Adiponectin: Characterization, Metabolic and Cardiovascular Action

Jefferson Petto¹,²,³,⁴, Alan Carlos Nery dos Santos¹,³, Marcelo Trotte Motta⁴, Roberto Santos Teixeira Filho⁴, Douglas Gibran Cerqueira do Espirito Santo¹,², José Lázaro Lins Ribas⁴, Ana Marice Teixeira Ladeia³

¹Grupo de Pesquisa Cardiovascular, Salvador, BA – Brazil
²Faculdade Social – Salvador, BA – Brazil
³Escola Bahiana de Medicina e Saúde Pública – Salvador, BA – Brazil
⁴Universidade Estadual de Feira de Santana, Feira de Santana, BA – Brazil

Abstract

In the last two decades, the understanding of adipose tissue biology underwent revolutionary changes, from a major energy storage site to an important endocrine organ responsible for the production and secretion of proteins, peptides and non-bioactive peptides. Among the proteins secreted by adipocytes, adiponectin (APN) is the most abundant, with important physiological actions in the cardiovascular and endocrine system, involving the sensitization of insulin action and regulation of body energy metabolism, including the heart. This review aims to describe the action of APN on the cardiovascular system. It includes original manuscripts with humans or animals. The databases PubMed and Medline, from years 1994 to 2013, were searched. Case reports, pilot studies or review studies have not been included. The health science descriptors and MeSH specific for Medline were used as keywords. The following cross searches were carried out: Adiponectin AND Obesity, Adiponectin AND Metabolism and Adiponectin AND Cardiovascular Disease. We found 303 manuscripts, excluded 204 and selected 31 manuscripts that were included this study. In the general context of this review, APN presents anti-inflammatory and ateroprotector effects in the vascular tissue and an insulin sensitizing action in tissues involved in glucose and lipid metabolism. It is thus considered an important biomarker for the development of cardiovascular diseases.

Keywords: Adiponectin; Adipose tissue; Basal metabolism; Cardiovascular diseases

Introduction

For a long time, adipose tissue was a considered an inert structure whose functions were partially limited to thermal insulation, mechanical support, as well as a virtually unlimited energy storage capacity. However, over the past two decades, the understanding of adipose tissue biology underwent revolutionary changes, from a major energy storage site to an important endocrine organ responsible for the production and secretion of proteins, peptides and non-bioactive peptides with autocrine, paracrine and endocrine actions.

The current concept of adipocytes as secretory cells gained importance with the discovery of leptin, a protein secreted by these cells, whose function is the regulation of appetite and energy balance and nutritional state homeostasis. Further advances have allowed the discovery of a growing list of other adipocyte-derived substances collectively termed adipokines, which include adiponectin (APN), tumor necrosis factor-α (TNF-α), resistin, visfatin, interleukin-6 (IL-6), angiotensinogen, plasminogen activator inhibitor-1 (PAI-1), among others less studied ones.

Among the adipokines secreted by adipocytes, the APN is the most abundant one, with important physiological actions in the cardiovascular and endocrine system, involving the sensitization of insulin action and regulation of body and cardiac energy metabolism.
Serum concentrations of APN are abnormal in various cardiovascular diseases and may have a prognostic value. As opposed to most adipokines, the circulating levels of APN are decreased in cardiovascular and metabolic disorders such as in coronary artery disease, hypertension, stroke, insulin resistance and diabetes mellitus type 27,9.

Literature data show that lower levels of APN (hypoadiponectinemia) is associated with higher prevalence and/or worse prognosis of cardiovascular diseases, regardless of other risk factors8,10. On the other hand, they suggest that the APN has cardioprotective effects in various cardiovascular diseases through its anti-diabetic, anti-inflammatory, antioxidant and anti-apoptotic properties10.

Until recently, it was believed that the APN was produced only by adipocytes; however, some findings suggest its production and secretion in other tissues such as cardiomyocytes and the muscle skeletal tissue12. Advances in research on this protein allow us to increasingly understand its physiology, which places it as a promising target for the prevention and treatment of cardiovascular and metabolic disorders13. However, there are still some counterpoints when the results of this work are considered and some doubts arise about the mechanisms of regulation and action of this adipokine, specifically on the cardiovascular system. In this context, the objective of this review is to describe and discuss the possible APN action mechanisms in the cardiovascular system.

A systematic review was conducted, without meta-analysis, describing the APN action on the cardiovascular system, composed only of original manuscripts conducted with animals or humans. The databases consulted were PubMed and Medline from 1994 (discovery of APN) to 2013. Case reports, pilot studies or review studies were excluded.

