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The multifaceted and controversial immunometabolic actions of adiponectin

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Adiponectin, one of the most abundant adipose-derived hormones, has variable actions in many tissues and organs. Although principally known for its insulin-sensitizing activity, recent data also highlight its homeostatic function, which is mediated both by direct actions on metabolic cells and indirectly through immunomodulatory effects on immune cells. Here we review the multifaceted immunometabolic actions of adiponectin and attempt to unify some of the contradictory reports on adiponectin function in inflammatory processes. We propose that a holistic understanding of adiponectin function can be garnered only from understanding its actions both on the immune system and on metabolism.

Introduction

Adiponectin is well recognized for the important role it plays in metabolic diseases. Most of the beneficial properties of this protein are attributed to its insulin-sensitizing and anti-inflammatory effects, despite evidence that it might increase inflammation in some contexts [1–3]. Here we discuss how the homeostatic functions of adiponectin are mediated by its direct actions on metabolic cells and also indirectly through immunomodulatory effects on immune cells. We attempt to unify contradicting reports in the literature on the biological function of adiponectin by integrating a breadth of current knowledge that encompasses immunology and metabolism.

Fundamentals of adiponectin and its receptors

Adiponectin

Adiponectin, also known as 30-kDa adipocyte complement-related protein (ACRP30), is an adipokine produced by adipocytes that plays important roles in glucose and lipid homeostasis and in insulin sensitivity [4]. Its genomic locus maps to chromosome 3q27, which is also a susceptibility site for early-onset diabetes and the metabolic syndrome [5]. Adiponectin exists as the full-length protein (fAd) or a proteolytic cleavage fragment known as globular adiponectin (gAd). Once synthesized, adiponectin forms trimers [low molecular weight (LMW)] that oligomerize to form hexameric middle molecular weight (MMW) and high molecular weight (HMW) forms. The HMW form appears to have the strongest insulin-sensitizing activity in hepatocytes [6].

The mRNA expression of adiponectin is reduced in obese and diabetic mice [7] and plasma protein levels are lower in obese compared with lean humans [8]. Adiponectin levels also inversely correlate with insulin resistance in mouse models of altered insulin sensitivity [9]. For example, in lipodystrophic mice, insulin resistance can be completely reversed by a combination of physiological doses of recombinant adiponectin and leptin but only partially by either adiponectin or leptin alone, attesting to the complex multifaceted regulation of insulin sensitivity [9]. Adiponectin is also effective in ameliorating hepatomegaly, steatosis, and alanine aminotransferase abnormalities in obese *ob/ob* mice. These therapeutic effects are partly related to the ability of adiponectin to enhance liver fatty acid (FA) oxidation and to suppress hepatic tumor necrosis factor alpha (TNFα) production [10].

Adiponectin receptors

Adiponectin acts via three receptors: AdipoR1, AdipoR2, and T-cadherin, the latter of which is to date known to have ligand-binding properties only. Mouse AdipoR1 encodes a protein of 375 amino acids with a predicted molecular mass of 42.4 kDa, whereas mouse AdipoR2 encodes a protein of 311 amino acids with a predicted molecular mass of 35.4 kDa. AdipoR2 shares 67% amino acid homology with AdipoR1 [11]. AdipoR1 and AdipoR2 are integral signaling membrane proteins with seven transmembrane domains that lack significant homology with other mammalian proteins, but are distantly related to other G protein-coupled receptors [12]. AdipoR1 is ubiquitously expressed, with the most abundant expression in skeletal muscle, whereas AdipoR2 is prominently expressed in the liver [12]. AdipoR1 has a high affinity for gAd and a low affinity for fAd, whereas AdipoR2 has intermediate affinity for both forms of adiponectin [12]. In mouse liver, AdipoR1 promotes AMP-activated protein kinase (AMPK) activation, whereas AdipoR2 mediates activation of peroxisome

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proliferator-activated receptor alpha (PPARα), both of which are involved in FA oxidation [13]. The HMW and hexameric forms of adiponectin, but not the trimer or globular forms, bind to T-cadherin expressed on endothelial and smooth muscle cells. The functional significance of this interaction is not completely defined [14].

In the normal liver, T-cadherin is present on the endothelial cells of large blood vessels and on myofibroblasts. It is also weakly expressed in the sinusoidal endothelial cells of the liver, but is not expressed by hepatocytes or Kupffer cells [15,16]. It has been reported that intraperitoneal injection of recombinant adiponectin in AdipoR1 and AdipoR2 double-knockout (KO) mice can induce inhibitor of nuclear factor kappa B (IκBα) degradation and interleukin-6 (IL-6) production in perigonadal white adipose tissues. Further investigations revealed that the IL-6 was produced in the stromal vascular fraction (SVF) of adipose tissue and IL-6 immunostaining demonstrated macrophages as the source of the IL-6. Because macrophages do not express T-cadherin, the existence of other adiponectin receptors has been suggested (Figure 1), but this remains to be proven [16].

