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Research Paper

The Effect of Physical Exercise on Expression of Endogenous Bioactive Peptides with Pro-and Anti-Atherogenic Properties

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Abstract

The prevalence of atherosclerotic cardiovascular disease (ASCVD) and its complications have increased substantially in recent decades. ASCVD is associated with various cardiovascular conditions and indirectly contributes to a high mortality rate in the general population. Salusin-α and salusin-β are two endogenous bioactive peptides that could serve as candidate biomarkers for ASCVD. Salusin-α protects against the development of ASCVD, and a decrease in its levels is associated with ASCVD. While salusin-β plays a role in the development and maintenance of ASCVD, with its exaggerated expression in atherosclerotic lesions, changes in people's lifestyles, especially sedentary behavior and lack of exercise, are recognized as critical risk factors for cardiovascular disease. Consequently, physical exercise (PE) has recently been identified as an effective strategy for lowering cardiovascular disease risk. In this review, we summarize the current knowledge on the effects of PE on ASCVD through the modulation of the expression of endogenous bioactive peptides with pro- and anti-atherogenic properties.

Keywords: Atherosclerosis, Cardiovascular Disease, Salusin-α, Salusin-β, Physical Exercise

Introduction

ASCVD is a complex, multifactorial disease that has been the leading cause of mortality and morbidity worldwide for the past 50 years. In recent years, ASCVD has become a chronic epidemic disease. [1]. Over the past years, ASCVD has become a chronicle epidemic disease [2]. Global reports of ASCVD from 1990 to 2019 showed that in 2019, nearly 197 million people suffered from ASCVD, and about 9.14 million people died due to ischemic heart disease [2].

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Despite significant progress in the prevention and treatment of ASCVD, it remains the leading cause of death worldwide and there still are challenges to be addressed.

Many factors affect ASCVD [3]; however, studies have emphasized the influence of the serum concentrations of the small peptide molecules salusin-α and salusin-β [4-6]. A reduction in the serum salusin-α concentration or an increase in the serum salusin-β concentration promotes the progression of ASCVD [7]. Salusin-α exhibits anti-atherogenic effects, as it reduces atherosclerotic plaques, and its expression is decreased in patients with hypertension and lipid profile disorders [8,9]. Conversely, salusin-β plays a pro-atherogenic role. The available literature has evaluated salusin-α and β as predictors for ASCVD, and salusin-β seems to be a better indicator of ASCVD development than salusin-α [8].

The mechanisms underlying the relationship between PE and health are numerous, with new evidence continually emerging to highlight additional benefits. PE can independently reduce the risk of ASCVD and has a positive, intensity-related impact on other cardiovascular risk factors, such as hyperlipidemia, hypertension, abdominal obesity, diabetes, and psychosocial factors . Additionally, there is a positive correlation between PE intensity and its protective effects against ASCVD. [13,14].

Although, PE had been commended by lots of guidelines and expert consensus for its prevention and protection effects on ASCVD, its underline mechanisms were still not well understood. Several beneficial effects that acted on ASCVD had been found. In this review, we aimed to summarize the current knowledge on the recent developments in salusins research, their emerging roles as promising biomarkers and therapeutic targets for ASCVD, and discusses on the effects of PE on ASCVD through modulation of expression endogenous bioactive peptides with pro-and-anti-atherogenic properties.

What is Salusins?

Salusins are two endogenous multifunctional bioactive peptides, salusin-α and salusin-β, first identified by Shichiri et al. (2003) from a human full-length enriched cDNA library [4]. Salusin-α, consisting of 28 amino acids, and salusin-β, consisting of 20 amino acids, are present in human plasma and urine, suggesting their possible role as peptide hormones derived from an alternative splicing product of the torsion dystonia related gene (TOR2A) [4]. Although

these two low-molecular-weight peptides have various functions [5,15,16], play important roles in ASCVD and cardiovascular disease [4,5,15].

