Minireview

Impact of spexin on metabolic diseases and inflammation: An updated minireview

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Impact statement

Spexin (SPX) is a short length bioactive peptide hormone encoded by Ch12:orf39 and secreted into circulation. After its initial discovery, SPX has been shown to be involved in several metabolic processes such as food intake, body weight, energy balance, glucose and lipid metabolism, lipid storage, salt-water balance, and arterial blood pressure. It was later found to have both central and peripheral actions, and plays an essential role in the development of diseases including diabetes, non-alcoholic fatty liver disease (NAFLD), metabolic syndrome (MetS), obesity, polycystic ovary syndrome (PCOS), cardiovascular diseases (CVDs), and kidney diseases. This review covers the regulation of metabolism by SPX and discusses novel insights into the role of SPX in metabolic and inflammatory diseases. There is promise that this peptide, which decreases with these diseases, can be used as a biomarker in the diagnosis of diseases, and also act as a potential therapeutic agent for metabolic and inflammatory diseases in the near future.

Abstract

Spexin (SPX) is a 14 amino acid length peptide hormone which was discovered using bioinformatic tools. It is extensively expressed in central and peripheral tissues and secreted into circulation in response to metabolic stress. Recent studies revealed that SPX acts as a multifunctional peptide in various metabolic processes such as body weight, food intake, energy balance, glucose and lipid metabolism, lipid storage, salt–water balance, and arterial blood pressure. Endogenous SPX is sensitive to metabolic changes, and circulating levels of SPX have been shown to be reduced in chronic diseases such as obesity, diabetes, and insulin resistance. Moreover, in fish and rodent models, systemic SPX treatment has positive effects on metabolism including reduced food intake, fat mass, lipid accumulation, and inflammation, improved insulin sensitivity, energy expenditure, and organ functions which are underlying mechanisms in diseases. Taken together, these findings suggest that SPX is a potential drug target for the development of new pharmacological strategies to cure metabolic diseases. This review focuses on metabolo-protective properties of SPX and discusses novel insights into the biology and mechanism of SPX in the pathogenesis of diabetes, obesity, non-alcoholic fatty liver disease, metabolic syndrome, polycystic ovary syndrome, cardiovascular diseases, and kidney diseases, which are considerable global health problems.

Keywords: Cardiovascular diseases, chronic kidney failure, diabetes, metabolic syndrome, inflammation, spexin

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Introduction

Peptide hormones have gained considerable attention due to their role in modulating many physiological functions in an endocrine-dependent manner.[1] Spexin (SPX) is a 14 amino acid (a.a.) peptide hormone which was discovered using bioinformatic techniques in 2007.[2] SPX is extensively distributed in peripheral and central tissues.[3] In addition to its wide range of tissue distribution, studies using fish, mice, and rats have shown that SPX regulates multiple biological functions such as food intake, body weight, glucose, and lipid metabolism.[4,5]

Circulating SPX levels were found to be lower in chronic diseases like insulin resistance, obesity, and type 2 diabetes (T2D).[6–9] Meanwhile, recent reports have shown that systemic SPX treatment has beneficial effects in several metabolic disease models, including reducing lipid accumulation,[10] fat mass and insulin resistance,[11] protecting cells from metabolic stress,[12] and improving metabolic parameters.[13]

In this minireview, we discuss the role of SPX in multiple organisms and diseases. It is expected that understanding the metabolic action of SPX will provide a new insight into the treatment of chronic diseases.

Biological functions of SPX

Structure and tissue distribution

SPX, also known as neuropeptide Q, was discovered in the human genome using the hidden-Markov model, a