Adiponectin, adiponectin receptors, and insulin sensitivity

The principal role of adiponectin in the liver is to promote insulin sensitivity, which may be mediated through multiple pathways (Figure 1). Berg *et al.* [17] demonstrated that, in wild type (WT) C57BL6J, *ob/ob*, nonobese diabetic, and streptozotocin-treated mice, in the basal state with low insulin concentrations, intraperitoneal injection of recombinant fAd but not globular adiponectin decreases blood glucose levels. This reduction of blood glucose in the postabsorptive state (low insulin levels) was related to the suppression of hepatic glucose production (HGP) and not mediated by muscle or adipose tissue glucose disposal. The latter effect appears to require higher insulin concentrations. Likewise, in isolated primary rat hepatocytes, 1–5 μg/ml of recombinant adiponectin, which is close to the normal range in serum (5–15 μg/ml), suppressed HGP in the presence of low concentrations of insulin (35 pM). Neither insulin nor adiponectin alone at these concentrations demonstrated any significant effect on HGP. On the basis of these physiological effects, the authors suggested that slight increases in adiponectin might have a strong insulin-sensitizing effect [17]. Other studies also support the notion that adiponectin reduces blood glucose by suppressing HGP without effects on hepatic glucose uptake or disposal [18]. It is noteworthy that the effect on liver is isoform specific, because only fAd reduces the activity of enzymes involved in hepatic gluconeogenesis.

Kadowaki *et al.* have demonstrated that adenovirus-mediated overexpression of AdipoR1 in the liver of leptin

