Section A-Research paper

Brief Insight about Biomarkers of Metabolic syndrome

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Abstract

Background: Metabolic syndrome (MetS) is a cluster of metabolic abnormalities that includes hypertension, central obesity, insulin resistance, and atherogenic dyslipidemia. American Heart Association (AHA) and National Heart, Lung, and Blood Institute (NHLBI) criteria are widely used for the diagnosis of MetS that require the presence of any 3 out of 5 metabolic traits for the diagnosis. These include: hypertension (>130/85 mmHg), abdominal obesity (a waist circumference of ≥102 cm in men, ≥88 cm in women, elevated triglycerides (TG ≥150 mg/dl), reduced plasma high-density lipoprotein cholesterol (HDL <40 mg/dl in men and <50 mg/dl in women), and impaired glucose tolerance (>100 mg/dl). The MetS prevalence was three times higher, marking about one- third of the American adult population. Recently, the National Health and Nutrition Examination Survey (NHANES) released recent data demonstrating declining numbers of the disease with 24% in men and 22% in women. The novel adipokine lipocalin-2 (LCN-2) is a glycoprotein consisting of 198 amino acids. Other names attributed to LCN-2 include siderocalin, neutrophil gelatinase-associated lipocalin (NGAL), and uterocalin. LCN-2 is a member of the lipocalin superfamily, a group of circulatory proteins that transport small and hydrophobic molecules such as steroids, fatty acids, retinoids, prostaglandins and hormones. The expression of LCN-2 is stimulated during 3T3-L1 adipogenesis in a CCAAT/enhancer-binding protein–dependent manner. White adipose tissue (WAT) was the major source of LCN-2 expression and reported its absence in brown adipose tissue (BAT) in wild-type male mice. They suggested that obesity regulates its expression. Fetuin-A, also referred to as α2-Heremans–Schmid glycoprotein (AHSG), is a protein with pleiotropic metabolic effects secreted by the liver. Fetuin-A is a potential adipokine, its expression and secretion levels have been increased in visceral adipose tissue humans with MetS. MiR-17-5p and miR-15a-5p, were found to be the strongest predictors of MetS presence, as their expression panel was decreased in individuals with MetS, independent of sex.

Keywords: Biomarkers, Metabolic syndrome

Introduction

Metabolic syndrome (MetS) is a cluster of metabolic abnormalities that includes hypertension, central obesity, insulin resistance, and atherogenic dyslipidemia. MetS, also is labeled as ‘insulin resistance syndrome’, ‘syndrome X’, ‘hypertriglyceridemic waist’, and ‘the deadly quartet’, MetS is strongly associated with an increased risk of developing atherosclerotic cardiovascular disease (CVD). Any patient diagnosed with metabolic syndrome should at least be seen as a high cardiovascular risk patient ***(1).***

The MetS prevalence was three times higher, marking about one- third of the American adult population. Recently, the National Health and Nutrition Examination Survey (NHANES) released recent data demonstrating declining numbers of the disease with 24% in men and 22% in women ***(2)***.