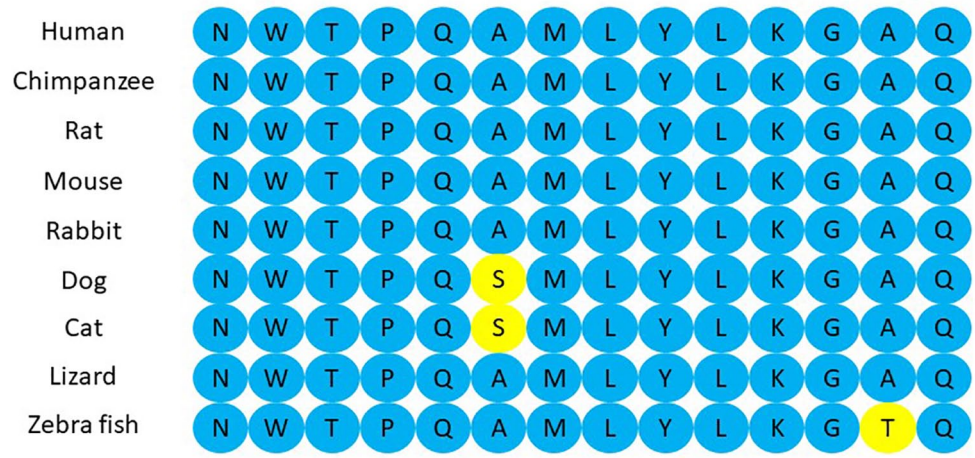

Figure 1. Amino acid sequences of SPX peptide in organisms: similar and different amino acid residues are shown in blue and yellow, respectively (A color version of this figure is available in the online journal.).
N: asparagine; W: tryptophan; T: threonine; P: proline; Q: glutamine; A: alanine; S: serine; M: methionine; L: leucine; Y: tyrosine; K: lysine; G: glycine; T: threonine.

bioinformatic approach frequently used for sequence analysis.[2,14] It is encoded by Ch12:orf39 (chromosome 12:open-reading framework 39) gene, and the highly conserved mature form of SPX is composed of a 14 a.a. after post-translational process.[2] SPX peptide sequences of different species are shown in Figure 1.

Recent studies have identified the tissue distribution of SPX in various types of organisms.[7,15] It was initially detected in mouse esophagus and stomach by *in situ* hybridization with antisense and sense probes.[2] Since then, high amounts of SPX transcripts were detected in liver, brain, ovary, hypothalamus, and thyroid in rats.[3] SPX mRNA was also found in brain, ovary, liver, and hypothalamus in goldfish.[16,17] Moreover, the presence of SPX was presented both in the central and peripheral tissues in humans.[7] Given its distribution in different tissues, SPX may be a key factor in multiple biological functions.

Mechanism of action

As a peptide hormone, prepropeptide SPX is cleaved through intracellular processes and released into circulation where it can bind to specific membrane receptors and modulate cellular function downstream of these receptors.[4] SPX exerts its function through binding to galanin receptor 2 (GAL2R) and GAL3R but not GAL1R, as determined by *in vitro* receptor–ligand interaction assay.[18] To confirm the cellular function of SPX that is mediated by GAL2R activation, the GAL2R antagonist, M871, was used in *in vitro* and *in vivo* experiments. In cardiomyocytes, impaired glucose and lipid metabolism was rescued by SPX treatment.[12] In the same experiment, it was also demonstrated that SPX treatment inhibited hypoxia-induced mitochondrial dysfunction and oxidative stress, while upon cells being co-treated with SPX and M871, these SPX-mediated positive effects were lost.[12] Supporting the conclusion that SPX activates GAL2R and modulates cellular functions, it was recently shown that high fat/fructose (HFF) diet-induced metabolic changes such as increased body weight, glucose and insulin, and homeostatic model assessment–insulin resistance (HOMA-IR) were reversed by SPX treatment.[19] While SPX improved these parameters, M871 suppressed enhancement of these metabolic markers induced by SPX.[19] Considering the cellular action elicited by SPX, much more study is needed to unveil the signaling pathways activated by SPX.

Metabolic function

Since its discovery, early studies have demonstrated the physiological function of SPX in smooth muscle contraction,[20] adrenocortical cell proliferation[21] and cardiovascular and renal modulation.[22] However, SPX was first identified in 2007, and Walewski *et al.*[13] initially revealed the metabolic functions of SPX on energy expenditure, body weight, and food intake in a rodent model. Since then, many studies have highlighted the essential role of SPX in metabolic diseases.[4,5] The metabolic diseases associated with SPX are shown in Figure 2.