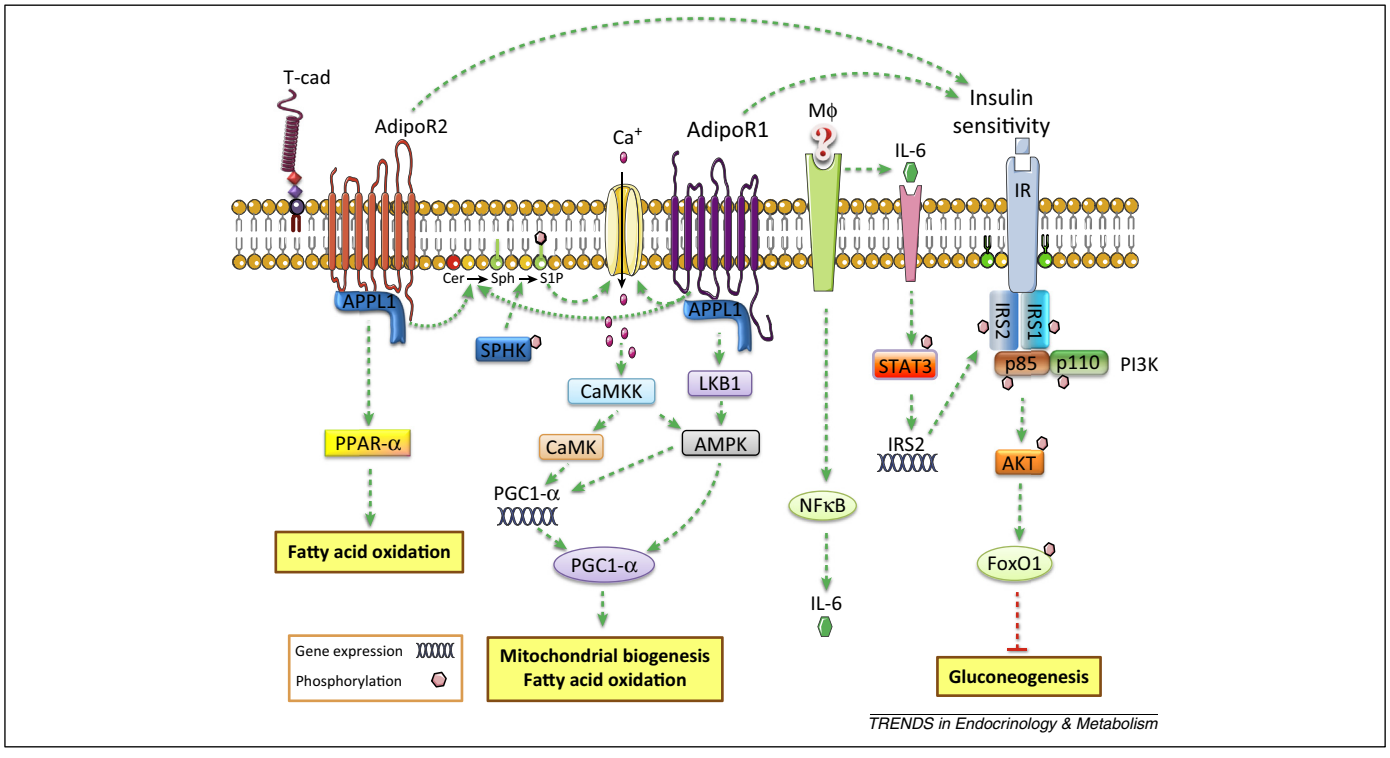

Figure 1. Adiponectin signaling. Adiponectin has three known receptors: AdipoR1, AdipoR2, and T-cadherin (T-cad). AdipoR1 and AdipoR2 are seven-transmembrane receptors, whereas T-cad does not have an intracellular domain. AdipoR1/2 can interact with adaptor protein containing pleckstrin homology domain, phosphotyrosine-binding domain, and leucine zipper motif (APPL1), which together with AKT and phosphoinositide 3-kinase (PI3K) increases insulin sensitivity [19]. AdipoR1 increases calcium influx to activate Ca^{2+}/calmodulin-dependent protein kinase kinase (CaMKK) and subsequent downstream kinases. Ca^{2+}/calmodulin-dependent protein kinase (CaMK) and AMP-activated protein kinase (AMPK) can increase peroxisome proliferator-activated receptor (PPAR) gamma coactivator 1 alpha (PGC-1α) mRNA expression. PGC-1α increases mitochondrial biogenesis and fatty acid (FA) oxidation. AdipoR1 activates liver kinase B1 (LKB1) and AMPK. AdipoR2 is predominantly expressed in the liver and can activate PPARα to increase FA oxidation and insulin sensitivity. AdipopR1/2 also has ceramidase activity and can catalyze the conversion of ceramide (Cer) to sphingosine, which can be phosphorylated by sphingosine kinase 1 (SPHK1) to produce sphingosine-1-phosphate (S1P). S1P has insulin-sensitizing and antiapoptotic properties and is involved in increasing calcium flux to cells. At least in macrophages, an unknown adiponectin receptor has been suggested that through fAd stimulation can activate nuclear factor kappa B (NFκB) to increase interleukin-6 (IL-6) and subsequently insulin receptor substrate 2 (IRS2) mRNA expression in hepatocytes through signal transducer and activator of transcription 3 (STAT3) activation [16]. Insulin-stimulated FoxO1 phosphorylation through PI3K and AKT can reduce hepatic gluconeogenesis. Figure prepared using templates on the Servier medical art website (http://www.servier.fr/servier-medical-art).

receptor null ($lepr^{-/-}$) mice reduces endogenous glucose production in clamp studies and hepatic gluconeogenesis measured by the pyruvate-challenge test (PCT) following injection of pyruvate, a precursor of gluconeogenesis. AdipoR2 overexpression did not have a similar effect. However, overexpression of both receptors increased the glucose-infusion rate (GIR), indicating increased peripheral insulin sensitization. Further investigation revealed that AdipoR1 decreases expression of the gluconeogenic enzymes glucose 6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase 1 (Pck1) and the lipogenic transcription factor sterol regulatory element-binding protein-1c (Srebp1c) in the liver, whereas AdipoR2 expression increased liver levels of glucokinase (Gck), the enzyme that facilitates phosphorylation of glucose to glucose 6-phosphate (G6P). Thus, both AdipoR1 and AdipoR2 improve insulin sensitivity and glucose homeostasis by targeting different aspects of glucose metabolism [13].

AdipoR1 and AdipoR2 interact with adaptor protein containing pleckstrin homology domain, phosphotyrosine-binding (PTB) domain, and leucine zipper motif (APPL1) [19], which has been suggested to act as an adaptor protein that tethers phosphoinositide 3-kinase (PI3K) and AKT and permits their recruitment to the cytoplasmic membrane [20]. Thus, Mao *et al.* demonstrated that knocking down APPL1 or using a mutant APPL1 ($APPL1^{\Delta PTB}$) in muscle abrogated both AKT and AMP-activated protein kinase (AMPK) phosphorylation, suggesting that the interaction of adiponectin with APPL1 is the mechanism for its insulin-sensitizing effects. In $APPL1^{\Delta PTB}$ hepatocytes, fAd-mediated phosphorylation of AMPK and p38 mitogen-activated protein kinase (MAPK) was abrogated [19]. Further, similar to the insulin dependence of adiponectin in shutting down HGP, treatment of C2C12 myoblasts with adiponectin did not increase AKT phosphorylation. However, a synergistic effect on AKT phosphorylation was observed when the cells were treated with both adiponectin and insulin [19].