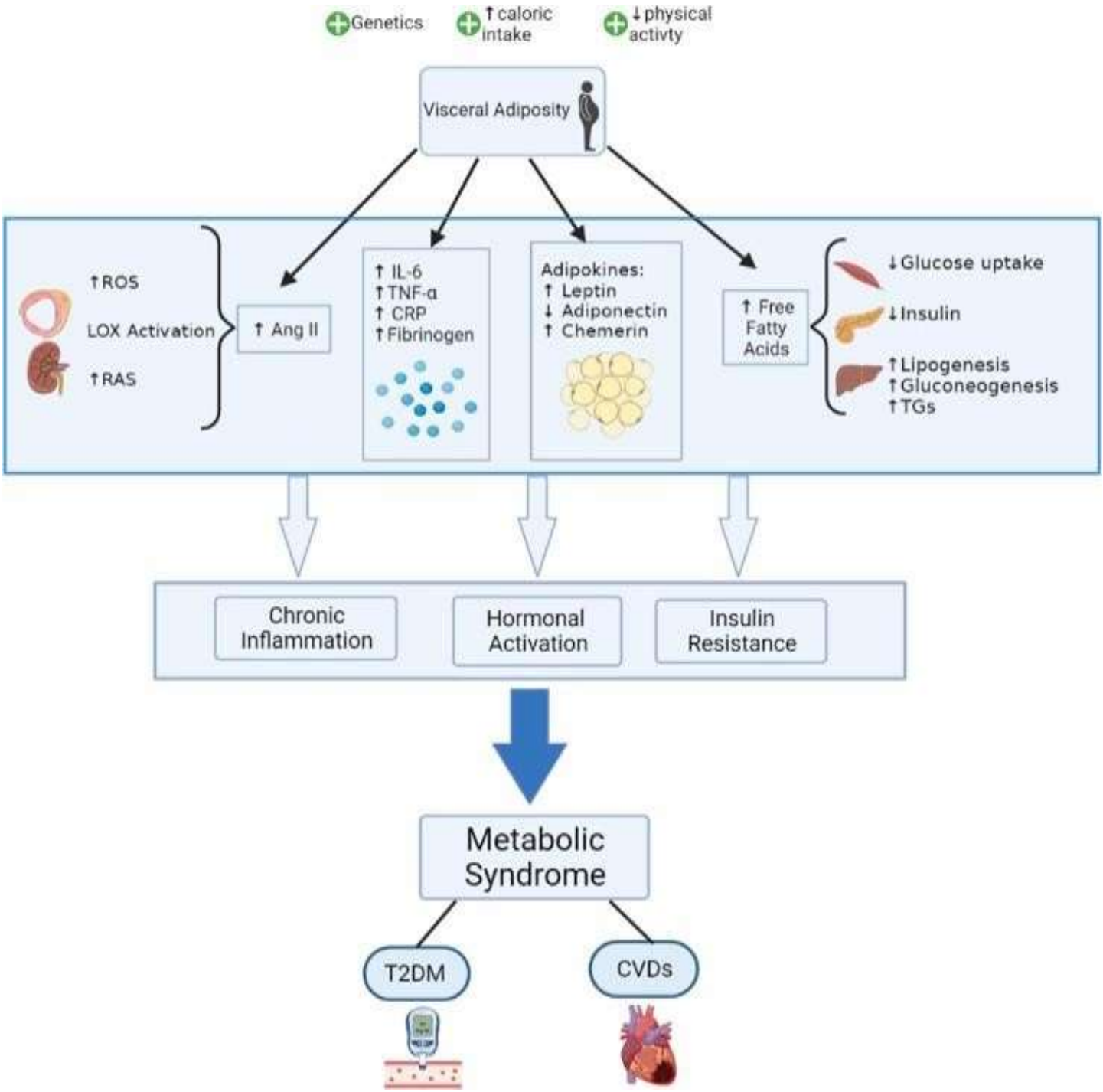

Figure 1: Mechanisms highlighting MetS pathophysiology **(3)**

lipocalin-2

The novel adipokine lipocalin-2 (LCN-2) is a glycoprotein consisting of 198 amino acids. Other names attributed to LCN-2 include siderocalin, neutrophil gelatinase-associated lipocalin (NGAL), and uterocalin. LCN-2 is a member of the lipocalin superfamily, a group of circulatory proteins that transport small and hydrophobic molecules such as steroids, fatty acids, retinoids, prostaglandins and hormones**.** LCN-2 is encoded by a gene, which is located at chromosome 9 locus 9q34.11. The *LCN-2* gene produces many functional transcripts that eventually encodes for a 198 amino acid secreted protein. LCN-2 was initially isolated from neutrophil granules released at sites of infection and inflammation in human ***(4)***.

LCN-2 has bacteriostatic properties that plays a key role in iron depletion during antibacterial innate immune response via sequestering bacterial ferric siderophores enterobactin**.** Besides its important role in innate immunity, this property gives it a protective role in infection, injury, inflammation**.** In addition, in human neutrophils it is able to interact with and stabilize matrix metalloproteinase 9 (MMP-9) which is known for its ability to breakdown basement membranes and the extracellular matrix. The LCN-2 and MMP-9 complex inhibit the auto degradation of MMP-9. This LCN-2/MMP-9 complex is suggested to aid in tumor progression and metastasis ***(5).*** In addition to its bacteriostatic properties, previous studies in animal models suggest important roles for LCN-2 in many physiological and pathological processes such as cell differentiation, apoptosis, organogenesis, inflammation, kidney damage and liver injury, Moreover, It is also suggested that LCN-2 has a role in cancer progression and metastasis. Recent research has illustrated that LCN-2 is upregulated in obese and type 2 diabetic patients ***(6)***.