Food intake

SPX plays a role in the regulation of food intake, since SPX −/− mutant zebrafish consumed less food than the wild type (WT).[23] It was also demonstrated that the mRNA levels of orexigenic agouti-related protein (AgRP) were increased in SPX −/− mutant fish compared to WT fish and these levels decreased after SPX treatment both in SPX −/− mutant and WT fish.[23] After central and peripheral SPX administration, basal, neuropeptide Y (NPY) – or orexin-induced food consumption were reduced as well as transcripts of orexigenic peptides such as NPY and AgRP in goldfish.[16] Moreover, SPX injection over 4 days resulted in decreased food intake, without any effects of SPX on taste, in female obese rats.[13] In addition, a single-dose SPX injection with different doses decreased food intake in mice during light and night period,[24] while chronic SPX injection (30 days) decreased food intake in diet-induced obese (DIO) mice.[11] These results suggested that in addition to these neural actions, SPX may act as a satiety factor. It was also demonstrated that short-term food deprivation (7 days) increased SPX mRNA levels in the hypothalamus, and this returned to baseline levels after refeeding.[25] In chicken broilers, serum SPX began to increase after 2 h fasting and a

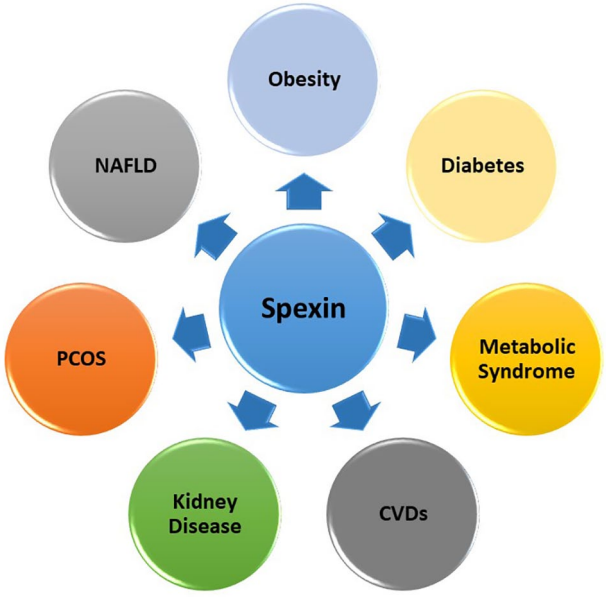

Figure 2. SPX and diseases: low SPX levels are associated with obesity, diabetes, polycystic ovary syndrome (PCOS), metabolic syndrome (MetS), non-alcoholic fatty liver disease (NAFLD), kidney disease, and cardiovascular diseases (CVDs) (A color version of this figure is available in the online journal.).

statistically significant increase was observed after 4 and 8 h.[26] Moreover, after fasting, SPX transcripts were increased in the liver and decreased in adipose tissue and muscle, which are important tissues in the coordination of energy homeostasis.[26]

Overall, SPX seems to be an orexigenic factor under negative energy balance. Further research needs to be done to define the role of SPX on food regulation and fasting conditions.

Body weight

In addition to its effect on food intake, SPX may be involved in the regulation of fat mass and body weight. For example, 19 days of SPX treatment reduced the body weight (25 µg/kg/day, intraperitoneally [i.p.]) of DIO mice.[13] Moreover, both healthy and DIO mice treated with SPX had lower body mass than their non-treated counterparts.[11] SPX also decreased fat tissue mass in healthy, DIO and T2D mice and increased lean mass only in DIO mice.[11] In addition, short- and long-term SPX treatment decreased body weight and body mass index (BMI) in rats treated with high-fructose and HFF diets.[10,19,27–29]

Glucose and lipid metabolism

Impaired glucose and lipid metabolism are associated with chronic diseases.[30] Accumulation of lipids and its metabolites in skeletal muscle, liver, and adipose tissue have been linked to insulin resistance.[31] SPX levels were lower in diabetic subjects, obese children, and adults.[6–9] Low SPX levels were observed in obese women and negatively correlated with BMI, insulin, and HOMA-IR.[32] The levels of SPX were inversely correlated with age suggesting a potential connection between SPX and age-related changes in metabolism.[33] T2D patients with high levels of glucose and hemoglobin A1c (HbA1c) had low amounts of SPX.[7] In contrast, serum SPX levels were increased after Roux-en-Y gastric bypass (RYGB) surgery and laparoscopic sleeve gastrectomy (LSG) up to 32% and 35%, respectively.[29,34]

