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Review

Sex hormone binding globulin as a potential drug candidate for liver-related metabolic disorders treatment

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ABSTRACT

Sex hormone binding globulin (SHBG) is a hepatokine that binds to circulating steroid hormones (testosterone, oestradiol) to regulate their concentration in the bloodstream. Recently SHBG was recognized as an essential biomarker for metabolic syndrome (MetS) and hepatic steatosis development. At the hepatic level, the production of SHBG is mainly regulated by sex steroids and thyroxine. Studies of various research groups, including ours, showed that SHBG could be considered a reliable marker of insulin resistance and, therefore, can serve as a predictor of type 2 diabetes. Moreover, increased levels of circulating pro-inflammatory mediators strongly correlate with lowered serum levels of SHBG. This review paper emphasizes the role of SHBG as a potential drug candidate in the course of various metabolic dysfunctions, including non-alcoholic fatty liver disease (NAFLD), obesity, diabetes mellitus and insulin resistance. The studies related to SHBG and its role in the course of metabolic disorders are very limited. Here, we have summarized the most current knowledge about SHBG and its mechanism of action, indicating a novel concept for its possible therapeutic application in the management framework of commonly occurring metabolic dysfunctions.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) represents a group of liver disorders affecting several extrahepatic organs and regulatory pathways. It is characterized by excess fat and steatosis in the liver, occurring independently from alcoholic intake in more than 5 % of hepatocytes. It is estimated that about 20/10,000 people are affected by NAFLD per year. The statistics show that NAFLD prevalence reaches 30–40 % of the male against 15–20 % of the female population. For unclear reasons, men are more prone to NAFLD than women. Additionally, the estimates are higher in people with type 2 diabetes mellitus (T2DM), occurring in up to 70 % of this group of patients [1].

The involvement of insulin resistance, oxidative stress and subsequent lipid peroxidation, pro-inflammatory cytokines, adipokines and mitochondrial dysfunction in the pathological assent of NAFLD has been repeatedly demonstrated, which supports the link between this pathology and metabolic syndrome (MetS). Although the data are primarily epidemiological, the etiology of NAFLD and MetS appear to underline common pathophysiological mechanisms, emphasizing insulin resistance as a key factor during the development of both conditions. Thus, NAFLD is considered as a hepatic representation of the metabolic syndrome [2]. Insulin resistance is just as linked to the eventuality of the onset of type 2 diabetes mellitus (T2DM) as the accumulation of fat in the liver and this phenomenon is greatly increased in the presence of NAFLD. In addition, in patients with T2DM, the predominance of NAFLD (55–60 %) and non-alcoholic steatohepatitis (NASH) (± 37 %) is increased, which confers to T2DM the role of a predictor of hepatic disorders, morbidity and mortality in patients with NAFLD [3].

Indeed, one of the main features of NAFLD associated with obesity is the hepatic absorption of fatty acids from blood plasma. The *de novo* synthesis of fatty acid becomes more significant than the oxidation and the export of fatty acids including lipotoxicity, which causes insulin resistance and pancreatic beta-cell dysfunction. In fact, increased circulating lipid levels and alterations in fatty acid utilization and

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intracellular signaling have been linked to hepatic insulin resistance [4,5].

Infiltration of fat into the liver outcomes are associated with hepatocellular inflammation and the occurrence of fibrosis. Several factors mediate this process, including tumor necrosis factor (TNF-α). This pleiotropic cytokine is produced by cells of monocytic lineage and by immunomodulatory stromal cells. The overexpression of TNF-α leads to increased oxidative stress, as well as cell death in the liver. Consequently, this potentiates the development of hepatic fibrosis and ultimately progresses to a more severe condition, *i.e.* non-alcoholic steatohepatitis (NASH). In the course of NAFLD, the expression of TNF-α also increases at the hepatic level, highlighting the link between the development of insulin resistance and hepatic steatosis [6].

The liver is responsible of the production of fasting plasma glucose and very-low-density lipoprotein (VLDL) serum triglycerides. In patients suffering from NAFLD, the ability of insulin to suppress the production of glucose and VLDL is impaired [7]. In addition, NAFLD is not only limited to advanced liver disease but also affects other extrahepatic organs leading to severe complications. In fact, patients with NAFLD die more frequently from cardiovascular diseases and extrahepatic malignancies than from NAFLD or other resulting liver conditions [8].

In analogy to the functional proteins released from adipose tissue and skeletal muscle, liver-derived proteins are known as hepatokines. Ongoing studies aimed at hepatokines molecular mechanism of action, may lead to the selection of novel promising biomarkers for new regiments of treatment dedicated to metabolic disorders and type 2 diabetes management.

Several proteins are exclusively or predominantly secreted by the liver and are now known to play a crucial role in maintaining metabolic homeostasis. Among them, sex-hormone binding globulin (SHBG), which had been identified as a protective molecule against metabolic syndrome, acting on macrophages and adipocytes suppressing inflammation and lipid accumulation [9–11].

SHBG, also known as the sex steroid-binding protein (SBP) or oestradiol testosterone binding globulin, is mainly secreted by hepatocytes. However, as an extracellular plasma glycoprotein, SHBG binds to circulating steroid hormones, including testosterone, dihydrotestosterone and oestradiol, in order to regulate their free concentrations in blood plasma; where this glycoprotein will act as a transporter of these sex steroids in order to regulate their bioavailability and access to target tissues and cells [12]. Epidemiological studies indicate that serum levels of SHBG are altered in the course of various metabolic dysfunctions including obesity, diabetes mellitus and insulin resistance and that patients suffering from obesity excrete low levels of SHBG compared to normal-weight patients. This allows considering SHBG a good molecular predictor of MetS and hepatic steatosis development [12]. At the hepatic level, the production of SHBG is mainly regulated by sex steroids and thyroxine; it was suggested that insulin also acts as an important regulator, and that low levels of SHBG can be considered as a marker of insulin resistance and therefore a predictor of type 2 diabetes; furthermore, previous studies showed that overweight people, with a high BMI index, exhibited higher insulin levels and lowered circulating SHBG levels [13,14]. The molecular mechanism of T2DM lies in the dysfunction of phosphoinositide 3-kinase (PI3K) activation, which plays a key role in signal transduction during metabolism. Negative regulation of insulin receptor substrate 1/ insulin receptor substrate 2 (IRS1/IRS2) is therefore linked to a reduction in insulin sensitivity leading to a significant drop in the expression of SHBG. Besides hormones transportation (testosterone and oestradiol), SHBG also acts as a signal transduction factor by regulating the expression of phosphoinositide 3-kinase/serine-threonine-protein kinases (PI3K/AKT) pathway components and consequently participating in the modulation of insulin signaling [14]. Recent epidemiological studies have demonstrated a reversed correlation between the plasma levels of several cytokines and SHBG in people with inflammatory diseases such as obesity and type 2 diabetes. In addition, when circulating pro-inflammatory cytokines levels increases, the plasma levels of SHBG decrease as well. This phenomenon is governed by pro-inflammatory cytokines (Tumor necrosis factor alpha (TNF-α)) which are decreasing SHBG expression at the mRNA level through the suppression of its specific hepatocyte nuclear factor 4 alpha (HNF-4α) promotor activity in liver cells. However, the effect of TNF-α is indirect and is mediated by nuclear factor kappa B (NF-kB) [15].