In contrast to the studies that propose an AMPK-dependent pathway for the insulin-sensitizing effects of adiponectin [11–13], Miller *et al.* [21] have demonstrated that liver-specific deletion of liver kinase B1 (LKB1), the upstream kinase that activates AMPK, partially blocks the effects of adiponectin in reducing HGP. Nevertheless, adiponectin decreased glucose output in $LKB1^{-/-}$ mice. Thus, adiponectin can regulate hepatic gluconeogenesis through LKB–AMPK-dependent and -independent signaling pathways. One possible AMPK-independent pathway could be through AdipoR2-mediated activation of PPARα, which has been proposed to augment insulin sensitivity [22,23]. Another mechanism is related to effects on sphingolipid metabolism. It has been suggested that both AdipoR1 and AdipoR2 have ceramidase activity that is enhanced on ligand binding. This removes a fatty acyl chain from ceramides to produce sphingosine, which can be phosphorylated by sphingosine kinases to generate sphingosine 1-phosphate, thereby improving glucose homeostasis (Figure 1) [24,25].

The insulin-sensitizing effects of adiponectin through modulation of the immune response are less well addressed. Recently, Awazawa *et al.* [16] demonstrated that adiponectin increases hepatic insulin sensitivity by promoting a transient increase in IL-6 levels in macrophages and subsequent signal transducer and activator of transcription 3 (STAT3)-mediated expression of insulin receptor substrate 2 (IRS-2) in the liver of obese mice (Figure 1). This adiponectin effect was blocked by IL-6-neutralizing antibodies in C57BL/6J WT mice and in both IL-6-KO and liver-specific STAT3-KO mice. Further investigation of the origin of IL-6 demonstrated exclusive expression by the SVF of visceral white adipose tissue but not adipocytes. Indeed, fAd was shown to activate nuclear factor kappa B (NFκB) and IL-6 expression in macrophages independently of its *bona fide* receptors AdipoR1 and AdipoR2, whereas the globular form did not. Of note, immunoblotting of protein from liver IRS-2 immunoprecipitation showed increases in p85 but not in p-Akt or p-FoxO1 in mice injected with adiponectin. By contrast, insulin injection alone or in combination with adiponectin increased phosphorylation of Akt and FoxO1, consistent with the insulin-dependent role of adiponectin, as discussed above.

Adiponectin and inflammation

Anti-inflammatory actions of adiponectin

There is extensive evidence that adiponectin acts as an anti-inflammatory mediator in metabolic diseases, including type 2 diabetes, fatty liver, and cardiovascular disease. For example, plasma levels of adiponectin in atherosclerosis are negatively correlated with levels of the inflammatory marker C-reactive protein (CRP) [26] in humans and adiponectin-KO mice have higher levels of TNFα mRNA expression in adipose tissues and TNFα protein in plasma [27]. Further, administration of adiponectin to *ob/ob* mice improves fatty liver disease through suppression of TNFα production and these anti-inflammatory effects might play a role in reversing metabolic dysfunction [10].

Adiponectin may also downregulate inflammation through alterations in macrophage function. For example, adiponectin inhibits transformation of human monocyte-derived macrophages into foam cells in cell culture [28] and attenuates Toll-like receptor-mediated activation of NFκB in RAW 264 macrophages [29]. Furthermore, adiponectin stimulates production of the anti-inflammatory cytokine IL-10 by human monocyte-derived macrophages and promotes the transition of macrophages from a proinflammatory M1 to an anti-inflammatory M2 phenotype in both human monocyte-derived macrophages and SVF cells isolated from human adipose tissue [30,31], as well as in peritoneal macrophages and SVF cells isolated from WT mice. These latter cells, when isolated from adiponectin-KO mice, displayed increased M1 markers [30,31]. Adiponectin also facilitates the uptake of apoptotic cells by binding to calreticulin in concert with its coreceptor CD91 [32]. Accordingly, macrophages in adiponectin-KO mice have a reduced ability to clear early apoptotic cells. This has important ramifications because the ability to take up apoptotic cells promotes an M2-like phenotype.