A recent study showed that glucose-treated pig pancreatic islets showed increased SPX secretion, and this increase was negatively correlated with insulin, since insulin inhibited SPX secretion.[35] Also SPX inhibited basal and insulin-stimulated glucose uptake and lipogenesis in mouse fibroblast (3T3-L1) cells and human adipocytes.[36] To determine the effect of SPX on lipid and glucose parameters, a T2D mice model was used. It was demonstrated that cholesterol and glucose levels were lower in SPX-treated T2D mice than in the control (only T2D).[11] Moreover, the elevated liver triglyceride levels and adipocyte size were reduced after 10 days of SPX treatment in fructose-induced mice,[37] and insulin sensitivity was improved after SPX injection in DIO and T2D mice.[11] Rats with MetS had elevated BMI, fasting glucose, insulin, total cholesterol, triglycerides, and HOMA-IR, while SPX injection significantly reduced these metabolic parameters when compared to rats with MetS.[27] HFF diet-induced metabolic changes (insulin, triglycerides, total cholesterol, and HOMA-IR) were decreased after SPX treatment in rats[19] and long-term (8 weeks) SPX injection resulted in a decrease of insulin, free fatty acids, triglycerides, and HOMA-IR and attenuated hepatic steatosis in DIO rats.[10]

The connection between metabolic diseases and SPX

Role of SPX on obesity, diabetes, and MetS

SPX is widely detected in endocrine and epithelial tissues[2,3,7,38] and is expected to be involved in metabolic disorders such as obesity, diabetes, and MetS. Because of its potential regulatory roles in energy intake[13] and inhibition of satiety,[16] SPX mRNA expression is affected in the forebrain region under different feeding status or metabolic states.[39]

The regulation of SPX in diabetes is controversial. Gu *et al.*[7] showed no correlation between SPX levels and glycemic parameters in diabetes (type 1 and 2). However, Karaca *et al.*[40] found low SPX levels in diabetic patients. It is not surprising that SPX has a role in the glycemic index and lipid metabolism.[7,41]

Sassek *et al.*[42] showed a correlation between insulin and SPX. In obese children, SPX concentrations were correlated with insulin and HOMA-IR.[43] Glucose levels and insulin sensitivity were affected by SPX levels in pregnant women without being related to gestational diabetes.[44] Consistent with these effects in diabetes, SPX has critical roles in obesity because it affects appetite and energy regulation and also carbohydrate consumption and lipid oxidation. Studies conducted on goldfish showed that SPX inhibited obesity-related hormones such as NPY and orexin.[16] SPX levels are also correlated with leptin and blood lipids.[44] Obese subjects had low serum SPX levels, and it is negatively correlated with leptin levels.[13] Controversially, Al-Daghri *et al.*[44] found no relationship between obesity and SPX levels. In another study, a negative correlation was found between SPX and obesity parameters such as BMI, HOMA-IR, and serum levels of insulin and glucagon in obese women.[32] Meanwhile, some studies found a positive correlation with obestatin, leptin, triglycerides, and adiponectin.[6,10,40,43,45] In an obese mouse model with T2D, SPX reduced body weight and decreased insulin resistance and HbA1c.[46]

MetS is the combination of risk factors associated with obesity, CVDs, and diabetes.[47] SPX levels were lower in patients with MetS. Also, an inverse correlation was found between SPX and glucose, blood pressure, and blood lipids (triglycerides and high-density lipoprotein [HDL]) in the MetS group.[11] Moreover, SPX treatment reduces fatty acid uptake into the hepatocytes.[46] Circulating levels of SPX were lower in female participants with MetS but not in male participants.[44] Subcutaneous (s.c.) injection of SPX reduced appetite and led to a reduction of caloric intake by nearly 32% in rats.[13] Moreover, Behrooz *et al.*[43] found an inverse relationship between SPX levels and dietary fat intake among obese children. SPX has a potential regulatory role in metabolic status, despite studies where the findings are so controversial.

