Medical Sciences

Adiponectin and its Hydrolase-Activated Receptors

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The relevance of adiponectin to insulin sensitivity has been elucidated over the last two decades. As a promoter of ceramide degradation, it works through its cognate receptors, AdipoR1 and AdipoR2, to alter bioactive sphingolipid species. Adiponectin diminishes the accumulation of ceramide, a lipid metabolite which can play a causal role in obesity-induced insulin resistance. Concurrently, adiponectin stimulates the production of sphingosine-1-phosphate (S1P), a cyto-protective molecule that accentuates adiponectin’s positive metabolic effects. This review focuses on recent work that solidifies knowledge of the adiponectin signaling pathway, gives new insight into some notable characteristics of adiponectin’s receptors, and most importantly, affirms adiponectin receptor agonism as a viable therapeutic tool to combat elevated ceramide levels and improve insulin sensitivity in obese patients with type II diabetes.

Ceramide | ceramidase | AMPK | S1P | insulin resistance

Introduction

As obesity has become more common, its associated risks, such as insulin resistance and cardiovascular disease have followed its trajectory. This incredibly relevant relationship has been the focus of extensive research. Many mechanisms detailing this link have been presented and one compelling explanation involves ceramides, which are simple sphingolipids and act as precursors to other sphingolipids. Ceramide biosynthesis, which has been detailed comprehensively [1], involves the initial condensation of serine and palmitoyl-CoA by serine palmitoyltransferase (SPT) to produce 3-oxosphinganine. The abnormal accumulation of ceramides in various tissues has already been implicated in numerous pathologies, including but not limited to, atherosclerosis [2], cardiomyopathy [3], vascular dysfunction [4], lipotoxic cell death [5,6,7], and most importantly for the purposes of this review, insulin resistance [8,9]. Elevated circulating ceramides have also been reported in human subjects with type II diabetes [10,11,12], pointing either to a causative effect or implying at the very least that ceramide is an effective marker of insulin resistance. Concurrently, adiponectin stimulates the production of sphingosine-1-phosphate (S1P), a cyto-protective molecule that accentuates adiponectin’s positive metabolic effects. This review focuses on recent work that solidifies knowledge of the adiponectin signaling pathway, gives new insight into some notable characteristics of adiponectin’s receptors, and most importantly, affirms adiponectin receptor agonism as a viable therapeutic tool to combat elevated ceramide levels and improve insulin sensitivity in obese patients with type II diabetes.

Ceramides promote insulin resistance

Work by Holland et al. in 2007 demonstrated that atypical, elevated ceramide synthesis contributes to insulin resistance [8]. Dexamethasone is a synthetic glucocorticoid that stimulates the expression of ceramide synthesis genes and increases overall ceramide content in serum and tissues. It was shown to impair glucose homeostasis and insulin signaling via in vivo studies that involved measuring serum glucose levels, glucose/insulin tolerance tests, and insulin stimulated pSerine(473)-AKT/total AKT ratios. All parameters were markedly worse in dexamethasone treated rodents. Hyperinsulinemic-euglycemic clamps confirmed this impairment as well. Dexamethasone treatment led to a dramatic decline in the glucose infusion rate needed to maintain euglycemia (~150 mg/dL) under hyperinsulinemia; this difference was brought upon by insulin’s inability to effectively suppress hepatic glucose output and promote glucose uptake in the skeletal muscle of dexamethasone treated rodents. However, the concurrent administration of myriocin, a fungal antibiotic that potently inhibits serine palmitoyltransferase (SPT), significantly mitigated dexamethasone’s insulin desensitizing effects. Myriocin treatment neutralized dexamethasone-induced disruption in glucose homeostasis and restored all the previously mentioned metabolic parameters to levels representative of vehicle treatment. Further studies in Zucker diabetic fatty (ZDF) rats corroborated the anti-diabetic effects of myriocin, solidifying ceramide as central to lipotoxicity-mediated insulin resistance [8]. Myriocin’s insulin sensitizing effects have been affirmed by others as well [27,28,29].