Health sciences descriptors and Medical Subject Headings (MeSH) specific for the Medline were used as keywords. The following terms were adopted and the following cross searches were performed: Adiponectin AND Obesity, Adiponectin AND Metabolism and Adiponectin AND Cardiovascular Disease.

The manuscripts were selected by two independent reviewers who have researched on pre-determined databases using the cross searches described for choosing the manuscripts from the eligibility criteria proposed for this study.

The eligibility criteria adopted for choosing the articles were: being publicly available and published from 1994 to 2013. Something to be assessed would be the cardiovascular outcomes associated with reduction or maintenance of physiological levels of APN or the physiological effects of APN in vascular inflammation or the effects of APN in the pathophysiology of atherosclerosis or cardiovascular remodeling or metabolism. When the study involved humans, these should be ≥ 18 years of age.

The reviewers evaluated the titles and abstracts of all manuscripts found by individually creating a database of manuscripts composed of studies that have a direct relation to the theme proposed. Each reviewer had six months to perform the research. After the composition of databases of manuscripts, two consensus meetings were scheduled to compare and select the manuscripts that make up this review. Figure 1 shows the methodology for the selection of studies.
To make it easier to understand, the discussion was divided into two parts. In the first part, a historical approach was applied to the discovery of APN, and its molecular structure, synthesis, secretion and regulation were briefly described. Finally, the second part of the discussion addressed the APN action on the cardiovascular system.

Following the pre-established methodological criteria, 303 manuscripts were found; of these, 204 were excluded because they do not fit the established criteria or do not have any connection with the theme. Therefore, 31 manuscripts that specifically addressed the APN action on the cardiovascular system were analyzed.

History

The APN was originally described as a protein expressed and produced by 3T3-L1 adipocytes from mice, called adipocyte complement-related protein of 30 kDa (Acrp30)14. In 1996, its human counterpart was identified and characterized, receiving the name of adipose most abundant gene transcript 1 (APM1). In the same year, two other studies described the APN with the names Adipo Q15 and gelatin binding protein of 28 kDa (GBP28)16. The divergence of names is possibly because only one year after the publication of the first report in which the APN was described as Acrp30, there were three other manuscripts published about the same time, describing the protein with different names17.

Molecular structure

The gene encoding the human APN is located in the chromosome 3q27. It has three exons and two introns and is more abundantly expressed in the adipose tissue18. Researchers demonstrated, in 2002, that this locus is associated with susceptibility to type II diabetes mellitus and cardiovascular diseases19.

Structurally speaking, the APN is a protein of approximately 30 kDa comprising 244 amino acids and four distinct domains: a signal sequence at the amino-terminal area, a variable region of 27 amino acids, a collagen-type domain - homologous to the collagens VIII and X19 and a globular domain in the C-terminal region14. The latter presents a sequential homology with the complement factor C1q. Therefore, the APN is included in the family of proteins of globular domain C1q14. In addition, this domain also presents structural homology with the TNF-α family of cytokines as demonstrated by X-ray crystallography, thus suggesting an evolutionary link between the members of the TNF-α family and APN20. The globular domain in the C-terminal area seems to be a functional domain that interacts with other proteins or receptors21.

The APN, in its complete form, is made from monomers, appearing in three forms: low molecular weight trimers, hexamers of medium molecular weight and multimers (with 12-18 monomers) of high molecular weight22,23. Thus, the basic unit of APN is composed of three monomers (trimer) connected by globular domains. These trimers are then bound by collagen-like domains to the pairs, forming hexamers or multimers of four or six trimers20.

Besides this, the APN may undergo a proteolytic cleavage process, forming a globular fragment comprising the C-terminal globular domain known as globular APN. It is believed that leukocyte elastase secreted by activated monocytes and/or neutrophils intermediates this process, thus generating a globular APN, which may form trimers, but not oligomers24.

Synthesis and secretion

The APN is the most abundant adipokine identified in human plasma. Its average concentration ranges from 5 to 30 mg/ml, which accounts for 0.01% of plasma proteins in adult individuals17,18. Interestingly, in newborns, APN values are significantly higher compared to maternal APN (61 mg/mL vs. 18 mg/mL)25. However, the physiological mechanisms that regulate the APN in newborns are poorly understood.

As stated previously, it was believed that adipocytes were the only APN secreting cells14. However, the progress of studies demonstrated its production and secretion in other cells and tissues, including fetal cells, myocytes, cardiomyocytes, salivary gland epithelial cells, endothelial cells of blood vessels and liver sinusoid vessels26, and the cardiomyocytes of atria27, ventricles27 and in the skeletal muscle of rodents12,28.