CVD and SPX

CVDs is a set of disorders combined with vascular and cardiac functions such as cerebrovascular disease, rheumatic heart disease, myocardial infarction, stable-unstable angina, coronary heart disease, heart failure, and atherosclerosis.[48] CVDs is also the leading cause of death globally. According to the World Health Organization (WHO) report, an estimated 17.9 million people died from CVD in 2019, representing 32% of all global deaths. Dyslipidemia defined as increased serum low-density lipoprotein (LDL) cholesterol, total cholesterol, or triglycerides and decreased serum HDL cholesterol concentration is an independent predictive factor for progression of CVD.[49] The cardiomodulatory properties of SPX were first observed when administered to rats intracerebroventricularly (i.c.v.). In this study, it was found that SPX affects arterial pressure, heart rate, urine flow rate, and salt–water balance.[22] In an *in vitro* study using rat cardiomyocytes (H9C2) and primary neonatal rat ventricular myocytes (NRVMs), SPX treatment protected cells from mitochondrial damage induced by hypoxia.[12] In addition to this cytoprotective function, it was observed that SPX treatment significantly decreased total cholesterol, LDL, triglycerides, and atherogenic index in a high-fructose diet-induced MetS model in rats.[27] Also, it has been shown that streptozotocin-induced T2D rats have ameliorated effects of SPX treatment both on biochemical parameters (triglyceride, LDL, atherogenic index, and creatine kinase-myocardial band [MB]) and damage to cardiac tissue.[50]

Similar to animal studies, it was shown that triglyceride, LDL, and blood pressure were negatively correlated with SPX in obese type 2 diabetic patients.[9] Furthermore, SPX showed a negative correlation with total cholesterol, LDL, and triglyceride values in gestational diabetes mellitus (GDM) patients compared to controls.[51] Similarly, studies in both obese and healthy women found that SPX and lipid levels were inversely related.[33,43] In a study performed in obese children, low SPX levels were connected with vasculopathy independent of age and lipid profile.[52] Interestingly, serum SPX levels were found to be positively correlated with lipoprotein (a) in women.[53] Overall, there are limited studies on the effect of SPX on cardiac functions, but the effect of this peptide on the lipid profile should be considered as a treatment option for CVDs in the future.

Kidney disease and SPX

Chronic kidney disease (CKD) is a global health problem, and is associated with morbidity and mortality in all countries. In addition, the prevalence of CKD is estimated to be 15% of the adult population worldwide.[54] CKD is associated with many risk factors including diabetes, hypertension, obesity, autoimmune diseases, urinary tract infections, kidney stones, and inflammation. Albuminuria, glomerular filtration rate (GFR), creatinine clearance, or markers of kidney damage are used to diagnose for CKD.[55] There are a few studies that have examined the relationship between SPX and kidney diseases.

MetS rat models induced by high-fat diet showed that SPX treatment reduced serum uric acid levels.[27] Furthermore, it has been reported that SPX treatment has a protective effect on kidney tissue by reducing tubular dilatation, inflammatory infiltration, necrosis, apoptosis, and fibrosis in high-fat diet-induced rats.[19] Similarly, SPX treatment reduced tissue inhibitor of metalloproteinase 1 (TIMP-1) and kidney injury molecule-1 (KIM-1) levels in an adenine-induced chronic renal failure (CRF) rat model. Also, SPX treatment reduced metalloproteinase-2 (MMP2) levels, which regulates the fibrotic process.[56] These results indicate the protective effect of SPX on renal damage.

Protective effect of SPX on NAFLD

NAFLD is a metabolic condition which is characterized by the excess accumulation of fat in the liver independent of viral infection or alcohol consumption and is associated with metabolic comorbidities such as dyslipidemia, insulin resistance, obesity, and (T2DM).[57,58] In DIO mice, SPX treatment decreased hepatic lipid storage, aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Also, long-chain fatty acids (LCFAs) uptake in hepatocytes is diminished by SPX.[46] Similarly, another study in DIO mice showed that SPX reduced lipid deposition and glycogen levels.[11] Moreover, hepatic glucose production is reduced by SPX in DIO rats, and CRISPR/Cas9-mediated silence of SPX in human liver cancer cells (HepG2) has prompted gluconeogenesis.[10] Similar to the findings of animal studies, plasma SPX levels were lower in NAFLD patients compared to controls.[59] These findings demonstrate a potentially therapeutic value of SPX for hepatic steatosis/NAFLD treatment.