Further studies have shown that salusin-α suppresses human foam cell formation by down-regulation of acylcoenzyme A: cholesterol acyltransferase-1 (ACAT-1), which promotes cholesterol ester accumulation in macrophages and helps prevent the progression of ASCVD [6-9,15]. The regulation of ACAT-1 expression by salusins was mediated through the G-protein/c-Src/PKC/MAPK signaling pathway [17]. Serum salusin-α levels are decreased in patients with coronary artery disease compared with healthy volunteers [8,9]. Further, Serum salusin-α has also been shown to be associated with ASCVD and left ventricular diastolic dysfunction in essential hypertension [7]. These data suggest that salusin-α, as an anti-atherogenic peptide, may contribute to the prevention of ASCVD progression . In contrast, salusin-β is a pro-inflammatory agent that promotes human foam-cell formation by up-regulation of ACAT-1 [6-9,15], and stimulates the proliferation of vascular smooth muscle cells (VSMCs) [4], which are key events in ASCVD. Therefore, salusin-β is considered to be a potential pro-atherogenic factor [9].

Biosynthesis of salusins

Using bioinformatic analysis of human stem cell-derived full-length cDNA provided sequences of an alternatively spliced product of TOR2A were identified, which can lead to an endogenous bioactive peptide precursor with the potential to become preprosalusin [4,9,15]. TOR2a has 5 exons, is located at 9q34.11, and encodes a protein of 321 amino acids [18] Its splice variant, preprosalusin (PSEC0218, HEMBA 1005096, AK075520), contains a frameshift resulting from the deletion of exon 4 that results in a deduced 242-amino acid protein with an alternate C terminus [4]. Preprosalusin has 242-amino acid sequences, and after removing 26-amino acids of the signaling peptide at the N-terminal, prosalusin is produced, consisting of 216 amino acids. [4]. Prosalusin is a commonly occurring alternatively spliced product of the TOR2A gene, which has structural homologies to torsion dystonia genes (DYT1 and DQ1) [18]. The proteolytic processing of prosalusin at the C-terminal causes the biosynthesis of two peptides of 28 and 20 amino acids called salusin-α and salusin-β [4]. Carboxypeptidase E likely removes the resultant C-terminal dibasic amino acids between salusin-β and salusin-α to create the 20-amino acid salusin-β [4]. Peptidylglycine α-amidating monooxygenase converts the glycine residue at the C-terminal end to NH_2, yielding the 28-amino acid salusin-α with C-terminal amidation [4].

Biological Functions of Salusins in Cardiovascular System

The biological functions of these two endogenous bioactive peptides, with pro-and-anti-atherogenic can be generally classified into systemic and local activities. Prominent systemic bioactivities of salusin-β include its potent hemodynamic effects. Salusin-β induces rapid and transient hypotension, bradycardia, and cardiac dysfunction by a cholinergic mechanism [19]. It promotes cardiomyocyte growth and anti-apoptosis [20] and has anti-apoptotic effects but does not have a direct vasodilator effect. [4,19]. These effects are mediated mainly by parasympathetic stimulation rather than direct suppression of cardiac contraction, as they are almost completely abolished by pretreatment with atropine sulfate [19]. However, in addition to its potent effects in facilitating vagal outflow to the heart, salusin-β also exerts negative inotropism through a direct myotropic effect [19]. Since salusin-β is found in its native form in the human circulation [21], it likely contributes to the maintenance and/or regulation of cardiovascular homeostasis. on the other hand, Salusin-α may also lower blood pressure, but to a much lesser extent: Systemic hemodynamic effects of salusin-α are not as evident compared to salusin-β [4].