Many lines of evidence indicate that low circulating levels of SHBG can be considered as an appropriate biomarker for insulin resistance and inflammation diagnosis. What is more, it could be a promising agent in terms of new therapeutic strategies aiming at regulating various metabolic pathways. In this review, we will discuss the main physiological functions of SHBG glycoprotein, with particular attention to its implication in cellular metabolism. We will also discuss its differential circulating levels in the body under normal and pathologic conditions. We will then focus on the implication of SHBG in the course of metabolic syndrome development (*i.e.*, insulin resistance, liver inflammation and NAFLD) and consider the possible therapeutic use of SHBG in the framework of metabolic disorders management.

The review was prepared based on 38 original experimental studies and 23 review papers. The articles were selected in the PubMed collection based using keywords: "NAFLD", "metabolic syndrome", "SHBG'' and its combinations. To this date, no review study has been published highlighting the link between the phenomena mentioned above. The present review will provide an overview of common pathophysiological mechanisms underlying NAFLD and MetS development. It will also show the role of SHBG as a hepatokine hampering the onset of insulin resistance, lipotoxicity as well as chronic low-grade inflammation. Moreover, the possible use of SHBG as a therapeutic candidate in the treatment of MetS-related liver diseases, notably NAFLD will be emphasized.

2. Sex hormone binding globulin (SHBG): a hepatokine mainly produced by the liver

SHBG is a homodimeric plasma glycoprotein with a molecular mass of approximatively 95 kDa, which is largely synthesized in the liver and secreted into the bloodstream. Its molecular weight depends partly on its glycosylation status. This extracellular plasma glycoprotein consists of two laminin G (LG)-type domains [10]. Two subunits are encoded by a gene located on the short arm of chromosome 17. The non-dimerized SHBG subunit comprises 373 amino acids, with three oligosaccharide side chains and two disulfide bonds. The homodimeric SHBG has two active and distanced steroid sites whose role is to bind to DHT, testosterone or oestradiol. Each monomer is composed of two β sheets, linked by eight hydrogen bonds, essential for forming the two continuous 14-strand b sheets of the mature homodimer [16].

2.1. SHBG synthesis pathway: transcription regulators

For the first time, liver SHBG secretion has been reported by Khan et al., who studied the binding of testosterone- oestradiol-binding globulin in a human hepatoma-derived cell line (HepG2). The cell line has been widely used as an *in vitro* model to study the regulation of SHBG synthesis [17].

Various studies, including those performed on HepG2 models, established the potential factors influencing the SHBG levels (Fig. 1). Furthermore, circulating levels of SHBG are affected by different hormonal factors - for example, thyroid hormones influence plasma levels of SHBG by altering its production. However, SHBG plasma levels are high in patients with hyperthyroidism and this can be explained by the poor functioning of thyroid hormones in the liver. Indeed, HepG2 cells express iodothyronine deiodinase type I, which converts the prohormone thyroxine (T4) into active receptor triiodothyronine (T3) in the healthy liver; however, the key enzyme responsible for deactivating thyroid hormones in the liver and other tissues, iodothyronine deiodinase type III, is lacking. On the other hand, HepG2 cells express glucuronidases

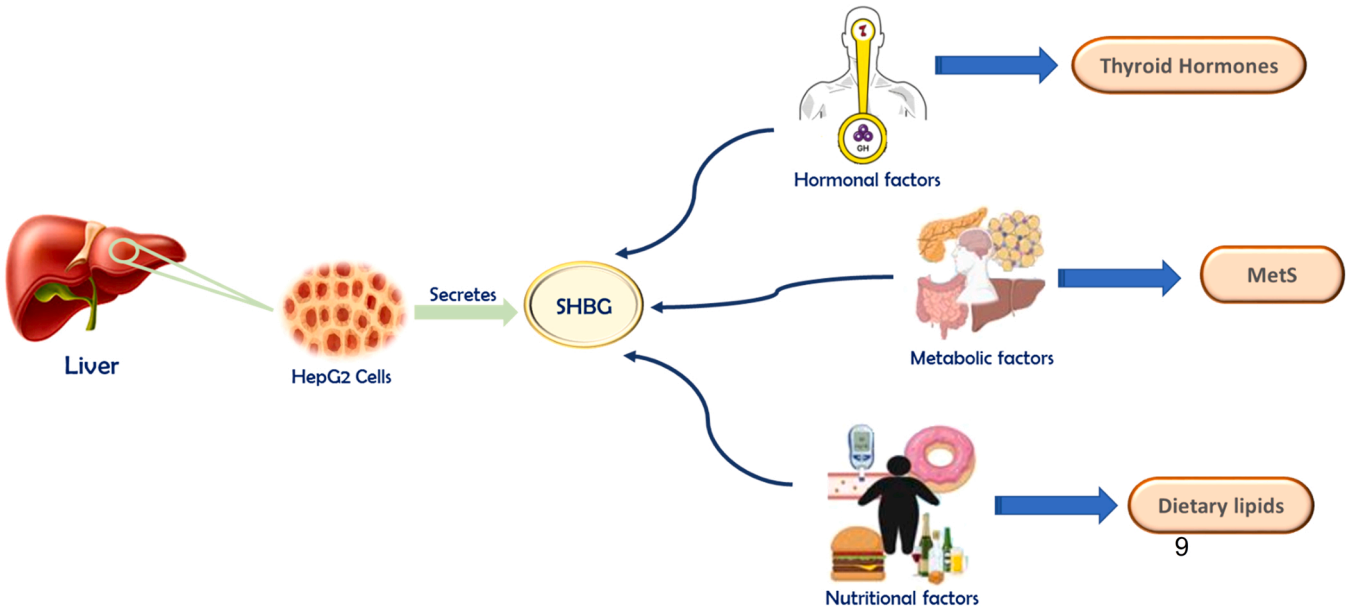

Fig. 1. Site of sex hormone binding globulin (SHBG) synthesis and the factors influencing its production.

responsible for the metabolic clearance of thyroid hormones in the liver. The human SHBG gene is also expressed in HepG2 cells, so its production is enhanced by thyroid hormone treatments which increase the levels of SHBG mRNA [18]. Other factors have also been demonstrated to influence the bioavailability of SHBG, including dietary lipids; in fact, it was noted that increased cholesterol levels correspond with decreasing SHBG levels [19,20].

Serum SHBG levels in new-borns are approximately ten times lower than those in maternal blood. This level increases slightly thereafter in children of both sexes (a concentration of about 100 nM), due to the production of thyroid hormones until puberty. During pubescence, the levels of SHBG begin to gradually decline. This phenomenon could be explained by the fact that these thyroid hormones act indirectly on the expression of SHBG by increasing the hepatic levels of the HNF-4α transcription factor, which is considered as a critical regulator of SHBG transcription in the liver. The mechanism of SHBG promoter activation involves the binding of HNF-4α to a cis-element like DR1, which then stimulates the production of the SHBG protein. Competing with HNF-4α at a third site on the promoter, peroxisome proliferator-activated receptor gamma (PPARγ2) reduces the copy of the gene to ribonucleic acid (RNA). If HNF-4α levels are low, nuclear receptor subfamily 2, group F, member 1 (Nr2f1), also known as COUP Transcription Factor 1 (COUP-TFI) binds to the first site and stops the synthesis of SHBG [20] (Figs. 2 and 3).