PCOS and SPX

PCOS is a widespread endocrine disorder particularly for women in their reproductive period.[60] The global prevalence of PCOS is anticipated to be 4–20%.[61] PCOS is characterized by oligomenorrhea/anovulation, excessive androgen secretion, and polycystic ovaries (more than 12 follicles).[61] Recent studies have shown that PCOS patients have several comorbidities, including insulin resistance, obesity, and disruption of glucose and lipid metabolism.[62] Since the metabolic balance is disturbed in PCOS, it is thought that SPX levels may also be affected in patients with PCOS.

SPX levels were found to be lower in the serum of PCOS patients compared to the control group. Furthermore, SPX levels were negatively correlated with insulin resistance,

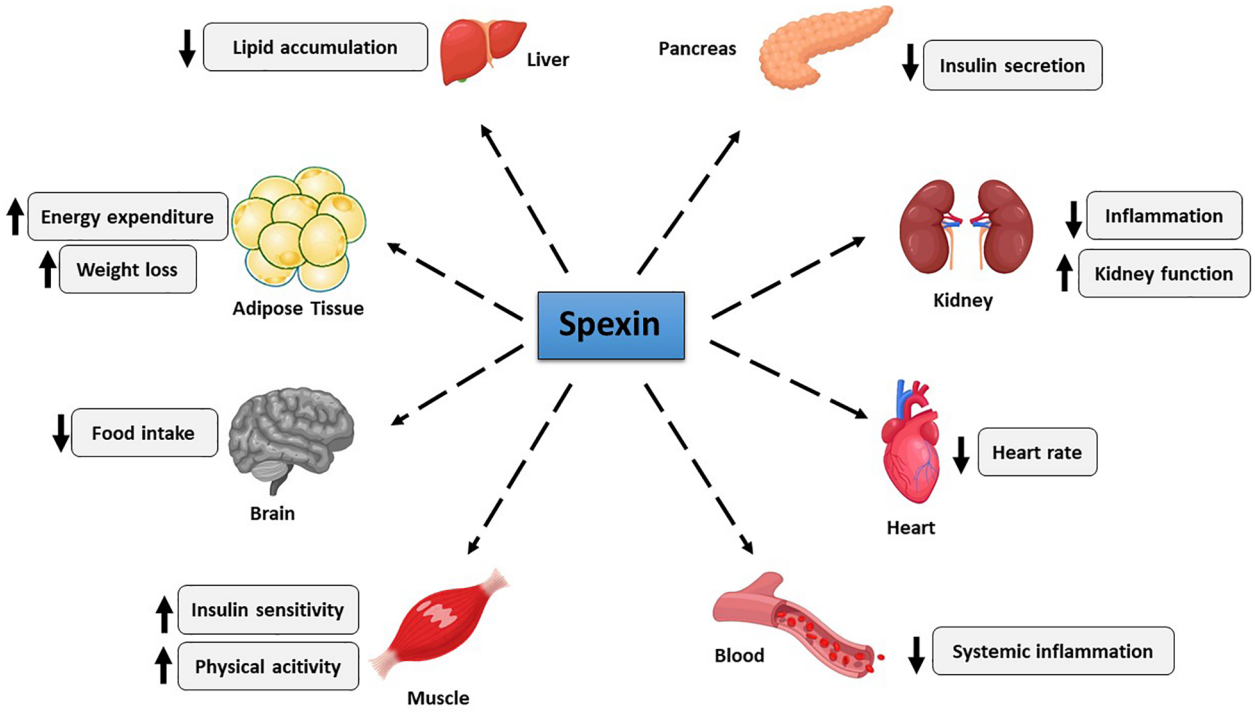

Figure 3. SPX functions in metabolism: Exogenous SPX treatment improves several metabolic disorders in various central and peripheral tissues (A color version of this figure is available in the online journal.).