The deleterious effects of ceramide were further delineated by Xia et al. in 2015. In their studies, they generated transgenic mice that overexpressed acid ceramidase (ASAH1) in a liver or white adipose tissue specific, titratable, and doxycycline-inducible manner. These mice demonstrated the effectiveness of localized ceramide degradation in protection against HFD-induced insulin resistance, metabolic dysfunction, and tissue lipotoxicity. Both transgenic models, which had significantly lower amounts of several ceramide species (namely C16:0, C18:0, and C20:0 fibrotic [22], anti-inflammatory [23,24], anti-lipotoxic [25], promotes ceramide reduction, and is insulin sensitizing [26]. In light of recent advances, we will look to summarize ceramide’s insulin desensitizing role, overview adiponectin’s ceramide lowering and insulin sensitizing effects, and bring attention to new knowledge that reveals the dynamic nature of adiponectin’s receptors, especially in regards to combatting insulin resistance.

Conflict of Interest: No conflicts declared.

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ceramides) in both the targeted tissues and serum, demonstrated greatly improved glucose homeostasis/tolerance, enhanced whole body insulin sensitivity, and an overall decline in lipotoxicity triggered complications (such as fibrosis and inflammation) in comparison to their wildtype counterparts under an extended high fat diet (HFD) challenge. Most evaluations took place after at least 8 weeks on HFD (doxycycline was administered with the HFD after ~2 months of age) [9]. Interestingly, it seems that localized ceramide degradation in either the liver or white adipose tissue was sufficient to protect against hepatic steatosis and improve hepatic insulin sensitivity in transgenic mice under HFD challenge. This indicates, as Xia and colleagues put it, a “cross-talk” of sorts between the liver and white adipose tissue; degrading ceramides in one tissue significantly lowers ceramides in both tissues, with the white adipose tissue specific degradation acting as a far more potent regulator of ceramide content in both tissues [9]. In 2016, Chaurasia et al. also looked at the effects of lowering ceramides in white adipose tissue by knocking out SPT long chain base unit 1 (Sptlc1) in obese mice. They observed reduced levels of several ceramide species and changes in fat pad weight that mirrored observations made by Xia and colleagues in 2015. However, the Sptlc1 knockout mice also displayed improvements in white adipose tissue energy expenditure and overall beiging [30]. This tissue remodeling was not seen in the Art-AC (which overexpress ASAHI in white adipose tissue) mice used by Xia et al. This is likely because the lysosomal degradation of ceramide does not produce sphingosine that can be phosphorylated by sphingosine kinase (SK). However, the accumulated sphingosine in the Sptlc1 knockout mice is available for phosphorylation. The sphingosine-1-phosphate (S1P) that results may play a role in the thermogenic differences between these two transgenic models.

Figure 1: Adiponectin-mediated agonism of adipoR1 and adipoR2 enhances receptor-intrinsic ceramidase activity. This degrades ceramide, a bioactive sphingolipid that blunts insulin signaling via PKCζ and PP2A. Specifically, this catalytic activity is driven by the nucleophilic cleavage of ceramide’s defining amide bond by a zinc-stabilized hydroxide ion. The resulting sphingosine is available for conversion into S1P by sphingosine kinase (SK). S1P is further involved with AMPK activation, while sphingosine and free fatty acid (FFA) are available to serve as ligands for PPARα activation. Together, these activities promote lipid oxidation, mitochondrial biogenesis, glucose utilization, and other anti-apoptotic modifications to culminate in adiponectin’s metabolic benefits within target cells.
visceral white adipose tissue [39]. In vivo deletion of CerS6 in mice also leads to decreased C16:0 ceramides and much improved glucose tolerance/insulin sensitivity under high fat diet challenge (HFD) challenge [39]. C18:0 ceramide has also been widely correlated with insulin resistance [40,41,42,43]. Taken together, the aberrant accumulation of ceramide (especially C16:0 and C18:0 ceramides) in tissues not primarily designed to store fats is critical for the development of obesity-induced type II diabetes. Ceramides help maintain the link between obesity and diabetes.

