Consistent with these findings, expression of uncoupling protein 1 (UCP1) in brown adipose tissue (BAT) was significantly decreased after i.v. injection of adiponectin. We then administered the hexameric form of adiponectin, the predominant form in the CSF, directly into the lateral cerebral ventricles and examined the direct effects of adiponectin in order to rule out the possibility that the actions of adiponectin on the peripheral organs participate in the AMPK activation in the ARH and stimulation of food intake. The suppression of AMPK and ACC phosphorylation after refeeding was indeed reversed by intracerebroventricular (i.c.v.) administration of the hexameric form of adiponectin. Intracerebroventricular injection of the hexameric form of adiponectin also significantly stimulated food intake after refeeding, along with increasing the AMPK and ACC phosphorylation levels. Moreover, oxygen consumption was significantly decreased following i.c.v. injection of the hexameric form of adiponectin. Intravenous injection of adiponectin decreased energy expenditure and UCP1 expression in BAT. Taken together, these findings indicate that adiponectin directly regulates AMPK activity in the ARH and food intake.

Scherer’s group has generated adiponectin-transgenic ob/ob mice that show serum adiponectin levels 2–3-fold higher than ob/ob mice [18]. These mice also show markedly increased body weight due to decreased energy expenditure, as manifested by lower body temperature and lower oxygen consumption, consistent with our observations that adiponectin decreases energy expenditure.

7. Adiponectin knockout mice exhibit decreased AMPK activity in the ARH, increased oxygen consumption, and greater loss of fat during fasting

In order to further elucidate the physiological role of adiponectin in the CNS, we investigated the effects of adiponectin deficiency on AMPK activity, food intake, and energy homeostasis in adiponectin knockout mice. AMPK phosphorylation in the ARH was significantly suppressed in adiponectin knockout mice after fasting. Expression of neuropeptide Y (NPY) in the ARH after fasting was also significantly lower in adiponectin knockout mice, while the expression of pro-opiomelanocortin (POMC) in the ARH was increased after fasting in these mice. Consistent with the decreased AMPK activity and decreased NPY expression in the ARH, adiponectin knockout mice consumed significantly more oxygen than their wild-type littermates under fasting conditions. Expression of UCP1 in BAT was significantly increased in adiponectin knockout mice as compared with wild-type mice. Moreover, despite the adiponectin deficiency, AMPK phosphorylation in skeletal muscle was significantly increased in adiponectin knockout mice compared to their wild-type littermates. These increases in UCP1 expression and AMPK phosphorylation, which may account for the increased energy expenditure in adiponectin knockout mice, cannot be explained by the peripheral actions of adiponectin.
nnectin but may presumably be explainable by its central actions. Despite the similar body weight of wild-type and adiponectin knockout mice, body fat mass, as measured by dual energy X-ray absorptiometry (DEXA), was significantly lower in adiponectin knockout mice than in wild-type mice. The reduction in visceral and subcutaneous fat mass after fasting was greater in adiponectin knockout mice than in wild-type mice.

8. Adiponectin knockout mice exhibit reduced food intake and increased oxygen consumption and appear to be protected from high-fat diet-induced obesity

Adiponectin knockout mice were found to be more resistant to high-fat diet (HFD)-induced obesity than wild-type mice. The visceral WAT mass and subcutaneous WAT mass were both significantly smaller in adiponectin knockout mice fed a HFD. Histological analysis of WAT and quantitation of adipocyte size in the mice revealed significantly smaller adipocytes in adiponectin knockout mice than in wild-type mice. Additionally, after 2 weeks of HFD, when the two groups were indistinguishable by body weight, we examined AMPK and ACC phosphorylation status, AdipoR1 and AdipoR2 expression, food intake, and oxygen consumption in the two groups. AMPK and ACC phosphorylation in the ARH was significantly suppressed in adiponectin knockout mice after fasting, although the expression levels of AdipoR1 and AdipoR2 in the ARH were not significantly different between the two genotypes. Daily food intake was significantly lower in adiponectin knockout mice than in wild-type mice, and oxygen consumption was significantly greater.