Once synthesized, the APN undergoes post-translational modifications, such as signaling, glycosylation and hydroxylation, giving rise to eight different isoforms, six of which are glycosylated in the collagen-like domain, thus suggesting that such changes are important in the oligomerization of such protein29. Furthermore, it was demonstrated that glycosylated APN is more potent than non-glycosylated bacterial APN, indicating that these post-translational modifications may be required for an optimal biological activity29.
APN secretion by adipocytes appears to be held by exocytosis after being transported through the Golgi apparatus and endosomal system and can occur constitutively or in response to stimuli. Literature data show, in vitro, that insulin stimulates the secretion of APN. In contrast, studies have found a correlation between the decrease of adiponectin RNAm levels with increasing insulin. A study by Delporte et al. evaluated the relationship between β-adrenergic agonists and circulating levels of APN and indicated an association between catecholamines and insulin resistance, since the β-adrenergic agonists and analogues of cyclic adenosine monophosphate (cAMP) inhibit the production and secretion of APN. Therefore, in that respect, there is inconsistency in the scientific literature.

TNF-α greatly reduces the synthesis and secretion of APN through the suppression of the APN gene promoter. Besides TNF-α, glucocorticoids, IL-6 and endothelin inhibit APN production.

**Receptors, interaction and regulation**

The APN actions are mediated by two specific types of receptors: AdipoR1, which has high affinity for globular APN and low affinity for APN of high molecular weight; and AdipoR2, with intermediate affinity for both forms of APN.

AdipoR1 is abundantly expressed in the skeletal muscle, while AdipoR2 has higher expression in the liver. In the skeletal muscle, the AdipoR1 actions are mediated by peroxisome proliferator-activated receptor (PPAR), and by activated adenosine monophosphate kinase (AMPK), stimulating the uptake of glucose and β-oxidation. The regulation of the APN activity in both AdipoR1 and AdipoR2 are mediated by the action of the protein adapter protein containing PH domain, PTB domain, and leucine zipper motif (APPL) that has two isoforms: APPL1 and APPL2.

APPL2 negatively regulates the signaling of APN in the skeletal muscle. However, APPL1 is the only one that interacts with activated protein kinase (Akt), mediating the insulin sensitizing effects by the APN. The APPL2 binding to AdipoR1 hinders the action of APPL1, thus inhibiting AMPK activation and the activation of mitotic activator protein kinase (MAPK). APPL2 inhibits the sensitizing effect of insulin by APN in the muscle skeletal cells.

In addition to its expression in well-documented tissues, evidence in rats indicate that AdipoR are abundantly expressed in the brain, especially in the hypothalamus, brainstem and endothelial cells. During the differentiation period of pre-adipocytes 3T3-L1, APN receptor expression increases significantly compared to non-differentiated adipocytes, with a 1.5-fold increased expression of AdipoR1 in a nine-day period, while AdipoR2 increases expression four to five times from the sixth through the ninth day. The biological mechanisms and the expression of APN receptors in tissues are not yet completely understood and require further studies.

The literature reports that several hormones promote insulin resistance, including the growth hormone (GH). However, the molecular mechanisms involved in this process are still unclear and require elucidation. Interestingly, it has been demonstrated that GH treatment increases AdipoR2 expression in differentiated 3T3-L1 adipocytes by 2.4 times compared to untreated cells. However, the removal of GH during the 24-hour period decreased expression of APN type-2 receptors compared to untreated cells. These findings suggest that AdipoR2 may be responsible for mediating some of the metabolic effects of GH in the adipose tissue. Furthermore, upregulation of AdipoR2 by GH possibly contributes to the improvement of insulin sensitivity, promoted by the APN in the adipose tissue. The cellular and molecular mechanisms involved in the regulation and expression of APN receptors are increasingly clear. However, new studies are required to clarify these mechanisms.

Some of the molecular mechanisms involved in the improvement of insulin sensitivity by the APN action are related to the suppression of hepatic gluconeogenesis and regulation of fatty acids metabolism via activation of AdipoR1 and AdipoR2, in addition to AMPK and PPARα. Interestingly enough, the study demonstrated that the actions by which the APN improves insulin sensitivity go beyond those already described and seem to involve IL-6.

