Section A-Research paper

Serum Adropin Levels in Hemodialysis Patients

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Abstract

Adropin is a novel pleotropic peptide which is encoded by the ENHO gene whose expression was found in the liver and brain, but its presence was also established in the muscle, heart, pancreas, and kidneys. However, studies have showed that adropin has a wide range of diverse effects, among which the most prominent one is maintaining energy homeostasis through glucose and lipid metabolism regulation. Since CKD/HD settings are associated with a high cardiovascular risk, hyperlipidemia, hyperinsulinemia, chronic low-grade inflammation, and malnutrition, it is reasonable to presume a potential link with adropin. A recent study conducted on HD patients determined that serum adropin levels are lower in HD patients compared to healthy controls. However, two other studies did not find any significant difference between HD patients and healthy controls. There is growing evidence demonstrating that circulating adropin levels depend upon diet preferences. It was shown that in women but not in men, serum adropin concentration positively correlated with fat intake.

Keywords: Adropin Levels, Hemodialysis Patients

In 2008, It was identified a new hormone called adropin. The authors of this pioneer work studied expression of genes in C57BL/6J (B6) melanocortin-3 receptor-deficient (Mc3r−/−) mice, which allowed for identification of an unknown liver transcript downregulated in obesity. Using bioinformatics and molecular biology it was found that this transcript encodes a secreted protein that was termed adropin (this name originated from Latin “aduro” which means “to set fire to” and “pinquis” which means “fats or oils”) **(1).**

Secreted adropin protein is composed of 43 amino acids, and is produced by proteolytic cleavage of 76 amino acid precursors. Notably, the amino acid sequence of adropin is highly conserved among the species and is identical in rat, mouse, human, and pig. Unfortunately, the plasma half-life of adropin is still unknown and remains to be determined.Adropin is encoded by the energy homeostasis-associated gene (Enho), which is expressed mainly in the brain and the liver. However, it is also detected in peripheral tissues such as in the heart, lung, kidney medulla, muscles, peripheral blood mononuclear cells, and breast cancer cells **(2).**

Furthermore, adropin protein is present in the circulatory system of animals and humans**,** and the biological effects of adropin are mediated via direct interaction with the G protein-coupled receptor—GPR19 receptor **(2).**it is . found that in rats, adropin suppresses water intake via activation of GPR19 in the brain **(2).**

Moreover, it was found that adropin modulates E-cadherin expression in breast cancer cells via activation of GPR19. In addition, another study reported that adropin modulates pyruvate dehydrogenase in cardiac cells in a GPR19- dependent manner However, it should be pointed out that a recent study failed to confirm an interaction of GPR19 receptors with adropin. Nevertheless, it must be noticed that there is evidence indicating that adropin is a plasma membrane protein modulating physical activity and motor coordination

via NB-3/Notch signaling in the brain. Thus, adropin may represent a protein with multiple functions, acting as a secreted factor and/or membrane protein **(3).**

Functions of Adropin

Adropin enhances glucose oxidation and ameliorates metabolic inflexibility of utilizing glucose in obese and insulin-resistant mice. The underlying mechanisms appear to involve suppressions of carnitine palmitoyltransferase-1B (CPT-1B) and CD36, two key enzymes in fatty acid utilization. Adropin treatment activates pyruvate dehydrogenase (PDH), a rate-limiting enzyme in glucose oxidation, and downregulates PDH kinase-4 (PDK-4) that inhibits PDH. Adropin can up-regulate the endothelial nitric oxide synthase (eNOS) expression through VEGFR2-PI3K-Akt or VEGFR2-ERK1/2 pathway, increase the release of NO, improve endothelial cell function, and promote the neovascularization, thereby protecting the cardiovascular system **(4)**.

In recent years, the role of adropin in the central nervous system (CNS) has also been studied. It has been shown that adropin acts as a plasma membrane-binding protein in CNS, interacts with brain specific Notch1 ligand NB3, regulates physical

activity and motor coordination through the NB3/Notch signaling pathway, and plays a pivotal role in cerebellum development in mice **(5)**.

It also exerts neuroprotective effects by reducing oxidative damage. In studies of the association of adropin with atherosclerosis and insulin resistance, in addition to its role in regulating metabolism and improving functions of endothelial cells, the immunological effects of adropin have gradually attracted scholars' attention **(3)**.

Metabolic Disorders Caused by the Immune Regulation of Adropin:

Obesity results from a persistent energy imbalance. Adipose tissue is increasingly considered as a key regulator of energy balance and is a "crossroad" of energy homeostasis, inflammation, and atherosclerosis

If the number of free fatty acid (FFA) exceeds the storage capacity of the adipose tissue, it may overflow and may be accumulated in metabolic tissues, such as skeletal muscle, liver, and pancreas; excessive FFA can activate inflammatory pathways and damage immune system and adipose tissues, thereby leading to cell dysfunction.Therefore, fatty acid can regulate the function and inflammation phenotype of immune cells, playing a substantial role in causing metabolic disorders, such as insulin resistance and type 2 diabetes. Numerous studies demonstrated that visceral adipose tissue is associated with macrophages in chronic inflammatory conditions around the adipocytes, and infiltration of visceral adipose tissues by proinflammatory macrophages is a key event driving adipose-tissue inflammation and insulin resistance (**6**).