Tissue distribution of lipocalin-2

LCN-2 was first identified, and located in the azurophilic granules of neutrophils **(**7**).**Following this, several researches were conducted to investigate the expression of LCN-2 in various tissues. LCN-2 is expressed in several normal tissues including kidney, lung, bone marrow, liver, adipose tissue, macrophages, thymus, non-neoplastic breast duct, prostate, small intestine and trachea. Although LCN-2 is absent in the normal brain, heart, skeletal muscle, spleen, testes, ovary and colon, it is expressed in the pathological state **(**7**).,** The expression of LCN-2 is strongly upregulated in many tissues as well as in the bodily fluids in various pathological disorders such as inflammatory and metabolic ones. Organs that contain LCN-2 after the onset of pathological conditions include the liver, heart, lungs, bone marrow, kidney, and spleen **(**7**).**

Adipose tissue

LCN-2 expression in adipose tissue has been reported ***(8)*** The expression of LCN-2 is stimulated during 3T3-L1 adipogenesis in a CCAAT/enhancer-binding protein–dependent manner. White adipose tissue (WAT) was the major source of LCN-2 expression and reported its absence in brown adipose tissue (BAT) in wild-type male mice. They suggested that obesity regulates its expression ***(9).***

Liver

The liver is considered as the main LCN-2 source during infection or post-partial hepatectomy. the blood level of LCN-2 and expression of liver LCN-2 mRNA increased significantly after partial resection of the liver **(10).**

Astrocytes

Astrocytes are important neuroglia cells found in the central nervous system. They are responsible for modulating synaptic activity and supporting neurons metabolically and trophically. Astrocytes reactively

respond to brain injury by undergoing astrocytosis. LCN-2 acts as an autocrine mediator of this astrocyte response in numerous ways, regulating movement, morphology, and death in astrocyte cells. In cultured astrocytes, there was increased LCN-2 expression and secretion following inflammatory stimulation. As shown by western blot analysis in C6 glia cells and primary astrocytes, the expression and secretion of LCN-2 was markedly increased after treatment with different inflammatory stimuli ***(11).***

Brain

LCN-2 expression is upregulated in patients with traumatic brain injury (TBI) in both the injured tissue of the brain as well as the plasma and serum of the patients. And can be used as a biomarker for the severity of TBI. This is very important since this disease can result in serious complications in the patients, leading to disability and mortality**.** In animal models, LCN-2 is expressed in astrocytes, endothelial cells, basal ganglia, and corpus callosum after cerebral ischemia ***(12).***

Kidney

LCN-2 expression is upregulated in both acute and chronic kidney damage. LCN-2 has been demonstrated as a potential early biomarker for kidney injuries in several studies ***(13)*** **.** Recently, neutrophils and macrophages as other possible producers of systemic LCN-2 **(**7**).** Chronic kidney disease (CKD) in addition is also associated with marked upregulation in LCN-2 levels in tissue and body fluids (blood and urine) **(**7**).**

Bone marrow

A strong expression of LCN-2 mRNA is detected in human bone marrow cells (myelocytes and metamyelocytes). It was found that LCN-2 expression prevents normal bone formation throughout osteoblast differentiation ***(14).***

Skin

The activated keratinocytes in the epidermis and infiltrated neutrophils in the dermis of psoriatic human patients and murine showed higher mRNA and protein expression levels of LCN-2 than detected in normal controls ***(15)*** . LCN-2 expression did drop when the lesions were repaired, suggesting that LCN-2 expression is regulated by the disease process **(7).**

Neutrophils

LCN-2 is constitutively expressed in human neutrophils and was initially isolated as a 25-kDa neutrophil protein. During inflammation, LCN-2 is secreted by prepackaged neutrophil granules **(7).** This indicates that the azurophilic granules of neutrophils are specialized to store LCN-2, which may play a role in increasing the pool of LCN-2 in the plasma and connective tissues, where they may help in the regulation of body metabolism **(7).**