2.2. Physiological role of SHBG: a crucial hormone transporter

Sex steroids testosterone and oestradiol are hormones that modulate various aspects of sexual differentiation, growth, functional maturation and development of reproductive tissues. These molecules can also influence the maturation of other organ systems (such as the lungs and kidneys) [20]. SHBG binds biologically and specifically to estrogens and androgens, with an affinity four to five times stronger compared to that of albumin. The steroid binding specificity of SHBG may be different among species; nevertheless, the androgens are generally characterized as a "favorite" ligand for SHBG in mammals, and due to this biological action, SHBG was previously also termed an androgen binding protein (ABP) [20,21].

Circulating SHBG can be internalized into target tissue cells by the low-density lipoprotein-linked protein 2 receptor, also called megalin receptor, which is actually a high molecular mass (600 kDa) protein located in the epithelial cell membranes, which is part of the LDL lipoprotein receptors and has a high homology with the beta2-macroglobulin receptors and intervenes with many ligands in the transport of low density lipids, vitamins A and D, in immunology and in the development and function of many organs, and thereby facilitates the uptake of several ligands, many of which are cataloged as intracrine, including SHBG. Megalin is encoded by the low-density protein receptor-related Protein 2 (LRP2) gene locus at 2q31. The human megalin promoter gene has PPAR-responsive elements, suggesting metabolic regulation in protein expression; this is also why its expression is reduced in Ren2 rats, which is used as a model of metabolic syndrome [22].

On the other hand, the biologically active dimerized SHBG complex acts as a ligand for a specific plasma membrane high-affinity receptor (RSHBG). The unoccupied SHBG molecule (*i.e.*, not bound to sex steroids) can associate with RSHBG. After the unoccupied SHBG binds to the cell surface receptor, the anchored SHBG – RSHBG complex will be activated by the release of sex steroids of varying biological potencies. This complex can act as an agonist or an antagonist, depending on the nature of the targeted tissue containing the RSHBG and the specific sex steroid [19]. By measuring the concentrations of pure SHBG as well as its steroid-binding capacity, it was concluded that an equimolar relationship between SHBG homodimer and steroid ligand exists. This explains the fact that a single steroid-binding site is located in the dimer interface of the glycoprotein. In addition, SHBG monomers interact very strongly and can only be effectively dissociated under denaturing conditions. However, it has been reported that dimer formation is favored when a steroid ligand is present. Furthermore, the crystal structure of the laminin G (LG)-type domain provides information on the mode of dimerization of SHBG; in fact, this phenomenon occurs when the strands between the strands 7 of one monomer and 10 of another pair up [23].

Despite the fact that the primary biological function of SHBG is to regulate the bioavailability of free sex hormones in target tissues, mounting evidence shows that this glycoprotein initiates signaling pathways modulating cellular absorption and the biological action of the sex steroids limiting their diffusion into target tissues [24]. For instance, Laurent et al. demonstrated that SHBG did not affect free testosterone concentrations but primarily increased total androgen and estrogen concentrations. In this study, two potential mechanisms of SHBG action were identified. The first mechanism decreases testosterone concentration *via* hypothalamic-pituitary feedback, while the second is related to a

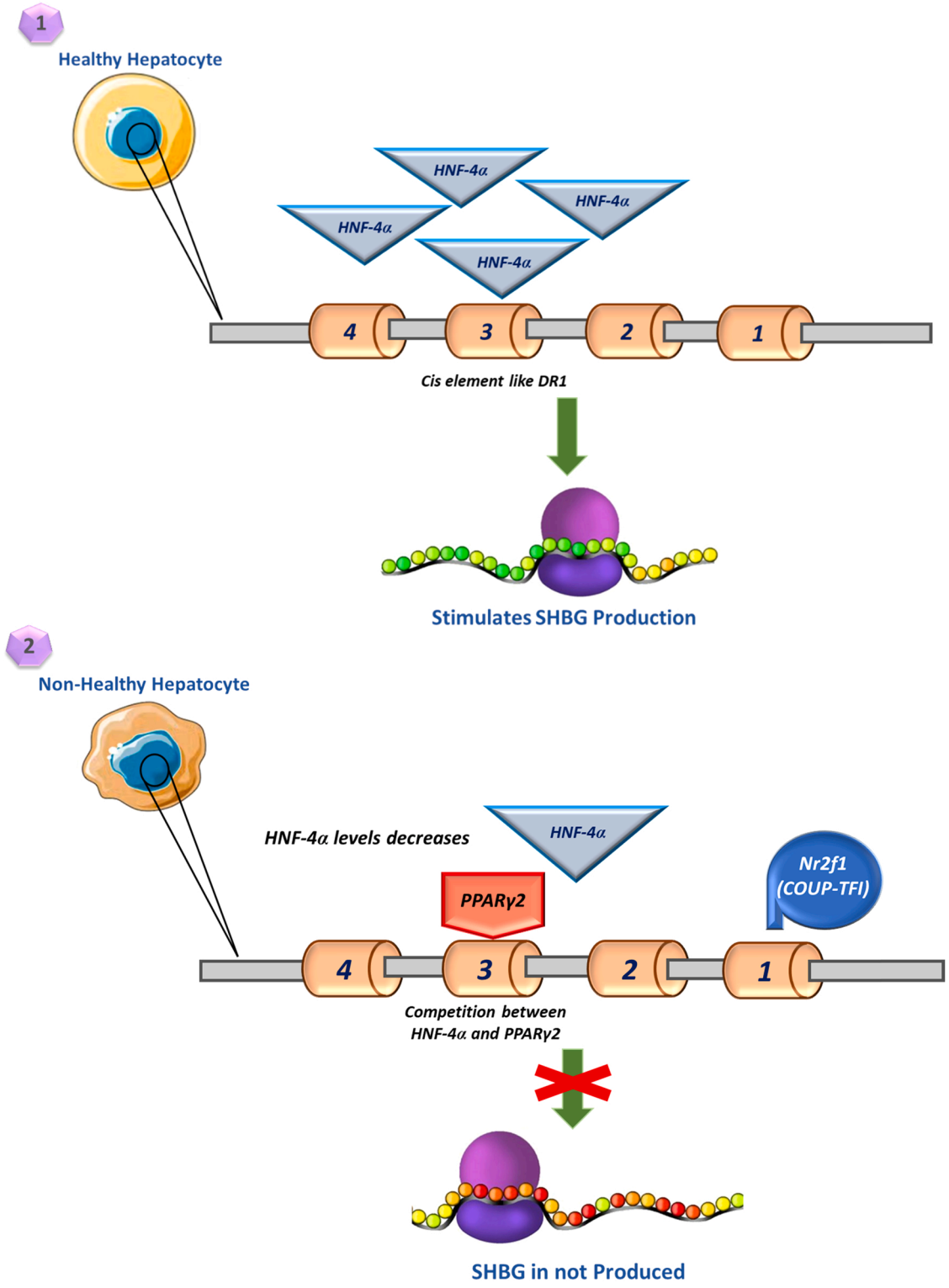

Fig. 2. Human sex hormone binding globulin (SHBG) proximal promoter sequence and transcription factors. 1- HNF-4α factor binds to the SHBG promotor called cis-element like DR1 to stimulate and regulate SHBG synthesis. PPARγ2 compete with HNF-4α at the cis-element like DR1 site, which will reduce the copy of RNA, and because of the low HNF-4α levels, the Nr2f1 (COUP-TFI) will bind to the first site, and this will decrease and stop the production of SHBG [20].

prolonged circulating half-life of ligand [25].