The macrophages in the adipose tissue are the main source of inflammatory cytokines, such as tumor necrosis factor α (TNF-α), a multifunctional proinflammatory cytokines that plays a significant role in the inflammatory process. The fat content is positively correlated with the number of macrophages, and the ablation of adipose tissues leads to a decrease in systemic inflammation. Adropin can regulate the expressions of lipogenic genes and peroxisome proliferator-activated receptor γ (PPAR-γ) in the adipose tissues and liver, and is a main regulator of lipogenesis as well. Besides, PPAR-γ was found to be significantly decreased in mice with overexpression of adropin**.** adropin promotes the proliferation of 3T3-L1 preadipocyte via mediating ERK1/2 and AKT, and inhibits differentiation of preadipocytes into mature adipocytes by reducing lipid accumulation and expressions of adipogenic genes in 3T3-L1 cells and rat preadipocytes. Thus, adropin can reduce macrophage infiltration by decreasing fat accumulation, thereby improving inflammation **(7).**

Association between Adropin and Other Inflammatory Diseases:

In addition to metabolic disorders and cardiovascular diseases, adropin has been shown as a potential antiinflammatory factor in other inflammatory diseases. It is well known that Treg cell is a subset of T cells that control autoimmune reactivity, and their deficiency can lead to autoimmune diseases. In the lung tissues of AdrKO mice, the number and ratio of Treg cells were found to be significantly reduced. At the same time, there was a sharp increase in CD3, CD20, and CD38 positive cells in the lung tissues of AdrKO mice. The neutrophil recruitment and neutrophil- endothelial cell interactions caused by ENHO mutation/adropin deficiency were associated with lung injury related to MPO-ANCA. It was previously revealed that adropin can inhibit hepatic cell inflammation in hyperlipidemia rats. In AdrKO mice, the more the accumulation of hepatic lipid, the more severe the inflammatory response, and the expressions of inflammation- related genes (IL1b, IL6, and Tnf) were remarkably elevated **(8)**.
This may be attributed to the regulatory effects of adropin on the accumulation of hepatic lipid. However, it also suggested that in a variety of inflammations, various tissues, and even blood, the level of adropin is associated with inflammation- related genes (especially IL6 and Tnf). In patients with knee osteoarthritis, the level of adropin is negatively correlated with TNF-α level, white blood cell (WBC) count, and neutrophil-lymphocyte ratio (NLR) **(9)**.
The underlying mechanism may be related to the upregulation of eNOS activity by adropin, and the produced NO can negatively regulate inflammatory mediators. Furthermore, it can impede the leukocyte extravasation and movement process regulated by TNF-α, thereby applying its anti-inflammatory effects **(10)**.

Adropin has an antioxidative stress effect. Study has shown that adropin deficiency correlates with increased oxidative stress associated with endothelial dysfunction in the brain of rats **(11).**
Meanwhile, adropin can activate ERK 1/2 through VEGFR2, and ERK 1/2 activation induces Nrf2 and protect neurons from oxidative stress. Inhibition of ERK 1/2 may reduce DNA repairing ability, accelerate cell apoptosis, and aggravate neuron loss **(12).**
The antioxidative stress effect of adropin is also related to its immune regulation function. Adropin activates Nrf2 signaling in nonalcoholic steatohepatitis (NASH) and plays a role in decreasing reactive oxygen species (ROS) production from liver mitochondria. So, it may protect mitochondrial function to alleviate oxidative stress and apoptosis and thus protect against liver injury and prevent the NASH progression (**13**).
Excessive reactive oxygen production can cause inflammation. The study indicated that the increase of oxidative stress in a fatty liver caused the apoptosis of Tregs, reduced the number of hepatic Tregs, and led to a lowered suppression of inflammatory responses. This is because increased fatty acid metabolism leads to increased mitochondrial respiratory activity and excessive production of mitochondrial ROS in the liver, which can reduce the expression of bcl-2 in Tregs and selectively affected a subpopulation of T lymphocytes (Tregs) **(14).**

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Adropin is a novel pleotropic peptide which is encoded by the ENHO gene whose expression was found in the liver and brain, but its presence was also established in the muscle, heart, pancreas, and kidneys. However, studies have showed that adropin has a wide range of diverse effects, among which the most prominent one is maintaining energy homeostasis through glucose and lipid metabolism regulation **(15).**
In a study by **Kumar et al., (16**) it was presented that mice with dietary induced obesity have a higher glucose tolerance and reduced insulin resistance after peritoneal treatment with adropin. Another study conducted on obese patients and healthy controls showed that serum adropin levels were lower in participants with obesity and insulin resistance whereas lower body mass index (BMI) was linked with the rise of serum adropin levels **(2).**
Additionally, a recent study showed that adropin treatment downregulated the expression of gluconeogenic regulatory enzymes in the liver which consequently led to inhibition of hepatic glucose production and

improved hepatic insulin sensitivity. Growing evidence also suggest that adropin possibly plays a significant role in the cardiovascular system. Several studies pointed to a possible association between adropin and blood pressure, neovascularization, and vascular protection, while it was indicated that adropin induces production of nitric oxide by regulating endothelial nitric oxide synthase and vascular endothelial growth factor receptor 2 (VEGF2) **(17)**.