Adiponectin, an insulin-sensitizing “friendly adipokine”, stimulates ceramidase activity via adipor1 and adipor2

Adiponectin’s ceramide lowering and anti-diabetic effects were covered by Holland and colleagues in 2011 [26]. In that paper, acute administration of recombinant adiponectin to ob/ob mice, which are leptin deficient and normally display elevated ceramides due to fat overload, universally lowered all ceramide species in livers. Furthermore, when obese mice on a long term HFD were acutely given recombinant adiponectin, they displayed significant declines in hepatic ceramide content in comparison to obese mice that were administered PBS. These observations affirmed the connection between adiponectin and ceramide reduction. Evidence for adiponectin’s insulin sensitizing effects was also overwhelming. Adiponectin administered ob/ob mice displayed markedly improved insulin response during hyperinsulinemic-euglycemic clamps; they required a far greater glucose infusion rate to maintain euglycemia (~150 mg/dL) and exhibited increased suppression of hepatic glucose output under hyperinsulinemia. Overall, recombinant adiponectin treatment allowed ob/ob mice to have enhanced glucose homeostasis and whole body insulin sensitivity in comparison to control ob/ob mice.

The paper also described the potential manner in which adiponectin exerts its effects. This brings us to adiponectin’s cognate receptors, adipor1 and adipor2. Though both are ubiquitously expressed, AdipoR1 and adiporR2 are specifically abundant in skeletal muscle and liver, respectively. They are highly conserved members of the PAQR family and reverse g-protein coupled receptors with seven transmembrane domains [45,46]. For this review, their relevance is tied to their involvement in insulin sensitization via adiponectin’s ceramide lowering effects. Before 2011, various PAQRs had been implicated with enhancing ceramidase activity [45,46]. For this review, their relevance is tied to their structural differences between the two receptors, confirmed by the extraordinary characteristics of its receptors, adipor1 and adipor2. In 2015, Tanabe and colleagues reported on the receptors’ structures. Using crystallography, they verified the seven transmembrane spans in both receptors, identified extensive structural differences between the two receptors, confirmed adipor1’s role in AMPK activation, and most notably, identified several large cavities and zinc binding sites in the transmembrane domains of both receptors [56]. The incredible nature of this discovery cannot be understated. Prior to Tanabe et al., only the members of the site-2 protease family were known to contain zinc ions in their transmembrane domains [57]. Though Tanabe et al. did not fully specify the nature of these cavities and the zinc binding site, they had potentially ascribed an intrinsic catalytic role to the adiponectin receptors; zinc ions have been associated with ceramidase activity [58].

Even more recently, this discovery has been built upon by Vasiliauskaité-Brooks et al. Using in meso crystallization and fluorescent spectroscopy, they described the crystal structures of both receptors, validating previous knowledge and revealing new information [59]. Most importantly, they showed, using fluorescent spectroscopy and fluorescent size exclusion chromatography (FSEC), that adipor2 can bind and hydrolyze C18:0 ceramides into free fatty acid and sphingosine. This observation was substantiated when the purified crystal structures of adipor2 crystals grown in a ceramide-doped lipidic cubic phase had free fatty acid molecules located within their intermembrane zinc binding cavity [60]. Further computational simulations and analyses conveyed a cavity designed to facilitate lytic activity, in particular the nucleophilic cleavage of...
ceramide’s defining amide bond by a zinc-stabilized hydroxide ion. Though the enzyme kinetics of adipor2’s hydrolase activity are physiologically slow, they are consistent with those of other intramembrane proteases. This activity, as measured by spectroscopically detectable sphingosine, is also massively amplified in adiponectin’s presence (25-fold). Adipor1’s crystal structure revealed a catalytic area that is surprisingly similar to adipor2’s. This contrasts with the numerous other structural differences between the two. Though they were not able to show a bound free fatty acid molecule in the crystal structure, identical experiments and LC-MS analyses demonstrated adipor1’s ability to bind and hydrolyze ceramide. This ceramidase activity, as measured by spectroscopically detectable sphingosine, was also greatly elevated in adiponectin’s presence. With their work, Vasiliauskaité-Brooks et al. have described the catalytic nature of adipor1 and adipor2, providing an assertive response to our earlier questions. Their data, though not completely conclusive, insinuate that there is not much independent, “basal” ceramidase activity; adiponectin’s presence prompts a massive, 20-25 fold increase in ceramidase activity from both receptors. Vasiliauskaité-Brooks et al. also indicate that though adipor2 can universally hydrolyze various ceramide species, from C6:0 to C24:0, it seems to have a binding preference for C18:0 ceramides. As explained prior, this species plays a crucial role in the development of hepatic insulin resistance and non-alcoholic fatty liver disease (NAFLD) [40,41,42,43,44].