IL-6 is a cytokine hitherto described as having inflammatory properties involved in insulin resistance. Study with rodents reported that acute elevation of IL-6 contributed to the improvement of insulin resistance. In an attempt to further elucidate this finding, researchers described the pathway in which the APN regulates the expression of type-2 insulin receptors (IRS-2) in the liver tissue through mechanisms involving IL-6 and signal transducer and activator of transcription-3. It is believed that regulation of IRS-2 by APN suppresses gluconeogenesis, but does not increase lipogenesis. In addition to these findings, the authors suggested the existence of a new APN receptor activated by a pathway involving the signal transducer and activator of transcription-3 and IL-6.
Recent progress in APN point out to a future apparently more promising than expected, suggesting that the adipocyte-derived protein is involved in more complex metabolic processes. In fact, according to Semple et al.\textsuperscript{45}, APN levels in patients with severe insulin resistance provides a simple and inexpensive means to discriminate the loss of function of APN receptors, that is, normal or high levels of the protein present a major predictive value for impairment or loss of function of insulin receptors\textsuperscript{45}. The explanation for this association appears to involve mutation of Akt2, a key protein for insulin receptor transduction signals.

In its action in regulating lipid levels, studies show that reductions in APN circulating levels are related to accumulation of lipids in the liver, as well as increase in circulating levels of very low density lipoproteins (VLDL) and chylomicrons\textsuperscript{46-50}.

Another study shows that APN increases the expression of apolipoprotein-A and lipase activity, both important in the catabolic process of triglyceride-rich lipoproteins, both in the adipose tissue and in the skeletal muscle. The same study shows that decreased levels of APN promote increased hepatic lipase activity by lowering high-density lipoprotein levels (HDL), with a consequent increase in the levels of dense and small low-density lipoproteins (LDL)\textsuperscript{51}.

**Cardiovascular actions**

Over the past few years, studies have indicated the importance of APN in human diseases, including metabolic and cardiovascular disorders\textsuperscript{52-54}. In fact, an increasing number of epidemiological investigations based on different ethnic groups have repeatedly documented a close connection between APN deficiency and the development of nearly all stages of vascular disease\textsuperscript{54}. In this context, hypoadiponectinemia has been described as a significant predictor of endothelial dysfunction in coronary and peripheral arteries, regardless of body mass index, insulin resistance and dyslipidemia\textsuperscript{52-55}.

At vascular levels, APN plays an anti-inflammatory action due to the following mechanisms: inhibition of activation of kappa B nuclear transcription factor (NF-kB), attenuation of the expression of adhesion molecules induced by TNF-\(\alpha\), induction of the production of anti-inflammatory cytokines such as IL-10 and IL-1 receptor antagonist on monocytes and macrophages and the suppression of the production of interferon alpha (IFN-\(\alpha\)) by macrophages stimulated by bacterial lipopolysaccharide\textsuperscript{56,57}.

The mechanism that causes endothelial dysfunction occurs due to decreased production of nitric oxide and/or increased production of vasoconstrictors such as endothelin-1 (ET-1) and angiotensin II\textsuperscript{58,59}. Associated with decreased production of nitric oxide is inflammatory activation with the promotion of the synthesis of pro-inflammatory cytokines (TNF\(\alpha\), IL-8). These mechanisms promote increased adhesion molecule expression such as VCAM-1, ICAM-1 and E-selectin and, consequently, migration and binding of monocytes into the intima of the vessel. When monocytes reach the vascular intima, they are transformed into macrophages and express Class A scavenger receptors, which recognize oxidized low-density lipoproteins. This biochemical recognition favors the phagocytosis of low-density oxidized lipoprotein molecules and the resulting formation of foam cells, which subsequently form the necrotic core of atheromatous plaques\textsuperscript{56,60,61}.

The APN inhibits the expression of class-A scavenger receptors which, therefore, reduces the intracellular accumulation of lipids, decreases the action of acyl-CoA cholesterol aciltransferase1 — whose function is to catalyze the formation of cholesterol ester — and induces the secretion of anti-inflammatory cytokines such as IL-10, through macrophages\textsuperscript{52-54}. According to Kumada et al.\textsuperscript{64}, APN plays a role in the stabilization of atherosclerotic plaque when it stimulates metalloproteinase-1 inhibitor expression by inducing secretion of IL-10 by macrophages\textsuperscript{64}. Furthermore, the APN suppresses heparin-binding EGF-like growth factor in endothelial cells activated by TNF-like growth factor by inducing secretion of IL-10 by macrophages\textsuperscript{64}.