Proinflammatory actions of adiponectin

Reports of proinflammatory actions of adiponectin in metabolic diseases are surrounded by controversy. Key issues contributing to this confusion are: (i) the source of adiponectin used in *in vitro* experiments; and (ii) the avid

binding of adiponectin to lipopolysaccharide (LPS). Thus, some of the reported proinflammatory effects of adiponectin, particularly those obtained using recombinant protein produced in *Escherichia coli*, may be the result of contamination with LPS. Other discrepancies may be due to: (iii) the use of different molecular weight and/or truncated forms of the adipokine; or (iv) experimental rodent models with strain-specific differences. Finally, an additional level of complexity stems from the notion that exposure to adiponectin might result in: (v) an initial inflammatory response followed by desensitization of cells to subsequent inflammatory stimuli. Below we discuss some key findings and attempt to reconcile the controversies.

Haugen *et al.* [33] reported that HMW adiponectin can activate the NFκB pathway in monocyte cell lines. Thus, treatment of U937 monocytes with recombinant HMW (from HEK cells) or globular adiponectin (from *E. coli*) for 18 h increased NFκB reporter activity and the expression of proinflammatory cytokines, whereas treatment with mutated recombinant adiponectin (the trimer isoform), which is unable to form HMW complexes, did not. However, treatment with HMW adiponectin reduced LPS- and TNFα-mediated activation of a NFκB reporter. Time-course experiments showed that the NFκB reporter activity peaked at 6 h and declined by 24 h of incubation with adiponectin. The possibility of LPS contamination was not addressed in this study; however, TLR4-neutralizing antibody did not reduce gAd-mediated NFκB activity. Tsao *et al.* [34] reported similar findings in C2C12 cells treated with hexameric adiponectin but not with globular adiponectin. As a control for LPS contamination, they treated the adiponectin with heat and proteinase K to inactivate adiponectin (but not LPS) and demonstrated that NFκB reporter activity was lost. This suggests that the demonstrated adiponectin-mediated NFκB reporter activation was not the result of LPS contamination. Similar effects of adiponectin have also been suggested by Cheng *et al.* [35], who reported that recombinant adiponectin (HEK293; endotoxin content about 40 pg/μg) induces a proinflammatory profile in human macrophages and T cells.

If adiponectin can induce a proinflammatory response, this raises the question of how it can also modulate anti-inflammatory responses. To answer this question, some earlier work on the regulation of inflammatory responses by bone marrow-derived macrophages might provide a clue. Thus, Medzhitov *et al.* demonstrated that stimulating macrophages with LPS propagated an initial phase with increases in the expression of proinflammatory genes, followed by a class switch from a harmful cytokine milieu to an increase in protective, antimicrobial, prorepair, and metabolic regulators by 24 h [36]. When macrophages were restimulated with LPS, the authors did not detect any increase in the expression of proinflammatory genes (tolerizeable genes) such as IL-6 or IL-1b, but instead found a higher and faster response in the production of non-harmful antimicrobial tissue-repair genes dubbed non-tolerizeable genes (e.g., Rantes, Msr1, Irak3) [36].

To better understand these data, it must be recognized that cells have short-term memory and record prior signals to adapt to their environment [37]. The idea that immune, and probably nonimmune, cells have short-term memory is best illustrated in the context of sepsis, which results in a systemic inflammatory response. In sepsis, the monocytes of patients in the recovery phase do not mount an effective immune response to a second bacterial insult. Indeed, this is one reason why a principal cause of death in patients recovering from sepsis is secondary bacterial infections. Teleologically, this might be to prevent collateral damage from over-reaction of the immune system to repeated inflammatory stimuli. Thus, short-term environmental memory may be a safety mechanism that persists for a limited time after the initial environmental challenge that affects the intensity or type of subsequent immune cell responses to similar or different stimuli [37].

In the context of adiponectin, the question is whether the anti-inflammatory effects of adiponectin are governed through first inducing an inflammatory memory effect. This idea is supported by Park *et al.* [38], who demonstrated that adiponectin-mediated induction of inflammation induces TNFα (peak at 2 h), followed by increases in IL-10 (peak at 5 h). The IL-10 subsequently induced tolerance to LPS in Raw 264.7 macrophages. In Table 1 we provide more evidence from the literature demonstrating that the conditions chosen to conduct experiments critically determine the outcome of adiponectin activity, whether pro- or anti-inflammatory. Additionally the type and ratio of the different isoforms of adiponectin could play a pivotal role, and this needs further study. As illustrated in Table 1, stimulating immune cells in cell culture without prior exposure to adiponectin (considering that the cells are often serum starved) can result in an inflammatory response that helps to limit the effects of subsequent inflammatory stimuli like LPS exposure. However, within an organism the immune system is constantly exposed to high levels of adiponectin. This potentially can mediate a memory effect that may induce a systemic anti-inflammatory response when encountering environmental challenges. Such a hypothesis may account for the divergent *in vitro* and *in vivo* data reported in the literature on the actions of adiponectin.