These revelations represent a sprouting interest in harnessing adiponectin receptor signaling for the treatment of diabetes. While these recent studies help to solidify a revised view of adiponectin and its ceramide-reducing effects, much of the earlier work on this subject pointed to AMPK and PPARα as the main envos of adiponectin’s effects on lipid regulation within cells. Collectively, these studies place ceramidase activity further upstream in adiponectin’s accepted signaling pathway, in its membrane receptors, as the initiating event in adiponectin signaling. Notably, blocking ceramidase activity prevents downstream activation of AMPK and PPAR [26].

The elevated importance of adipor1 and adipor2 now make them the targets of transgenic manipulation and therapeutic intervention. A recent paper in Molecular Metabolism typifies this trend. Transgenic mice that overexpress either adipor1 or adipor2 in a tissue-specific, doxycycline-inducible, titratable manner were generated. Liver (Alb-R1/R2) and white adipose tissue (Art-R1/R2) were targeted in these studies. All mice were placed on HFD containing doxycycline after ~2 months of age. Under extended HFD challenge (all measurements were made after 8 weeks of HFD), all the mice became obese. Both Alb-R1/R2 mice displayed numerous physiological and metabolic advantages over their wildtype counterparts. They had better glucose homeostasis/tolerance and insulin tolerance, as measured by serum glucose levels, glucose/insulin tolerance tests, insulin stimulated pSerine473-AKT/total AKT ratios, and glucose infusion rates during hyperinsulimemic-euglycemic clamps [44]. During clamps, there was a dramatic suppression of hepatic glucose output in alb-R1/R2 mice, indicating improved hepatic insulin sensitivity in particular. These improvements were coupled with lowered hepatic steatosis and hepatic ceramide content (namely C16:0, C18:0, and C20:0 ceramides) in the alb-R1/R2 mice. The art-R1/R2 mice displayed the same differences. As in Xia et al., there is evidence of a “cross-talk”; degrading ceramides in one tissue lowers ceramide content in the other [44]. Though the reason for this “cross-talk” is not completely clear, it may involve ceramide transport between tissues.

It is difficult to know if overexpression of either receptor in both tissues better protects against HFD-induced insulin resistance and steatosis - titrating equivalent levels of receptor expression would be critical for such an analysis [44]. With recent work describing the various structural differences between the receptors [56,59], it would not be surprising if there is also a difference in the overall efficacy of ceramide degradation between the two receptors. Indeed, in Vasiliauskaité-Brooks et al., it is shown that adipor1’s catalytic cavity is exposed to the cytoplasm in its open conformation. On the other hand, adipor2’s open catalytic cavity is positioned farther within the plasma membrane [59]. Perhaps, the divergent abundance of the receptors may play a role in any supposed difference in catalytic efficacy. Though they are expressed ubiquitously, adipor1 is abundant in skeletal muscle and adipor2 is abundant in liver [45]. Maybe the receptors’ roles developed to cater to their specific environments.