Plasma levels of SHBG are elevated throughout childhood to limit potentially harmless actions of sex steroids derived from adrenal androgen metabolism. However, SHBG will be progressively down-regulated during puberty, indirectly leading to maturation of the hypothalamic-pituitary-gonadal axis (HPG axis). During puberty, the blood levels of SHBG decrease in girls and boys about 2 and 4 times, respectively. It implies that the increased production of adrenal and gonadal androgens explains this phenomenon of changing levels of SHBG by directly influencing its production in pubescent boys. Thus, when the secretion of SHBG decreases, the amounts of free testosterone in the blood circulation steadily increases, which is necessary for the

maturation of the accessory sex organs and the normal development of the whole body [20].

2.3. SHBG levels in the course of metabolic disorders

The conventional roles of SHBG involve transporting sex hormones and regulating hormonal dynamics within the body. However, it has been extensively demonstrated that there is a relationship between serum SHBG concentration and metabolic syndrome occurrence [10]. The low SHBG circulating levels is considered as a risk factor for obesity, diabetes and insulin resistance and is also correlated with higher levels of serum inflammatory markers [10]. Moreover, decreased levels of SHBG were noted in patients suffering from NAFLD, insulin resistance, metabolic syndrome and T2DM. Notably, restored SHBG levels were inversely correlated with liver fat accumulation reduction [26]. In turn, generalized adiposity ultimately leads to insulin resistance, particularly in patients with type 2 diabetes. However, hepatic insulin signaling is achieved when the binding of insulin to the hepatic insulin receptor occurs, this will cause the downstream activation of the insulin receptor substrate (IRS) and phosphoinositide 3-kinase (PI3-kinase), initiating protein kinase B (Akt) which acutely activates glycogen synthase and inactivates fork-box-containing protein O subfamily 1 (FOXO1). However, this insulin binding phenomenon leads to the activation of glycogen synthase and the negative regulation of the transcription of gluconeogenic enzymes, the latter mediated by the nuclear export of FOXO. Based on results obtained from RNA sequencing of liver samples from obese individuals with NAFLD and NASH, demonstrated global downregulation of insulin signaling genes compared to lean or obese but not suffering from steatosis. However, the link between increased liver triacylglycerol (TG) content and impaired insulin signaling remains unknown to date. That said, it has been shown that in severely obese people with steatosis, hepatic diacylglycerol (DAG) content correlates positively with hepatic TG levels but negatively with suppression of glucose production by insulin, and it lipids as well as DAG associated with droplets from liver samples have been shown in obese individuals to induce activation of protein kinase C ε (PKCε), which is the major isoform of PKC in human liver, thereby altering the insulin signaling. Thus, these studies conducted on different pathological sources of liver, support the idea that increased accumulation of TG and DAG at the hepatic level activates PKC, thereby altering insulin signaling, which adds results from more general transcriptional downregulation of insulin signaling [27]. Although low plasma concentrations of SHBG have an independent role in the pathogenesis of insulin resistance and predisposition to type 2 diabetes, the latter condition is undeniably linked to a decrease in plasma levels of SHBG [28].

Previous epidemiological reports highlighted the strong correlation between the secretion of several cytokines and SHBG in humans. Indeed, it can be observed that when an increase in circulating pro-inflammatory cytokines appears in certain patients, in particular those with chronic low-grade inflammatory diseases such as obesity, diabetes, arthritis, rheumatism as well as osteoarthritis, they exhibit concomitant low plasma levels of SHBG [29]. Ramon-Krauel et al. reported that during obesity the plasma levels of TNF-α or interleukin 1β (IL-1β) are higher. In addition, the molecular mechanisms by which TNF-α reduces hepatic production of SHBG has recently been elucidated. Indeed, changes in hepatic levels of HNF4-a are at the origin of the decrease in the synthesis of the SHBG glycoprotein by HepG2 cells; because not only does TNF-a inhibit the expression of the HNF4A gene; but also inhibits its transcriptional activity *via* NF-kB [12,29].

Inflammatory diseases are characterized by increased levels of interleukin 1 (IL-1) mainly produced by macrophages, that activates NF-κB related pathways. The interleukin (IL)-1 cytokines and receptors family are associated with innate immunity and play a vital role in the induction and the regulation of the organism defense and inflammation. The IL-1 family includes pro-inflammatory cytokines (IL-1α/β, IL-36α/β/γ), anti-inflammatory cytokines (IL-37, IL-38), activating receptors (IL1-R1, IL-36R), decoy receptors (IL-1R2, IL-18BP) and other regulators, kinases and phosphatases which together are responsible for the IL-1 mediated response [30]. The mechanism underlying this phenomenon lies in the probable downregulation of HNF-4α transcription factor by the MAPK kinase (MEK-1/2) and the MAPK signaling pathways of the N-terminal kinase c-Jun (JNK) *via* activation of c-Jun transcription factors. It is explained by the interaction occurring between the pro-inflammatory cytokines (TNF-α and IL-1β) and their receptors which mediate various signaling cascades through the MAPK kinase and the MAPK as well as c-Jun transcription factors leading to alterations in HNF-4α levels, thereby decreasing the expression of SHBG [15] (Fig. 3).

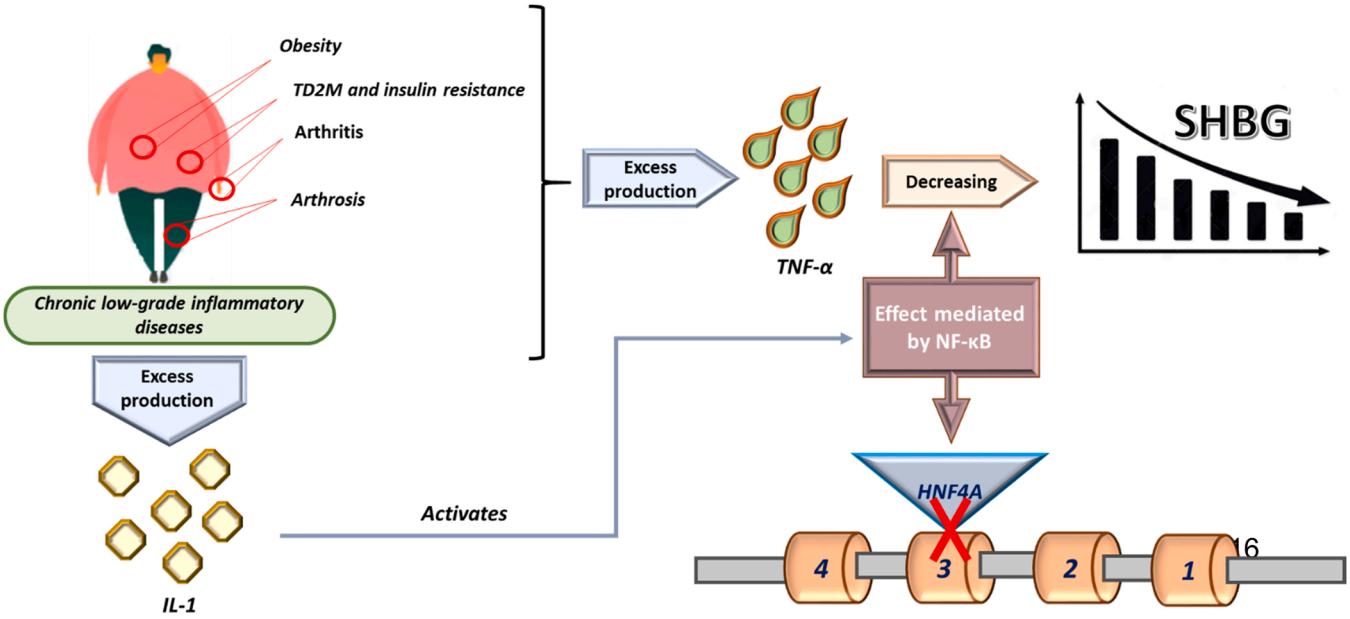

Fig. 3. Effect of some inflammation factors on sex hormone binging globulin (SHBG) expression. During chronic low-grade inflammation diseases, the organism overexpresses cytokines such as IL-1 and TNF-α which play a key role in decreasing SHBG levels production. The action of IL-1 is mediated by the NF-κB factor which downregulates HNF-4α transcription leading to the suppression of SHBG synthesis.