The weight of *in vivo* data suggests that adiponectin principally has anti-inflammatory effects. However, there is also a body of literature suggesting that adiponectin levels are paradoxically increased in some inflammatory diseases in humans such as rheumatoid arthritis (RA), chronic obstructive pulmonary disease (COPD), and end-stage renal disease, as reviewed elsewhere [1,2], and also with cardiovascular events and cirrhosis (Table 2). The underlying mechanism for this effect is unknown and warrants further study. Whether this is a consequence of differential immune responses between tissues or the fact that the same stimuli can have opposite effects and different outcomes in a context- and tissue-specific manner is unknown [39–41].

Next, we explore adiponectin-specific immunomodulatory effects in metabolic organs like the liver and in adipose tissue and their role in maintaining metabolic homeostasis.

Adiponectin regulation of metabolic homeostasis through immune cells

The proximity of metabolic and immune-responsive cells in metabolically critical organs like liver and adipose tissue

Table 1. Paradigms of adiponectin action: pro- versus anti-inflammatory effects[a]

In vitro	Treatment		Read out	Outcome	Proof for lack of LPS effect in ADN[b] treatment	Source of ADN	Refs
	1st hit	2nd hit					
Myocytes	fAd	–	NFκB reporter	Activity increased	Loss of activity with proteinase K and heat treatment of ADN but not LPS	Recombinant ADN from *Escherichia coli* and HEK293T cells	[34]
	gAd	–		No change			
Human synovial fibroblasts	fAd	–	IL-6	Activity increased	No change in activity with 1 μM polymyxin B pre-incubation for 30 min in ADN-treated cells but decrease in activity with LPS treatment	Recombinant ADN	[63]
Macrophages	fAd	–	IL-6 and NFκB	Activity increased	LPS contamination less than 1 pg/mg in *E. coli* ADN and both sources of ADN induced IL-6	Recombinant ADN from *E. coli* and HEK293 cells	[16]
Kupffer cells	fAd and gAd	LPS	TNFα	Decreased	Overnight treatment of cells with 10 μg/ml polymyxin and ADN before LPS treatment	Recombinant gAd from *E. coli* and recombinant fAd from HEK293 cells suppressed LPS proinflammatory effect but recombinant fAd from *E. coli* had no effect	[64]
Macrophages	gAd (18 h)	– LPS (2 h)	TNFα and IL-10	Initial increase in TNFα (2 h) with subsequent decrease in TNFα (5 h) and increase in IL-10 that suppressed LPS inflammatory effect	Pretreatment of cells with 10 μg/ml polymyxin B for 60 min	Recombinant ADN from *E. coli*	[38]
Macrophages	gAd	LPS	NFκB activity	Decreased	Affi-Prep polymyxin column was used to remove endotoxin	Recombinant gAd from *E. coli*	[29]
Kupffer cells	gAd	LPS	TNFα	Decreased	gAd contained less than 0.2 ng LPS/μg protein	Recombinant gAd from *E. coli*	[65]

[a] *In vitro* experiments show that adiponectin can be proinflammatory when added to cells (top), but that it can prevent the effects of a second proinflammatory hit (bottom).
[b] Adiponectin.

underpins the possibility for crosstalk between metabolism and the immune system [42]. It has been suggested that maintaining a homeostatic state requires reciprocal communication between these two systems [43], acting as an immunometabolic rheostat that sets the threshold or intensity of metabolic and immune function [44,45].

Providing support for this concept, Haschemi *et al.* [46] have reported that polarization of RAW264.7, mouse peritoneal, and bone marrow-derived macrophages to M1 or M2 requires reprogramming of glucose metabolism. Polarization to M1 involves a switch to aerobic glycolysis, whereas M2 cells adopt oxidative phosphorylation (OxPhos) [47]. Mandal *et al.* [48] proposed that recombinant fAd (from HEK293 cells) mediates an increase in OxPhos in Kupffer cells and RAW264.7 macrophages, thereby inducing a change to an M2 anti-inflammatory phenotype. Likewise, Stanya *et al.* [49] have suggested that the M1/M2 phenotype modulates hepatocyte metabolism: M1 induces hepatic insulin resistance and M2 reduces liver glucose production thus increasing insulin sensitivity. Odegaard *et al.* [50] also demonstrated that alternative activation of Kupffer cells and adipose tissue macrophages (ATMs) is important for the regulation of hepatocyte metabolism and systemic insulin sensitivity and Satoh *et al.* [51] reported that reduction of M2 macrophages in fat depots and the differentiation of macrophages to an M1 phenotype leads to reduced adipose tissue mass. They discovered tribbles homolog 1 (Trib1) to be a critical factor for the differentiation of tissue-resident macrophages to an M2 phenotype, and Trib1 deficiency in hematopoietic cells leads to a severe reduction of M2 macrophages in various organs, including adipose tissue. This can then lead to less fat mass and to lipolysis, even in mice on a normal chow diet.