BMI, and androgen in women with PCOS.[63] However, no change was observed in SPX levels in PCOS patients with obesity associated (*FTO*) gene polymorphism and fat mass compared to the control group.[64]

SPX modulates inflammatory response

Inflammatory response is part of the body's self-defense mechanism, including the recognition and elimination of harmful and foreign stimuli, whether acute or chronic.[65] Even though acute increases in inflammatory response are crucial for survival during infection and physical injury, some reports have revealed that many lifestyle modifications could promote systemic inflammation. When these acute states turns into chronic situations, it forms the basis for the pathogenesis of many diseases such as CVDs, stroke, chronic respiratory diseases, obesity, cancer, diabetes mellitus, CKD, NAFLD, autoimmune, and neurodegenerative diseases.[65,66] Pro-inflammatory molecules – such as C-reactive protein (CRP), interleukin 1 (IL-1), IL-1 receptor antagonist protein (IL-1RN), IL-6, IL-8, IL-13, IL-18, interferon α (IFNα) and IFNβ, transforming growth factor-β (TGF-β), tumor necrosis factor (TNF), and its soluble receptors – are produced in excess during the inflammatory response, and overproduction of these molecules is very important in the pathogenesis of these diseases.[67]

Studies on different diseases and different metabolic states indicate that SPX affects the inflammatory response. In a mouse model of T2D, SPX treatment reduced inflammation by lowering TNF-α and IL-6 levels both in serum and liver.[11] Also, SPX treatment decreased serum TNF-α and IL-6 levels in a high-fat diet-induced MetS animal model.[27] Furthermore, SPX treatment reduced serum IL-1β and TNF-α levels in a T2D rat model.[50] Consistent with these *in vivo* studies, SPX levels were negatively correlated with hs-CRP in adolescent patients with MetS compared to the control group.[68,69] Moreover, SPX levels were found to be associated with IL-1β, IL-6, and IL-10 in obese and normal weight children.[8,43] SPX showed a negative correlation with TNF-α and IL-6, while IL-1β showed a positive correlation, and this could be a positive predictor of SPX in patients with gestational diabetes.[51] Also, in a fructose-rich-diet obese mice model, SPX treatment decreased IL-1β, TNF-α, and IL-6 production in adipose tissue while increasing IL-10 production.[37] In DIO rats, SPX decreased IFN and IL-10 in kidney tissue, while it increased monocyte chemoattractant protein-1 (MCP1) expression.[19] Furthermore, SPX treatment decreased *IL-33* gene expression in kidney tissue in adenine-induced CRF in rats.[56] In the same experiment, SPX treatment reduced serum IL-5, granulocyte colony-stimulating factor (G-CSF), and IFN-γ levels.[70] Unlike animal studies, there was no observed correlation between SPX and hs-CRP in PCOS patients compared with the control group.[63] Overall, these results show that SPX has an anti-inflammatory action by altering cytokine levels in many different disease models.

Conclusion and future perspectives

SPX is a recently discovered peptide which is involved in the progression of metabolic diseases caused by metabolic dysfunctions. Circulating levels of SPX have been shown to be reduced in several diseases such as diabetes, obesity, MetS, CVDs, kidney diseases, NAFLD, and PCOS. Meanwhile, systemic SPX treatment has positive effects in fish and rodents including reduced food intake, fat mass, lipid accumulation, and inflammation, improved insulin sensitivity, energy expenditure, and organ functions which are associated with multiple metabolic diseases (Figure 3).

Based on recent findings, SPX might be an interesting target for the development of novel pharmacological strategies to cure metabolic diseases. However, there are still many open questions including which cellular mechanism coordinates SPX action. Future studies are needed to better understand the effect of SPX on tissue function and cell signaling in animal models. In addition, further clinical trials using SPX or its more potent analogs are needed to define their roles in health and diseases.

AUTHORS' CONTRIBUTIONS

I.T., G.M., and B.Y. contributed to writing, composing, and editing of the manuscript. All authors critically revised the manuscript and approved the final version of the submitted manuscript.

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