Moreover, it was found that patients with coronary heart disease have low serum adropin levels compared to the healthy controls and **Yu et al., (18)** also showed that a decrease of serum adropin levels in patients with coronary artery disease could predict the incidence of acute myocardial infarction. Furthermore, the most recent studies are linking adropin with chronic inflammatory states and are proposing a possible immunomodulatory effect. It has been showed that patients with obstructive sleep apnea, inflammatory bowel diseases, polycystic ovary syndrome and diabetes have significantly lower serum levels of adropin.The perceived link is still not understood but it is possible that endothelial dysfunction is the main driver for the serum adropin levels reduction in these disorders. Since CKD/HD settings are associated with a high cardiovascular risk, hyperlipidemia, hyperinsulinemia, chronic low-grade inflammation, and malnutrition, it is reasonable to presume a potential link with adropin. A recent study conducted on HD patients determined that serum adropin levels are lower in HD patients compared to healthy controls. However, two other studies did not find any significant difference between HD patients and healthy controls **(19).**

Modulation of Adropin by Body Mass Index (BMI), Diet, and Diabetes

Serum adropin levels are upregulated in mice fed a high-fat diet for 48 h (**4**). In contrast, in mice with high-fat diet-induced obesity, serum adropin levels are low ((<1 ng/mL). An inverse correlation of adropin levels and body mass index (BMI) was also confirmed by human studies, suggesting that a low level of adropin is a hallmark of obesity **(5)**.

In addition, serum adropin levels are also affected by sex. For example, women have lower circulating adropin levels as compared to men. Furthermore, it

was shown that in men, but not in women, circulating adropin is negatively associated with low-density lipoprotein (LDL) cholesterol levels **(2).** Interestingly, it was shown that glucose consumption suppresses adropin levels in circulation while fructose supplementation has an opposite effect. Importantly, stimulation of adropin by fructose was more pronounced in humans with higher triacylglycerol levels, suggesting a role of lipids in regulating circulating adropin **(2).**

Recently, it was shown that low levels of circulating adropin can be used as a predictor of body weight gain and metabolic dysregulation in Rhesus macaques challenged with a high fructose diet **(2).**

Animals with low adropin levels had higher fasting glucose as well as leptin levels in response to a fructose challenge. Additionally, the same study showed an inverse correlation between serum adropin levels and apolipoprotein C3 in animals fed a highfructose diet **(2).**

It is worth noting that high levels of apolipoprotein C3 are positively correlated with plasma triacylglycerol**(1)**. Consistently, increased levels of apolipoprotein C3 in animals with low levels of adropin, which were fed a high-fructose diet, were accompanied by more severe hyperglycemia **(2).**

There is growing evidence demonstrating that circulating adropin levels depend upon diet preferences. It was shown that in women but not in men, serum adropin concentration positively correlated with fat intake. In addition, humans with low adropin levels consume more carbohydrates (simple and complex carbohydrates). Nevertheless, it is unknown whether diet affects adropin levels or whether adropin influences nutritional habits. Therefore, an association of adropin with diet preferences needs to be discussed cautiously **(20).**

Role of Adropin in Endothelial Dysfunction

It has been well-established that the endothelium plays a central role in the maintenance of vascular homeostasis, and that impairment of endothelial function contributes to the development and progression of various pathologies, most notable with regard to the cardiovascular system **(8)**.
eNOS bioavailability is the most important representative of endothelial function. The former is regulated by at least three distinct mechanisms: transcriptional upregulation of eNOS, posttranscriptional activation of eNOS, and reduction in ROS-mediated breakdown of nitric oxide (NO) **(5)**.
Adropin exerts multiple effects in all of the three most important vascular cells participating in the pathogenesis of atherosclerosis (endothelial cells, macrophages and vascular smooth muscle cells (VSMCs)). It has already been discussed how adropin affects endothelial cells by alternating eNOS expression **(8)**.
Respecting the effects of adropin on NO metabolism, it was examined whether adropin has a role in blood pressure regulation. Multiple authors reported higher adropin levels in hypertensive patients compared with normotensive control **(21)**.

However, although chronic infusion of adropin at 5 μg/kg/h did not significantly impede the increase in aortic atherosclerotic lesion area and atheromatous plaque size, chronic infusion of adropin at 10 μg/kg/h significantly reduced the aortic atherosclerotic lesion area with a tendency to decrease the plaque size, as well as significantly decreasing the intra-plaque monocyte-macrophage and VSMC contents **(22).**

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