Holland et al. (2017) also illustrated the importance of adiponectin to its receptors’ ceramide-hydrolase activities. This corroborates previous observations and affirms that adiponectin and its receptors are mutually dependent on each other for their activities [26,44,59]. Alb-R1/R2 mice were crossed with adiponectin KO mice (APNKO), which do not endogenously produce adiponectin. All of the aforementioned improvements were neutralized in the absence of adiponectin. Alb-R1/R2APNKO mice did not have lower ceramide levels of any species in the liver in comparison to control APNKO mice. Any differences in glucose homeostasis/tolerance, insulin sensitivity, or lipid homeostasis/tolerance were also negated. Speculation about significant receptor ceramidase activity in the absence of adiponectin agonism has been, for now, put to rest. The anti-diabetic potential of adipor2 was also assessed [44]. These studies were conducted with ob/ob mice, which are leptin deficient, simulate type II diabetic conditions, and are known to have lowered expression of both adiponectin receptors [61]. Ob/ob mice were crossed with alb-R2, art-R2, alb-AC, and art-AC mice. For some reason, adipor1 overexpression on an ob/ob background was not evaluated. Though all overexpressing cohorts displayed lowered ceramide levels in their specific target tissues, the degradation was different, specifically between the ob/obgorob-R2 and ob/obgor-AC mice. Ob/obgor-AC targeted C16:0 ceramides at higher rates. Overall, the ob/obgor-AC mice had improved glucose homeostasis/tolerance when compared to their ob/ob counterparts. Ob/obgor-AC had no such improvements. The reason behind this may be that acid ceramidase, a lysosomal hydrolase, does not promote S1P accrual as a byproduct of ceramide degradation. In contrast, the sphingosine produced by adipor1 or adipor2 ceramidase activity is available for phosphorylation by sphingosine kinase (SK). S1P is known to be anti-apoptotic, cytoprotective, and stimulate AMPK activity, which can enhance adiponectin’s anti-diabetic effects [48,53,54,55].

Clinical relevance of adiponectin and its cognate receptors and overall outlook

The past few decades have established adiponectin as a unique adipokine, one that is both a vital marker and a highly active, almost universally positive protein. Amongst its many benefits, the adiponectin: adipor1/2 interaction can, under proper stimulation, potently degrade a bioactive species that links obesity and insulin resistance in peripheral tissues, a hallmark of type II diabetes. Moreover, adiponectin-induced ceramide degradation creates a pool of sphingosine that can be shuttled into the production of S1P, a molecule with well documented anti-apoptotic, cytoprotective effects [53,54,55]. All these observations point to a novel and effective therapeutic means to combat obesity-driven type II diabetes. Efforts could involve replenishing plasma adiponectin levels, which are significantly reduced during
obesity, and cultivating elevated adiponectin receptor agonism. However, as adiponectin is highly abundant, the use of recombinant adiponectin could never be a cost-effective solution to improve metabolism. Moreover, adiponectin’s complexity, size, and kinetics have made it difficult to produce en masse for therapeutic uses [62,63]. Small molecules may offer an alternative solution to propagate adiponectin signaling. AdiporON, an orally-bioavailable adiponectin mimetic that can bind to and stimulate adipor1 and adipor2, was identified by Okada-Iwabu et al. in 2013 [64]. It is able to improve insulin sensitivity in vitro, improves lifespan in severely diabetic mice, and like adiponectin, promotes ceramidase activity (diabetic ob/ob mice given AdiporON reductions depicted in hepatic ceramide levels) [44,64]. However, any translational effort should also proceed with caution. Data that correlates adiponectin with reduced bone density, infertility, and left ventricular hypertrophy has been produced [65,66,67,68] and must be taken into account as therapeutic advances are pursued. Still, adiponectin receptor agonism represents a fantastic opportunity to advance the clinical treatment of obesity-driven insulin resistance and type II diabetes.

Expression of adiponectin receptor in mammalian cells, offers minimal signal-to-noise for the evaluation of receptor agonism. In HEK293T cells it is difficult to achieve more than a 3-fold change in ceramidase activity. This is likely due to two reasons. First, culture of mammalian cells with serum additives provides a minimal signal-to-noise for the evaluation of receptor agonism. The authors wish to acknowledge support from the American Heart Association 15UFELO25090280 (AXS) and 12BGIA-8910006 (WLH) as well as the NIH grants R00DK094973 and R01DK108833 to WLH.

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