3. Non-alcoholic fatty liver disease (NAFLD) and metabolic syndrome (MetS) crosstalk

Over the past four decades, NAFLD has become the most common chronic liver disorder; and 25 % of the world's adult population is affected. This pathology is closely and bidirectionally related to the different aspects of MetS. Incidentally, NAFLD is now the fastest and the most ascending cause of liver-related mortality in the world; as this pathology is considered to be the basis of end-stage liver disease. And although fatty liver disease resulting in cirrhosis was described almost 20 years ago, the term "non-alcoholic steatohepatitis'' has been invented for the first time in 1980 by Ludwig et al. Furthermore, a patient is considered to be affected by NAFLD when steatosis is present in more than 5 % of hepatocytes, and obviously in association with features of MetS, in the absence of excessive alcohol consumption and/or other chronic liver diseases [31].

Overproduction of metabolic substrates, carbohydrates and fatty acids is an aspect that appears during both NAFLD and NASH occurrence and which leads to cellular dysfunction in liver tissue. Oxidative stress, reactive oxygen species (ROS) accumulation and deterioration of mitochondrial metabolism have been identified as causative factors in NAFLD initiation and progression [32]. According to the World Health Organization (WHO), metabolic syndrome refers to a combination of metabolic abnormalities including glucose intolerance or diabetes and/or insulin resistance together with two or more abnormal other conditions such as fasting plasma glucose, blood pressure and triglycerides and HDL-cholesterol [33]. MetS contributes to cardiovascular diseases and diabetes onset and significantly increases the risks of NAFLD development [34]. Silvia et al. had summarized the common disease mechanisms shared between NAFLD and MetS and showed that inflammation is a critical factor that is involved in the pathogenesis of NAFLD [35]. Furthermore, during NAFLD, the imbalance of cytokines production, including proinflammatory mediators such as TNF-a, IL-1, IL-6 released by T helper 1 subtype and anti-inflammatory cytokines such as IL-4, IL-10, and IL-13 secreted by T helper 2 subtype, was found to play an essential role during the development of the liver pathology [36,37]. The research of Silvia et al. also provided the most frequently overrepresented pathways in both NAFLD and MetS which are: interleukin-10 signaling, SUMOylation of intracellular receptors, interleukin-4, interleukin-13 signaling, the regulation of insulin-like growth factor transport, the uptake by insulin-like growth factor binding proteins and transcriptional regulation of white adipocyte differentiation. On the other hand, reported data indicated that, overall, the common signaling pathways underlying NAFLD and MetS relate to immune mechanisms. Likewise, clinical and experimental studies have shown that adipose tissue acts as a site of inflammation in the onset of type 2 diabetes; whose secretion of cytokines by macrophages and adipocytes play a role in its appearance. Therefore imbalance between lipid synthesis and lipolysis triggers acute inflammatory responses and ultimately results in oxidative stress, endoplasmic reticulum (ER) stress and mitochondrial dysfunction [38]. Otherwise, inflammasome are known to be cytosolic multiprotein oligomers including NOD-like receptor family (NLR), apoptosis-associated speck–like protein (ASC) and caspase-1 that are responsible of the activation of inflammatory responses. Indeed, inflammasome activation plays a crucial role in inflammation, which also may be an important link between the initial metabolic stress and subsequent hepatocytes death [39]. Activation of NLRP3 inflammasome by the association between inactive NLRP3, ASC and procaspase-1 induces caspase-1 maturation and release the IL-1β and the IL-18 pro-inflammatory cytokines leading to a downstream inflammatory response [40,41]. Therefore, inhibiting inflammasome activation or its mediators may be an attractive therapeutic target for NAFLD and MetS [37,38].

Insulin resistance is a common feature of both NAFLD and MetS that contributes to their pathogenesis [42]. Insulin resistance is characterized by impaired glucose metabolism in non-hepatic tissues, namely adipose tissue and skeletal muscle [39]. The exact mechanisms underlying insulin resistance are still not completely clarified. However, free fatty acids posses a crucial role in insulin sensitivity decreasing through the phosphorylation of protein kinase C epsilon, which subsequently reduces the activation of insulin receptor by suppressing the HMGA1 protein expression that binds to the insulin receptor promoter [43]. Some major risk factors contribute to insulin resistance, including obesity and diabetes. Obesity can also result in hypoxia, which influences adipocyte blood provision leading to the overproduction of free fatty acids (FFAs), TNF-α, IL-6, PAI-1 resulting in impaired insulin signaling [20,44]. TNF-α induces apoptosis of adipocytes and increases insulin resistance *via* the inhibition of insulin receptor substrate 1 signaling pathway (IRS-1), where IRS-1 is phosphorylated in response to insulin, insulin growth factor-1 and cytokines [45]. Thus, TNF-α downregulation strategy contributes to the improvement of IRS-1 phosphorylation through the activation of c-Jun and NF-*κβ* transcription factors, and subsequent insulin sensitivity restoration. IL-6, a cytokine that has bidirectional inflammatory and anti-inflammatory actions, can also affect the glucose uptake through IRS-1 phosphorylation [46,47]. Besides, IL-6 impairs insulin sensitivity and is also a major determinant of the hepatic production of C-reactive protein leading to insulin resistance *via* NF-κB signaling [45]. The activation of MAPK, which regulates cell processes including proliferation, gene expression, differentiation, mitosis, cell survival, and apoptosis, was also previously considered as a risk factor in the onset of the MetS including insulin resistance and obesity [15,48]. Also, JNK and ASK1 which are serine/threonine kinase enzymes belonging to the MAPK pathway, have the ability to regulate cell survival and cell death, also play a crucial role in liver damage, inflammation and fibrosis [49]. The inhibition of ASK inactivates JNK and protects against hypoxia/reoxygenation injury in steatotic hepatocytes, therefore ASK is considered as a potential treatment targeting patients with NASH [47]. In ERK signaling pathway, the hepatic lipid metabolism relates to circulating osteoprotegerin (OPG), a secretory protein and the OPG signaling can promote liver steatosis. Zhang et al. revealed that downregulation of OPG in NAFLD might be a compensatory strategy of the body to diminish excess hepatic fat accumulation in obesity [48]. Particularly, ERK phosphorylation decreases in the liver when OPG is overregulated, while OPG knockout (OPG-/-) mouse exhibit low lipid accumulation and reduces the expression of CD36 that increases FFA uptake and drives hepatosteatosis. In addition, the inhibition of PPARγ or the activation of ERK blocks the induction of CD36 expression by OPG, this demonstrates that OPG signaling promotes liver steatosis through the ERK-PPARγ-CD36 pathway [50].