9. Physiological and pathophysiological roles of adiponectin

These findings have brought new insights on adiponectin as an appetite stimulator, longer-term fat modulator, and a starvation signal. We propose a hypothesis that adiponectin regulates food intake in coordination with leptin. Under fasting conditions, the adiponectin signal in the ARH increases; consequently, hypothalamic AMPK is activated, which stimulates food intake. After food consumption, on the other hand, the leptin signal in the ARH increases; consequently, hypothalamic AMPK activity decreases, resulting in reduced food intake. Thus, the leptin signal is regulated inversely in relation to adiponectin signal in the hypothalamus. Adiponectin enhances hypothalamic AMPK activity and food intake, as opposed to the action of leptin.

In addition to the regulation of food intake, adiponectin and leptin may also participate in the maintenance of energy homeostasis. Several gut-derived hormones, such as ghrelin and insulin, have been discovered as the modulator of energy balance, which primarily act on appetite. Adipokines appear to be also involved in this; they mainly regulate body fat mass as the long-term modulator of energy balance. On facing the loss of adiposity, the adiponectin signal increases and leptin signal decreases in the ARH; consequently, hypothalamic AMPK is activated, which suppresses energy expenditure, promoting fat storage. On facing the excessive adiposity, on the other hand, the adiponectin signal decreases and the leptin signal increases in the ARH; consequently, hypothalamic AMPK activity decreases that stimulates energy expenditure, inhibiting fat accumulation. Thus the fundamental roles of leptin and adiponectin seem to be to preserve an adequate fat reserve: leptin acts as a satiety signal, and adiponectin acts as a starvation signal. These observations support the lipostat theory, and adiponectin is the first-identified adipokine that contributes to fat accumulation in response to depletion of adiposity (Fig. 3).

![Fig. 3](image.png)

Fig. 3. Body fat content is maintained by factors secreted from adipose tissue, as a result of feedback signals arising from the fat depots that are sensed by the brain.

![Fig. 4](image.png)

Fig. 4. Under a high-fat diet, adiponectin-induced AMPK in the brain remains elevated, while that in the liver and muscle is markedly attenuated, thus further worsening obesity, metabolic syndrome and type 2 diabetes.
Fig. 5. Adiponectin serves as a starvation gene. Adiponectin inhibits energy expenditure, promotes food intake centrally, and stimulates FFA utilization in peripheral tissues.

Once this energy regulation is disrupted, obesity begins to develop. Under an excessive fat reserve, serum adiponectin levels are decreased. The HMW form of adiponectin is known to be most active [13] and does not enter the CSF [23,22]. Under an obese condition, serum adiponectin levels, especially of active HMW multimers, are reported to decrease in an obese individual and murine models, which decreases muscle and hepatic AMPK activity and fatty acid combustion, exacerbating insulin resistance. In the CNS, on the other hand, although a HMW form of adiponectin in serum decreases under an obese condition, trimers and hexamers are present, maintaining the serum adiponectin levels relatively stable in the CSF. Thus, hypothalamic AMPK activity is not suppressed, not decreasing food intake and energy expenditure. This results in worsening obesity, metabolic syndrome and type 2 diabetes (Fig. 4).

Lastly, let us consider the role of adiponectin in the history. During the course of evolution, starvation signals were essential for the survival of an organism. Adiponectin levels were likely to be high under the scarce fat reserve of starvation. Trimeric and hexameric forms of adiponectin would increase appetite by affecting the brain to decrease energy expenditure and to promote fat accumulation. The HMW form of adiponectin was likely to derive energy for survival from combusting (adipocyte-secreted) FFA in the liver and skeletal muscle. The increase in peripheral fatty acid combustion may have contributed to the preferential supply of glucose to the brain. Adiponectin may have played an important role as an starvation gene (Fig. 5). This may explain why adiponectin receptors existed earlier than leptin receptors and have been conserved from yeast to humans [14].

References