In contrast to their systemic actions, the small peptide molecules salusin-α and salusin-β exert different local activities in the cardiovascular systems. Salusins can induce intracellular signaling and cellular responses. Salusin-β stimulates human macrophage foam cell formation, as well as the proliferation of vascular smooth muscle cells (VSMCs) and fibroblasts [4,17]. The proliferation of VSMCs and vascular fibrosis are closely linked with many clinical diseases including ASCVD, hypertension and diabetes, as well as their associated target organ damage [22]. In contrast, salusin-α reduces macrophage foam cell formation and has minimal mitogenic effects on VSMCs and fibroblasts [4,17]. Salusins have been shown to promote the growth of cardiocytes [20] and protect against their apoptotic death [23]. Specifically, salusin-β increases intracellular free Ca^{2+} by facilitating its influx through voltage-dependent Ca^{2+} channels and stimulates the release of vasopressin and oxytocin from the posterior pituitary in an autocrine/paracrine manner. [24]. Salusin-α and salusin-β stimulate protein synthesis through several signaling pathways, including calcium, calcineurin, mitogen-activated protein kinase (MAPK), and protein kinase C (PKC). These findings indicate that salusins have distinct functions in the cardiovascular system.

Salusins and ASCVD

Salusins play important roles in ASCVD. Despite the clear effects of salusin-α and salusin-β on ASCVD [4,5,15], further studies are needed to explore their underlying mechanisms. Early research has identified salusins as multifunctional hemodynamic regulators. [4]. Studies have shown that the concentration of salusin-α in patients with ASCVD is significantly lower compared to healthy individuals, while the concentration of salusin-β is higher [25,26]. Salusin-β can accelerate the development of ASCVD, while salusin-α does not have this effect [27,28]. Other reports indicate that salusin-α inhibits foam cell formation from monocytes, whereas the most pronounced pro-atherosclerotic effect of salusin-β is its ability to promote the formation of macrophage foam cells [15,17]. These studies suggest that salusin-α and salusin-β are associated with ASCVD and support their clinical application. Decreases in serum salusin-α levels have been observed in various human cardiovascular diseases, such as hypertension [7] and chronic renal failure [29]. Clinical investigations have shown that serum salusin-α concentrations are lower in patients with mild hypertension compared to in healthy subjects. This reduction in salusin-α levels may contribute to the development of mild carotid ASCVD. [30,31]. In patients with acute carotid syndrome, serum concentrations of salusin-α are inversely correlated with the severity of coronary ASCVD [17,32]. However, salusin-β plays a role in the hemodynamic regulation and pathogenesis of ASCVD in cardiovascular diseases. Studies have shown that elevated salusin-β serum concentrations are associated with the severity of cardiovascular disease [33,34]. This relationship between salusin-β and ASCVD has also been confirmed in patients with coronary artery disease (CAD). A study comparing patients undergoing transcatheter therapy found that salusin-β levels were significantly higher in patients with cardiovascular disease (CVD) before therapy compared to healthy controls [35]. Moreover, after therapy, salusin-β expression was significantly lower compared to pre-intervention levels and to the control group. Additionally, salusin-β levels were significantly higher in patients with stenosis or dilation observed in coronary angiography than in healthy volunteers [36,37]. A similar finding was observed in patients with slow coronary flow, which appeared to be associated with microvascular ASCVD [38]. Therefore, salusin-α and salusin-β are potential markers for ASCVD [39].

Salusins and ASCVD Risk Factors

ASCVD is a complex, chronic, and multifaceted lifelong process of vascular change, influenced by numerous factors, which ultimately leads to cardiovascular and cerebrovascular events [40]. Low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol play crucial roles in the development of ASCVD. Increased LDL cholesterol and decreased HDL