In turn, PAI-1 or plasminogen activator inhibitor-1, a serine protease inhibitor, gained attention as a functional biomarker of the MetS. The overexpression of plasma PAI-1 corresponds with abdominal obesity and inflammation, that increases the risks of cardiovascular diseases and NAFLD [51]. Recent research showed that PAI-1 directly regulates the transcriptional expression of genes such as PCSK9 and FGF21 involved in mammalian lipid homeostasis [51]. FGF21, which belongs to the fibroblast growth factor (FGF) family, plays an important role in reducing plasma glucose and insulin levels by lowering triglyceride and cholesterol levels, thus improving insulin resistance. Meanwhile, PCSK9 is a protein that functions as a regulator of cholesterol homeostasis; Yuan Guo- et al. showed in their study that FGF21 inhibits PCSK9 expression by suppressing the expression of sterol response element binding proteins 2 (SREBP2), which means that PCSK9 trans-activator therefore improve the level of low-density lipoprotein cholesterol in the liver [52].

4. Perspectives in the use of SHBG as a new therapeutic lead in NAFLD and MetS treatment

To date, the research about the role of SHBG in disease treatment has increased since many studies reported the inverse correlation between SHBG circulating levels and expression of markers involved in

inflammation and insulin resistance that contribute to both NAFLD and MetS onset and progression [53]. Although low circulating levels of SHBG have been correlated with increased insulin resistance, the exact interconnection between the mechanism underlying insulin resistance and SHBG suppression remains unclear. Simons et al. considered SHBG as a vital biomarker of *de novo* lipogenesis (DNL) in the research about the relationship between DNL and serum SHBG [54]. Indeed, Simons et al. showed that DNL induced by monosaccharide downregulates the expression HNF-4α expressed by hepatocytes. More interestingly, patients with polycystic ovarian syndrome (PCOS) – an endocrine disorder characterized by metabolic abnormalities and NAFLD - have high intrahepatic lipid levels of SHBG, while women with PCOS had significantly lowered levels of this hepatokine [54,55]. This reinforces previous research about the role of DNL in the observed decrease of serum SHBG levels and the implication of saturated fatty acid palmitate, which is a terminal product of DNL in reducing SHBG expression [56]. Xu et al. suggested that low hepatic SHBG production may be an essential step in the pathogenesis of PCOS and therefore, SHBG could represents an early biomarker and therapeutic target in PCOS [57]. Importantly, in NAFLD patients, insulin resistance plays a crucial role in the hepatic DNL stimulation through the increase in intrahepatic triglyceride levels in plasma. Therefore the improvement of insulin resistance by targeting DNL can be a potential contribution in NAFLD treatment [58]. Taken together, we hypothesize that administration of SHBG may lead to improved insulin sensitivity through stimulation of hepatic DNL.

Obesity, one of the most recurrent conditions linked to both NAFLD and MetS, is strongly associated with the overproduction of TNF-α and down-regulation of adiponectin [59]. Interestingly, TNF-α and adiponectin regulate hepatic SHBG production, particularly, TNF-α reduces SHBG level by inhibiting HNF-4α expression, while adiponectin increases the expression of SHBG that is mediated by HNF-4α expression [12]. Simó et al. described that in obesity cases, low-grade inflammation is an important regulator of plasma SHBG concentration as the increase of circulating proinflammatory cytokines is inversely correlated with low plasma SHBG production; although the exact implicated pathway is still undetermined [15]. Before that, the research by John et al. used a single nucleotide polymorphism (SNP) near the SHBG gene named rs1799941, that is strongly related to SHBG expression, on more than 27,000 type 2 diabetes patients and showed the genetic evidence that increased SHBG level could reduce the risk of type 2 diabetes [55,60]. For this reason, we hypothesis that administration of exogenous SHBG may reduce the low-grade inflammation that is in the center of obesity pathogenesis and thus restore proper body mass index in obese patients.

The close interplay between SHBG concentration and factors involved in NAFLD and MetS and prior evidence provides new insight into the use of SHBG as a potential candidate in the management of NAFLD as well as MetS. Though, clinical and experimental researches should be considered to examine the roles of SHBG in relative signaling pathways. Most recently, we have shown that SHBG mitigates the ER stress in HepG2 both *in vitro* and *ex vivo* [60]. The exogenous SHBG decreased the expression levels of several transcripts associated with unfolded protein response that adapts to ER stress, including inositol-requiring enzyme 1 (IRE1α), activated transcription factor 6 (ATF6), DNA damage-inducible transcript 3 (CHOP), and immunoglobulin heavy chain-binding protein (BIP) [60]. Among them, IRE1α is an essential activator of XBP1, which positively regulates ER chaperones and promotes lipid metabolism. Previously, the role of SHBG overexpression has also been demonstrated in protecting against high-fat diet-induced obesity in mice by preventing the development of obesity through lipolysis stimulation in white adipose tissue by Saez-Lopez et al. [61]. In this research, Saez-Lopez et al. showed that increased SHBG expression, reduced insulin, leptin, and resistin levels while enhancing adiponectin expression caused by a high-fat diet.

In this review, we have emphasized the vital role of SHBG and its regulation in the course of metabolic disorders. We have shown that SHBG is hampering ER stress in insulin-resistant hepatocytes, decreasing type 2 diabetes and counteracting the occurrence of high-fat diet-induced obesity, SHBG improved insulin sensitivity through stimulation of hepatic DNL. Collected data and our previous studies shed promising light on the potential significance of SHBG as a novel therapeutic target in the treatment of MetS and NAFLD.

Ethics approval and consent to participate

Not Applicable.

Consent for publication

Not Applicable.

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CRediT authorship contribution statement

Nabila Bourebaba: Writing – original draft, Writing – review & editing. **ThuHa Ngo:** Writing – review & editing, Data Collection. **Agnieszka Śmieszek:** Writing – review & editing. **Lynda Bourebaba:** Writing – review & editing. **Krzysztof Marycz:** Conceptualization, Supervision.

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Competing Interests

Not Applicable.

Data availability

Not Applicable.

References

[1] E. Cobbina, F. Akhlaghi, Non-alcoholic fatty liver disease (NAFLD) – pathogenesis, classification, and effect on drug metabolizing enzymes and transporters, Drug Metab. Rev. 49 (2) (2017) 197–211, https://doi.org/10.1080/03602532.2017.1293683.

[2] P. Paschos, K. Paletas, Non alcoholic fatty liver disease and metabolic syndrome, Hippokratia 13 (1) (2009) 9–19.

[3] E. Muzurović, D.P. Mikhailidis, C. Mantzoros, Non-alcoholic fatty liver disease, insulin resistance, metabolic syndrome and their association with vascular risk, Metabolism 119 (2021), 154770, https://doi.org/10.1016/j.metabol.2021.154770.

[4] E. Fabbrini, S. Sullivan, S. Klein, Obesity and nonalcoholic fatty liver disease: Biochemical, metabolic, and clinical implications, Hepatology 51 (2) (2010) 679–689, https://doi.org/10.1002/hep.23280.