Heart

Previous studies have proved that LCN-2 is upregulated in ischemia- reperfusion processes, coronary heart disease and myocardial infarction suggesting that it is a promising and useful biomarker of the severity and mortality of heart diseases. Another study demonstrated that *LCN-2* expression has been detected in the mouse cardiomyocytes. Moreover, hypoxia induced mRNA expression and protein levels of LCN-2 in HL-1 cells ***(16).***

Pancreas

LCN-2 protein levels were markedly higher in pancreatic juice in patients with chronic pancreatitis and pancreatic cancer **(17).** The serum biomarker that illustrated the most promise for pancreatic cancer in mice was LCN-2 **(7).**

Lipocalin-2, obesity and insulin resistance

The adipocytokine, LCN-2, plays a key role in regulating body fat mass and insulin resistivity, as well as regulating the metabolism of energy, glucose, and lipids. It has a regulatory importance in the stimulation of PPARγ, which mediates adipogenesis and lipogeneses in liver and adipose tissues**.** However, the clear mechanism of LCN-2 in the pathogenesis of obesity-related diseases is not clear. Both the mRNA expression and serum levels of LCN-2 in adipose tissue and liver are markedly increased in obese and diabetic murine as well as in mice fed high fat diet (HFD) ***(18).***

Circulating LCN-2 concentrations positively correlate with parameters of adiposity, hyperglycemia, hypertriglyceridemia, and the insulin resistance index in humans ***(6).***

LCN-2 expression and secretion are stimulated by pro-inflammatory cytokines such as IL-6, IL-1β, IFNγ and TNFα in cultured human and mouse adipose cells **(7).** IFNγ induces the expression of LCN-2 via activating STAT1 and TNFα needs NF-kB signaling pathway in order to mediate LCN-2 expression and secretion ***(19).***

Fetuin-A

Fetuin-A, also referred to as α2-Heremans–Schmid glycoprotein (AHSG), is a protein with pleiotropic metabolic effects secreted by the liver. Fetuin-A is a potential adipokine, its expression and secretion levels have been increased in visceral adipose tissue humans with MetS. The Circulating fetuin-A levels are significantly higher in patients with MetS, and increase the risk of MetS**.**Fetuin-A mediates macrophage migration and infiltration into the adipose tissue by way of chemo-attractants, thus inducing inflammatory cytokine release and subsequently contributing to the development of MetS. Adiponectin has recently been shown to have an inhibitory effect on the expression of fetuin-A, which can partly explain the increased circulating fetuin-A concentrations in hypoadiponectinemia seen with the MetS ***(20)***.

Fetuin-A or the AHSG (Heremans–Schmid glycoprotein) gene is located on chromosome 3q27, which has been identified as T2DM susceptibility locus and has been mapped as a quantitative trait locus for MetS. Moreover, single-nucleotide polymorphisms (SNPs) of the AHSG gene and their relationship to MetS features such as BMI have been reported in several studies ***(21)***.

Circulatory MicroRNAs

MicroRNAs (miRNAs) are small evolutionarily conserved noncoding RNAs with various biological functions, mainly negatively regulating post-transcriptional gene expression. MiR-17-5p and miR-15a-5p, were found to be the strongest predictors of MetS presence, as their expression panel was decreased in individuals with MetS, independent of sex ***(22)***.

Metabolomics

The role of various metabolites in the pathogenesis of MetS has also been recently assessed. Biogenic amines found in red meats, including choline, L-carnitine, and tri-methylamine-N- oxide, were shown to be associated with an adverse cardiometabolic profile and insulin resistance**.** In addition, several amino acids, including alanine, glutamate, glutamine, aspartate and asparagine, arginine, histidine, methionine, cysteine, and lysine have been shown to play a role in the pathogenesis ofMetS. Aromatic amines (phenylalanine, tryptophan, tyrosine, and phospholipids) and branch chain amino acids caneven serve as biomarkers to predict the start of MetS in patients who have not yet developed diabetes**.** On the other side, histidine and lysine were shown to have antioxidant properties, decreasing the inflammatory burden and oxidative stress ***(23,24).***

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