cholesterol levels are associated with a higher risk of ASCVD. Additionally, studies have shown that elevated plasma triglyceride (TG) levels, known as hypertriglyceridemia, are also associated with an increased risk of ASCVD and cardiovascular disease (CVD). [41]. Studies have shown that serum salusin-β levels positively correlate with triglycerides (TG), LDL cholesterol, and the TG/HDL cholesterol ratio. Additionally, in dyslipidemic patients undergoing hemodialysis, a negative correlation was observed between salusin-α and LDL cholesterol, while a positive correlation was found with the HDL/LDL cholesterol ratio [43]. Nagashima et al. [15] demonstrated that intravenous infusion of salusin-α increased serum HDL cholesterol and decreased serum total cholesterol (TC) levels without affecting CD36 expression in exudate peritoneal macrophages of apolipoprotein E-deficient mice. In Grzegorzewska et al.'s study, negative correlations between salusin-α and LDL cholesterol suggest that dyslipidemic hemodialysis (HD) patients who were not treated for lipid abnormalities at the start of the study could benefit from lower LDL cholesterol levels if they have higher plasma salusin-α levels. A positive correlation between salusin α and HDL cholesterol was observed only in dyslipidemic HD patients with HDL cholesterol of 40 mg/dl or higher [43]. Moreover, CD36 expression positively correlated with the plasma level of salusin-α, which is considered as an inhibitor of ASCVD [7,9,15], and with IgG anti‐oxLDL, which can reduce the progression of ASCVD [44].

ASCVD is a chronic disease where inflammation plays a crucial role in all stages. Inflammatory molecules have been identified as markers of disease activity [45]. Sato et al. found that salusin-β can be released from THP-1 and U937 human monoblastic leukemia cell lines. Stimulation of these cells with the inflammatory cytokines tumor necrosis factor-α (TNF-α) and lipopolysaccharide (LPS) results in increased secretion of salusin-β [46]. This suggests a potential relationship between salusin-β and inflammatory reactions. Given the potential relationship between salusins and inflammation, as well as the crucial role of inflammation in the development and progression of ASCVD, it is plausible that salusins may regulate ASCVD by modulating inflammatory responses. In fact, Koya et al. have indicated that salusin-β can accelerate inflammatory responses in vascular endothelial cells through nuclear factor-kB (NF-κB) signaling [27]. It has been suggested that salusins play a role in the formation and progression of ASCVD [28]. Previous studies have demonstrated that salusin-β, a pro-atherogenic peptide, stimulates the expression of monocyte chemoattractant protein 1 (MCP-1), interleukin-1 beta (IL-1β), nicotinamide adenine dinucleotide phosphate oxidase 2 (NOX2), and vascular cell adhesion molecule-1 (VCAM-1) in endothelial cells. This stimulation facilitates monocyte adhesion to endothelial cells, contributing to the progression of ASCVD [27].

It has been shown that inflammatory cytokines and growth factors regulate Jak-2 kinase in cells expressing salusin-α in atherosclerotic lesions. Activation of Jak-2 kinase inhibits the production of preprosalusin, leading to decreased serum levels of salusin-α [47]. Additionally, salusin-α and salusin-β have opposing effects on foam cell formation [39]. In an animal study, chronic infusion of antisalusin-β antiserum and salusin-α into ApoE-deficient mice prevented the development of atherosclerotic lesions. These findings support the idea that salusin-α has an anti-atherogenic effect, in contrast to the pro-atherogenic effect of salusin-β.

PE as a Potential Non-Pharmacological strategy

The management of ASCVD significantly increases healthcare costs worldwide, raising concerns among researchers and clinicians. This has led to the search for effective long-term strategies to enhance treatment efficacy by managing conventional risk factors [48]. Physical exercise (PE) is recognized for its physiological benefits that contribute to homeostasis and overall health. PE plays a crucial role in both the primary and secondary prevention of cardiovascular disease (CVD). It can independently reduce the risk of ASCVD, with its intensity-related positive effects extending to other cardiovascular risk factors, including blood lipids, hypertension, abdominal obesity, diabetes, and psychosocial factors. The greater the adherence to appropriate exercise intensity, the more substantial the benefits one can achieve [50]. In addition, there is a positive correlation between exercise intensity and protective effects against ASCVD [50]. Sedentary individuals have a significantly higher risk of cardiovascular disease compared to those who engage in regular physical exercise (PE). For instance, a person who exercises about five times per week has approximately 50 times lower risk of cardiac-related complications than a sedentary individual [52]. In addition to its crucial role in maintaining vascular endothelial function, exercise has significant anti-inflammatory and antioxidant effects. Physical exercise is an effective clinical tool for managing chronic inflammation by increasing levels of anti-inflammatory cytokines and reducing pro-inflammatory cytokines through decreased oxidative stress [53]. Hence, PE is considered as a potential non-pharmacological strategy due to its ability to enhance the anti-inflammatory phenotype and provide protection against a variety of diseases [54].