[5] D. Yazıcı, H. Sezer, Insulin resistance, obesity and lipotoxicity, in: A.B. Engin, A. Engin (Eds.), Obesity and Lipotoxicity. Vol 960. Advances in Experimental Medicine and Biology, Springer International Publishing, 2017, pp. 277–304, https://doi.org/10.1007/978-3-319-48382-5_12.

[6] P. Almeda-Valdés, D. Cuevas-Ramos, C. Alberto Aguilar-Salinas, Metabolic syndrome and non-alcoholic fatty liver disease, Ann. Hepatol. 8 (2009) S18–S24, https://doi.org/10.1016/S1665-2681(19)31822-8.

[7] H. Yki-Järvinen, Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome, Lancet Diabetes Endocrinol. 2 (11) (2014) 901–910, https://doi.org/10.1016/S2213-8587(14)70032-4.

[8] C.X. Cai, H. Buddha, S. Castelino-Prabhu, et al., Activation of insulin-PI3K/Akt-p70S6K pathway in hepatic stellate cells contributes to fibrosis in nonalcoholic steatohepatitis, Dig. Dis. Sci. 62 (4) (2017) 968–978, https://doi.org/10.1007/s10620-017-4470-9.

[9] A. Iroz, J.P. Couty, C. Postic, Hepatokines: unlocking the multi-organ network in metabolic diseases, Diabetologia 58 (8) (2015) 1699–1703, https://doi.org/10.1007/s00125-015-3634-4.

[10] H. Yamazaki, A. Kushiyama, H. Sakoda, et al., Protective effect of sex hormone-binding globulin against metabolic syndrome: in vitro evidence showing anti-inflammatory and lipolytic effects on adipocytes and macrophages, Mediat. Inflamm. 2018 (2018) 1–12, https://doi.org/10.1155/2018/3062319.

[11] N. Stefan, H.U. Häring, The role of hepatokines in metabolism, Nat. Rev. Endocrinol. 9 (3) (2013) 144–152, https://doi.org/10.1038/nrendo.2012.258.

[12] M. Ramon-Krauel, M.J. Leal-Witt, Ó. Osorio-Conles, M. Amat-Bou, C. Lerin, D. M. Selva, Relationship between adiponectin, TNFα, and SHBG in prepubertal children with obesity, Mol. Cell. Pediatr. 8 (1) (2021) 3, https://doi.org/10.1186/s40348-021-00113-z.

[13] U.M. Rajala, S.M. Keinänen-Kiukaanniemi, P.K. Hirsso, et al., Associations of total testosterone and sex hormone–binding globulin levels with insulin sensitivity in middle-aged finnish men, Diabetes Care 30 (4) (2007), https://doi.org/10.2337/dc06-1979 (e13-e13).

[14] C. Feng, Z. Jin, X. Chi, et al., SHBG expression is correlated with PI3K/AKT pathway activity in a cellular model of human insulin resistance, Gynecol. Endocrinol. 34 (7) (2018) 567–573, https://doi.org/10.1080/09513590.2017.1411474.

[15] R. Simó, C. Sáez-López, A. Barbosa-Desongles, C. Hernández, D.M. Selva, Novel insights in SHBG regulation and clinical implications, Trends Endocrinol. Metab. 26 (7) (2015) 376–383, https://doi.org/10.1016/j.tem.2015.05.001.

[16] T.N. Le, J.E. Nestler, J.F. Strauss, E.P. Wickham, Sex hormone-binding globulin and type 2 diabetes mellitus, Trends Endocrinol. Metab. 23 (1) (2012) 32–40, https://doi.org/10.1016/j.tem.2011.09.005.

[17] M.S. Khan, B.B. Knowles, D.P. Aden, R. Rosne, Secretion of testosterone-estradiol-binding globulin by a human hepatoma-derived cell line, J. Clin. Endocrinol. Metab. 53 (2) (1981) 448–449, https://doi.org/10.1210/jcem-53-2-448.

[18] D.M. Selva, G.L. Hammond, Thyroid hormones act indirectly to increase sex hormone-binding globulin production by liver via hepatocyte nuclear factor-4α, J. Mol. Endocrinol. 43 (1) (2009) 19–27, https://doi.org/10.1677/JME-09-0025.

[19] M. Pugeat, J.C. Crave, M. Elmidani, et al., Pathophysiology of sex hormone binding globulin (SHBG): relation to insulin, J. Steroid Biochem. Mol. Biol. 40 (4–6) (1991) 841–849, https://doi.org/10.1016/0960-0760(91)90310-2.

[20] G.L. Hammond, Diverse roles for sex hormone-binding globulin in reproduction, Biol. Reprod. 85 (3) (2011) 431–441, https://doi.org/10.1095/biolreprod.111.092593.

[21] N. Xita, A. Tsatsoulis, Genetic variants of sex hormone-binding globulin and their biological consequences, Mol. Cell. Endocrinol. 316 (1) (2010) 60–65, https://doi.org/10.1016/j.mce.2009.08.025.

[22] C. Basualto-Alarcón, P. Llanos, G. García-Rivas, et al., Classic and novel sex hormone binding globulin effects on the cardiovascular system in men, in: A. Ferlin (Ed.), International Journal of Endocrinology, 2021, 2021, pp. 1–13, https://doi.org/10.1155/2021/5527973.

[23] C. Guadarrama-García, M. Bello, M. Soriano-Ursúa, Molecular insights into how SHBG dimerization exerts changes on ligand molecular recognition, J. Steroid Biochem. Mol. Biol. 197 (2020), 105502, https://doi.org/10.1016/j.jsbmb.2019.105502.

[24] C. Chen, J. Smothers, A. Lange, J.E. Nestler, J.F. Strauss Iii, E.P. Wickham Iii, Sex hormone-binding globulin genetic variation: associations with type 2 diabetes mellitus and polycystic ovary syndrome, Minerva Endocrinol. 35 (4) (2010) 271–280.

[25] M.R. Laurent, G.L. Hammond, M. Blokland, et al., Sex hormone-binding globulin regulation of androgen bioactivity in vivo: validation of the free hormone hypothesis, Sci. Rep. 6 (1) (2016) 35539, https://doi.org/10.1038/srep35539.

[26] J. Ye, Z. Yao, A. Tan, et al., Low serum sex hormone-binding globulin associated with insulin resistance in men with nonalcoholic fatty liver disease, Horm. Metab. Res 49 (05) (2017) 359–364, https://doi.org/10.1055/s-0043-102690.

[27] A. London, A.M. Lundsgaard, B. Kiens, K.N. Bojsen-Møller, The role of hepatic fat accumulation in glucose and insulin homeostasis—dysregulation by the liver, JCM 10 (3) (2021) 390, https://doi.org/10.3390/jcm10030390.

[28] N. Abate, S.M. Haffner, A. Garg, R.M. Peshock, S.M. Grundy, Sex steroid hormones, upper body obesity, and insulin resistance, J. Clin. Endocrinol. Metab. 87 (10) (2002) 4522–4527, https://doi.org/10.1210/jc.2002-020567.

[29] R. Simó, A. Barbosa-Desongles, A. Lecube, C. Hernandez, D.M. Selva, Potential role of tumor necrosis factor-α in downregulating sex hormone–binding globulin, Diabetes 61 (2) (2012) 372–382, https://doi.org/10.2337/db11-0727.

[30] R.C. van Deuren, P. Arts, G. Cavalli, et al., Impact of rare and common genetic variation in the interleukin-1 pathway on human cytokine responses, Genome Med. 13 (1) (2021) 94, https://doi.org/10.1186/s13073-021-00907-w.