The phenotype reported by Stanya *et al.*, Satoh *et al.*, and Odegaard *et al.* has close similarity to the effects of adiponectin on liver and adipose tissue. As mentioned above, adiponectin may promote the transition of macrophages from a proinflammatory M1 to an anti-inflammatory M2 phenotype [30,31]. In addition, Scherer *et al.*

Table 2. Evidence of association of high levels of adiponectin with some diseases

Disease	Study group	Outcome	Refs
Cardiovascular events and mortality	Copenhagen City Heart Study: randomly selected men and women from the community without cardiovascular disease ($n = 5624$)	Increased mortality and an increasing number of major adverse cardiovascular events (cardiovascular mortality or nonfatal myocardial infarction or ischemic stroke)	[66]
Cardiovascular mortality	Group 1 ($n = 3272$): no cardiovascular disease, heart failure, or atrial fibrillation Group 2 ($n = 1030$): cardiovascular disease but no heart failure/atrial fibrillation Group 3 ($n = 383$): heart failure/atrial fibrillation	Group 1: lower mortality with total adiponectin level of 12.4 mg/l or less, higher mortality above this cut-off point Group 2: positive association of adiponectin level with mortality Group 3: positive association of adiponectin level with mortality	[67]
RA	91 healthy control versus 167 RA patients	Higher adiponectin levels in patients with RA	[68]
Biliary atresia (BA)	60 BA patients post-Kasai procedure versus 20 controls	High adiponectin levels associated with increased fibrosis and liver stiffness	[69]
Cirrhosis	87 cirrhotic patients versus 21 healthy controls	Higher adiponectin levels in cirrhosis, independent of its etiology	[70]
COPD	Non-smokers ($n = 51$), healthy ever-smokers ($n = 62$), COPD patients ($n = 71$)	High adiponectin levels in patients with COPD	[71]

proposed that adiponectin acts as a hunger signal for adipose tissues, because adiponectin overexpression can increase the capacity for storage of lipids and protects other organs, like liver and pancreas, from exposure to toxic free FAs [52], thus improving insulin sensitivity in a leptin-deficient background. These findings are also supported by Liu *et al.* [53], who demonstrated that adiponectin-KO mice possess smaller epididymal fat pads compared with their WT counterparts. However, whether adiponectin-mediated increases in fat mass are associated with its immune modulatory properties is unknown. Along these lines, Luo *et al.* [54] demonstrated that transgenic expression of AdipoR1 in macrophages reduces the number of M1 and increases the number of M2 macrophages in adipose tissue. This resulted in improved insulin sensitivity, lower body fat, and reduced hepatic steatosis and gluconeogenesis on a high-fat diet. Although contradictory, the lower body-fat mass in these mice may be explained by the higher AdipoR1 expression in macrophages and induction of M2 markers that might increase FA oxidation and energy expenditure, a hypothesis that needs to be proven. Interestingly, in adipose tissue, adiponectin and its receptors can be found in the mature adipocyte fraction (MAF) and the SVF, and adiponectin protein accumulates in the interstitial space of adipose tissue, especially at the sites of macrophages in obese adipose inflammation [55]. Thus, one may speculate that adiponectin's effects on liver and adipose tissue might involve modulation of the immune system.

Nonetheless, we raise caution in framing macrophages into two distinct M1 and M2 entities and a more informative classification based on the spectrum of their function is suggested. For example, Xu *et al.* [56] demonstrated recently that adipose tissue has a buffering role and this mechanism protects other organs from the lipotoxic effects of triglyceride and FA leakage from hypertrophic adipocytes. This buffering effect of adipose tissue is governed by adipose tissue-resident macrophages that increase in number with obesity. However, the authors did not demonstrate a switch from M2 to M1, but suggested that greater inflammatory cytokine expression in obese adipose tissue is secondary to a quantitative increase in the total number of ATMs rather than a change in their function to a more proinflammatory phenotype. These findings support the need for a change in our perceptions of macrophages as proinflammatory M1 or anti-inflammatory M2 to a greater understanding of changes in their functionality.