PE and Salusins

Today, physical exercise (PE) is recognized as an integral part of medical science. There is a strong correlation between regular PE and reduced all-cause mortality. PE offers a wide range of health benefits and is considered a crucial factor in both the primary and secondary prevention of cardiovascular diseases

[56]. Research findings indicate that both aerobic and high-intensity interval training (HIIT) regimens can significantly improve levels of salusin-α, an anti-atherosclerotic agent, and salusin-β, a pro-atherosclerotic agent, after a chronic period of training. This effect has been observed in obese and overweight children and in women with overweight/obesity following 3 sessions per week over a 12-week period [13,14]. Interestingly, HIIT has been shown to produce more significant improvements compared to moderate-intensity continuous training (MICT). Cross-sectional and longitudinal studies support that engaging in intense physical activities is associated with a reduction in cardiovascular disease risk factors [58-60]. It appears that combining exercise with mulberry leaf extract is effective in controlling inflammatory indicators and ASCVD related to diabetes in elderly individuals with Type 2 diabetes mellitus (T2DM). The study results demonstrated that salusin-β and interleukin-6 levels significantly decreased in the training, training + supplement (mulberry leaf), and supplement (mulberry leaf) groups by the end of the study. Meanwhile, salusin-α levels increased significantly in these same groups. Covariance analysis further revealed that salusin-β and interleukin-6 levels were significantly lower, while salusin-α levels were significantly higher in the training, training + supplement (mulberry leaf), and supplement (mulberry leaf) groups compared to the control group. [61]. On the other hand, lifestyle interventions, including a lipid-lowering diet, increased physical activity, or administration of atorvastatin, led to changes in serum salusin-α levels and lipid profiles in dyslipidemic hemodialysis patients. Improvement in the serum lipid profile due to lifestyle changes was associated with a decrease in plasma salusin-α levels, likely due to the reduction of salusin-α up-regulation caused by dyslipidemic conditions. Conversely, an increase in salusin-α during atorvastatin treatment was attributed to a specific effect of atorvastatin on salusin-α secretion [43].

The exact mechanisms by which the levels of salusin-α and salusin-β are affected are unclear and more research is needed to solve this issue. However, the decrease in salusin-β levels and the increase in salusin-α levels is a very important finding, because it shows that PEs, especially HIIT, can be successful in reducing and increasing a key pre- to anti-atherogenic factor [13,14,57,61]. It is important to note that the reasons why HIIT is more successful than aerobic training are not completely clear, but it may be related to the higher intensity of this training model. On the other hand, the time efficiency of this training model is one of the other advantages that can be mentioned. Also, HIIT intermittent mode with rest can contribute to the enjoyment and non-uniformity of the training session [62]. Therefore, PE is positively related to the improvement of

expression of endogenous bioactive peptides with pro-and-anti-atherogenic properties.

Conclusion

These findings suggest that salusins show contrasting effects on ASCVD; salusin-α and salusin-β possess anti-atherogenesis and pro-atherogenesis, respectively. Therefore, salusins treatments, especially PE as a potential non-Pharmacological strategy, could emerge as a new line of therapy against ASCVD and its related diseases. Although no direct evidence supports the premise that PE prevents ASCVD by modifying the salusins as two endogenous multifunctional bioactive peptides, many studies have confirmed this hypothesis. It is important to note that the decrease of salusin-β, which is considered as an endogenous bioactive peptide with pro-atherogenic properties, and/or the increase of salusin-α, which is considered as an endogenous bioactive peptide with anti-atherogenic properties, caused by PE in the circulating blood and cardiovascular tissues could be a promising candidate biomarker for predicting improvement ASCVD.

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