In recent years, there have been important changes in the understanding of the pathophysiological mechanisms related to heart function, beyond the traditional concepts involving hemodynamic, neuroendocrine and immune dysfunctions. Innovative studies have proposed interesting molecular mechanisms connected to the action of adipokines in cardiac function, including the APN\textsuperscript{56-69}.

At first, it was believed that this protein was exclusively secreted by adipocytes. Interestingly, in experimental studies conducted in humans and mice, it was shown that the APN is also secreted by cardiomyocytes, though in lower amounts compared to adipocytes\textsuperscript{16}. Worthy of note is that the APN produced by cardiomyocytes appears to directly regulate the cardiac metabolism through autocrine and paracrine actions by molecular pathways involving Adipok1 and AdipoR2 receptors and AMPK activation\textsuperscript{16,70}. Although there is no consensus as to the APN actions in cardiac pathophysiology, it is
known that circulating levels of APN are closely connected with both cardiovascular disorders\textsuperscript{71,72} and protection\textsuperscript{73,74}.

It has been recently suggested that the APN would be capable of influencing cardiac remodeling by regulating the AMPK\textsuperscript{75}. In fact, it would not be difficult to accept such a suggestion if it is considered that, in animal studies, hypoadiponectinemia results in concentric hypertrophy characterized by a decreased AMPK activity, increased regulated kinase by extracellular\textsuperscript{r75} signal and decreased production of vascular endothelial growth factor (VEGF)\textsuperscript{76}. In contrast, increased AMPK activity by APN\textsuperscript{75} has been described for inhibiting the action of EF2, extracellular signal-regulated kinase and for increasing the production of VEGF\textsuperscript{76}, possibly reverting and/or decreasing concentric cardiac hypertrophy.

Although in human studies the mechanisms that connect APN to cardiac remodeling are unclear, the results suggest that hypoadiponectinemia represents an important starting point for heart disease. In normotensive healthy individuals with normal ventricular function, plasma levels of APN have an inverse association with the left ventricular mass\textsuperscript{7,77}. In agreement with these results, the study by Jakson\textsuperscript{78} observed an inverse relationship between plasma levels of APN and left ventricular mass in normotensive black non-insulin-resistant volunteers. However, this association did not achieve significance after multivariate adjustment for obesity. However, in hypertensive individuals resistant to insulin, left ventricular mass was directly associated with plasma APN, suggesting that APN has a different prognostic value depending on the metabolic profile and risk factors associated\textsuperscript{79}.

It seems to be true that metabolic disorders represent a strong point in the pathophysiology of cardiac remodeling. In the study by Rutter et al.\textsuperscript{79}, glucose intolerance and insulin resistance were significantly related to increased left ventricular mass in women. In contrast, in men, the results were not as clear\textsuperscript{79}. Similar results were obtained in the study by Henry et al.\textsuperscript{80}. Taking these data together, it can be suggested that APN represents an important link between metabolic disorders and dysfunctions connected to the pathophysiology of cardiac remodeling, whereupon hypoadiponectinemia is, therefore, a possible predictor of cardiac hypertrophy in humans.

As research involving APN evolve, the molecular mechanisms that connect it to cardiac disorders become even more fascinating. An interesting study by Fujita et al.\textsuperscript{74} demonstrated that angiotensin II promotes cardiac fibrosis in rats with APN deficiency. The mechanisms involved in this process involve decreased AMPK activity, increased kinase activity regulated by extracellular signal and increased production of reactive oxygen species, which possibly favors the deterioration of cardiac function. However, in the same study, animals with APN deficiency, when treated with the same protein, were protected against cardiac fibrosis induced by the action of angiotensin II. The authors concluded that the APN protects against cardiac fibrosis and consequent muscle dysfunction, at least in part, through activation of molecular pathways involving AMPK and PPAR-\(\alpha\)\textsuperscript{75}.

In this review, the APN is an adipocytokine mainly secreted by adipocytes and has anti-inflammatory and ateroprotector effects in the vascular tissue as well as in the containment of myocardial remodeling. It also sensitizes insulin in skeletal and cardiac muscle tissues as well as in adipose tissue with an important action in the control of blood glucose and lipid levels. Thus, hypoadiponectinemia is considered an important biomarker for the development of cardiovascular diseases and metabolic disorders.

\textbf{Potential Conflicts of Interest}\nThis study has no relevant conflicts of interest.

\textbf{Sources of Funding}\nThis study had no external funding sources.

\textbf{Academic Association}\nThis study is not associated with any graduate programs.
References


Adiponectin: Metabolic and Cardiovascular Action