Adiponectin and glucose homeostasis

Recently, Iwabu *et al.* [57] demonstrated that muscle-specific AdipoR1-KO mice have reduced AMPK activation and mitochondrial biogenesis that is related to lower expression of PPAR gamma coactivator receptor 1 alpha (Pgc-1α). By stark contrast, in high-fat diet-fed mice a new AdipoR1/2 agonist (AdipoRon) reduced Pgc-1α expression in the liver and lowered hepatic gluconeogenesis [58]. It is noteworthy that, in hepatocytes, Pgc-1α can increase gluconeogenesis in a hepatocyte nuclear factor 4 (HNF4)-dependent manner [59] and it has been suggested that adiponectin regulates binding of the HNF4a isoform to DNA [53]. Nevertheless, there is little evidence that adiponectin can increase Pgc-1α expression in the liver when it is in a catabolic/fasted state. Recently, Handa *et al.* [60] demonstrated the role of adiponectin in mitochondrial biogenesis through regulation of Pgc-1α. They reported a reduction in serum adiponectin levels and decreased Pgc-1α expression in the liver of $Lepr^{-/-}$ mice fed a high-fat diet. Treatment with recombinant fAd increased Pgc-1α mRNA and protein expression and mitochondrial biogenesis in cultured hepatocytes.

Burgess *et al.* [61] also demonstrated that Pgc-1α is not essential for the full expression of gluconeogenic genes like Pck1 and G6Pase; however, in the fasting state Pgc-1α is required for upregulation of genes in the tricarboxylic acid cycle (TCA) and for beta oxidation, to provide substrates for gluconeogenesis. Consistent with this, HGP rates were reduced in the liver of Pgc-1α-KO mice because of mitochondrial defects and lower hepatocyte energy production. Likewise, Foretz *et al.* [62] have demonstrated that metformin-induced reductions in HGP were augmented in AMPK- and LKB1-KO hepatocytes and are related to the reduction in ATP levels, because gluconeogenesis is

an energy-consuming process. These reports suggest that adiponectin might be involved in enhancing gluconeogenesis in the fasted state, but to the best of our knowledge evidence in the literature supporting this claim is not available.

Concluding remarks and future perspectives

Numerous studies have provided strong evidence for the insulin-sensitizing effects of adiponectin in the prevention of metabolic diseases. However, much work remains to be done to understand its function from a holistic perspective. Considering the vast array of tissues that are affected by adiponectin and the wide range of activities of this protein, a systematic approach is warranted. Currently, most insights on the roles of adiponectin come from studies performed in the setting of the metabolic syndrome, a pathological host milieu. However, to dissect the context-dependent and homeostatic roles of adiponectin will require investigation in both pathological and normal physiological states (Box 1). For example, how can adiponectin increase mitochondrial biogenesis through Pgc-1α in muscle and have insulin-sensitizing activity when, if the same happens in liver, Pgc-1α would induce gluconeogenesis? If this is not the case, does it relate to differential mitochondrial function in muscle and liver? In this regard, a mitochondrial proteomic study has shown that muscle mitochondria resemble mitochondria from brown adipose tissue, whereas that from the liver is like white adipose tissue with different metabolic activities [63]. Similarly, one of the less explored effects of adiponectin is its immunomodulatory function and the mechanism for these effects. First, if adiponectin can increase NFκB activity independent of its authentic AdipoR1/2 receptors, what are the unknown receptors (Box 1)? More interestingly, mounting an inflammatory response needs activation of glycolysis and glucose uptake in immune cells [64]. Hence, does adiponectin do this (independent of insulin and in stark contrast to its insulin-dependent role in metabolic cells) and if so, how? Finally, Awazawa *et al.* [16] noted that adiponectin-mediated IL-6 expression in adipose tissue macrophages increased hepatic insulin sensitivity, suggesting that we are just at the beginning of the road to fully understanding tissue homeostasis, which might involve some 'physiological' levels of inflammation. However, if we accept adiponectin as having only anti-inflammatory activity, why are adiponectin levels higher in some diseases (Table 2)? This also begs the question of whether the predominantly anti-inflammatory effects of adiponectin have unwanted effects in some contexts or diseases. Considering the growing interest in using adiponectin or its receptor agonists for therapeutic applications, an integrated understanding of its isoform-specific, metabolic, and immune function is therefore essential.

Box 1. Outstanding questions

- Are there as-yet-unknown 'physiological' activities of adiponectin?
- Are there other signaling receptors for adiponectin in macrophages to account for its ability to increase NFκB activity and IL-6 production?
- Does adiponectin induce a proinflammatory response *in vivo* in some contexts?
- If adiponectin can induce a proinflammatory response, how can it also modulate anti-inflammatory responses?
- What is the mechanism for the association between raised adiponectin levels and inflammation and fibrosis in some systemic diseases?
- Do some of the controversies related to adiponectin biology arise from the use of recombinant adiponectin in experimental models?

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