[31] E.E. Powell, V.W.S. Wong, M. Rinella, Non-alcoholic fatty liver disease, Lancet 397 (10290) (2021) 2212–2224, https://doi.org/10.1016/S0140-6736(20)32511-3.

[32] M.E. Rinella, A.J. Sanyal, Management of NAFLD: a stage-based approach, Nat. Rev. Gastroenterol. Hepatol. 13 (4) (2016) 196–205, https://doi.org/10.1038/nrgastro.2016.3.

[33] K.G.M.M. Alberti, P.Z. Zimmet, WHO Consultation, Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO Consultation, Diabet. Med 15 (7) (1998) 539–553, https://doi.org/10.1002/(SICI)1096-9136(199807)15:7<539::AID-DIA668>3.0.CO;2-S.

[34] G. Targher, G. Arcaro, Non-alcoholic fatty liver disease and increased risk of cardiovascular disease, Atherosclerosis 191 (2) (2007) 235–240, https://doi.org/10.1016/j.atherosclerosis.2006.08.021.

[35] S. Sookoian, C.J. Pirola, Review article: shared disease mechanisms between non-alcoholic fatty liver disease and metabolic syndrome – translating knowledge from systems biology to the bedside, Aliment Pharm. Ther. 49 (5) (2019) 516–527, https://doi.org/10.1111/apt.15163.

[36] W.E. Zahran, K.A. Salah El-Dien, P.G. Kamel, A.S. El-Sawaby, Efficacy of tumor necrosis factor and interleukin-10 analysis in the follow-up of nonalcoholic fatty liver disease progression, Ind. J. Clin. Biochem. 28 (2) (2013) 141–146, https://doi.org/10.1007/s12291-012-0236-5.

[37] T. Nakayama, K. Hirahara, A. Onodera, et al., Th2 cells in health and disease, Annu. Rev. Immunol. 35 (1) (2017) 53–84, https://doi.org/10.1146/annurev-immunol-051116-052350.

[38] I. Hameed, S.R. Masoodi, S.A. Mir, M. Nabi, K. Ghazanfar, B.A. Ganai, Type 2 diabetes mellitus: from a metabolic disorder to an inflammatory condition, WJD 6 (4) (2015) 598, https://doi.org/10.4239/wjd.v6.i4.598.

[39] T. Csak, M. Ganz, J. Pespisa, K. Kodys, A. Dolganiuc, G. Szabo, Fatty acid and endotoxin activate inflammasomes in mouse hepatocytes that release danger signals to stimulate immune cells, Hepatology 54 (1) (2011) 133–144, https://doi.org/10.1002/hep.24341.

[40] L. Barbier, M. Ferhat, E. Salamé, et al., Interleukin-1 family cytokines: keystones in liver inflammatory diseases, Front. Immunol. 10 (2019) 2014, https://doi.org/10.3389/fimmu.2019.02014.

[41] M.K.h. ElMahdy, M.G. Helal, T.M. Ebrahim, Potential anti-inflammatory effect of dapagliflozin in HCHF diet- induced fatty liver degeneration through inhibition of TNF-α, IL-1β, and IL-18 in rat liver, Int. Immunopharmacol. 86 (2020), 106730, https://doi.org/10.1016/j.intimp.2020.106730.

[42] S. Ballestri, S. Zona, G. Targher, et al., Nonalcoholic fatty liver disease is associated with an almost twofold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from a systematic review and meta-analysis: NAFLD foreruns metabolic syndrome, J. Gastroenterol. Hepatol. 31 (5) (2016) 936–944, https://doi.org/10.1111/jgh.13264.

[43] S. Bhattacharya, D. Dey, S.S. Roy, Molecular mechanism of insulin resistance, J. Biosci. 32 (2) (2007) 405–413, https://doi.org/10.1007/s12038-007-0038-8.

[44] A. Engin, Adipose tissue hypoxia in obesity and its impact on preadipocytes and macrophages: hypoxia hypothesis, in: A.B. Engin, A. Engin (Eds.), Obesity and Lipotoxicity. Vol 960. Advances in Experimental Medicine and Biology, Springer International Publishing, 2017, pp. 305–326, https://doi.org/10.1007/978-3-319-48382-5_13.

[45] S. Wang, Y. Zhao, N. Xia, et al., KPNβ1 promotes palmitate-induced insulin resistance via NF-κB signaling in hepatocytes, J. Physiol. Biochem. 71 (4) (2015) 763–772, https://doi.org/10.1007/s13105-015-0440-x.

[46] R. Krogh-Madsen, P. Plomgaard, K. Møller, B. Mittendorfer, B.K. Pedersen, Influence of TNF-α and IL-6 infusions on insulin sensitivity and expression of IL-18 in humans, Am. J. Physiol.-Endocrinol. Metab. 291 (1) (2006) E108–E114, https://doi.org/10.1152/ajpendo.00471.2005.

[47] V. Rotter, I. Nagaev, U. Smith, Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor-α, overexpressed in human fat cells from insulin-resistant subjects, J. Biol. Chem. 278 (46) (2003) 45777–45784, https://doi.org/10.1074/jbc.M301977200.

[48] Z. Tang, N. Xia, X. Yuan, et al., PRDX1 is involved in palmitate induced insulin resistance via regulating the activity of p38MAPK in HepG2 cells, Biochem. Biophys. Res. Commun. 465 (4) (2015) 670–677, https://doi.org/10.1016/j.bbrc.2015.08.008.

[49] R. Loomba, E. Lawitz, P.S. Mantry, et al., The ASK1 inhibitor selonsertib in patients with nonalcoholic steatohepatitis: a randomized, phase 2 trial, Hepatology 67 (2) (2018) 549–559, https://doi.org/10.1002/hep.29514.

[50] C. Zhang, X. Luo, J. Chen, et al., Osteoprotegerin promotes liver steatosis by targeting the ERK–PPAR-γ–CD36 pathway, Diabetes 68 (10) (2019) 1902–1914, https://doi.org/10.2337/db18-1055.

[51] J.A. Levine, C. Oleaga, M. Eren, et al., Role of PAI-1 in hepatic steatosis and dyslipidemia, Sci. Rep. 11 (1) (2021) 430, https://doi.org/10.1038/s41598-020-79948-x.

[52] Y. Guo, Q. Liu, D. Xu, Shedding light on FGF21: a potential negative regulator of PCSK9, Int. J. Cardiol. 214 (2016) 75–76, https://doi.org/10.1016/j.ijcard.2016.03.165.

[53] R. Deswal, A. Yadav, A.S. Dang, Sex hormone binding globulin – an important biomarker for predicting PCOS risk: a systematic review and meta-analysis, Syst. Biol. Reprod. Med. 64 (1) (2018) 12–24, https://doi.org/10.1080/19396368.2017.1410591.

[54] P.I.H.G. Simons, O. Valkenburg, I. Telgenkamp, et al., Relationship between de novo lipogenesis and serum sex hormone binding globulin in humans, Clin. Endocrinol. 95 (1) (2021) 101–106, https://doi.org/10.1111/cen.14459.

[55] A.L.L. Rocha, L.C. Faria, T.C.M. Guimarães, et al., Non-alcoholic fatty liver disease in women with polycystic ovary syndrome: systematic review and meta-analysis, J. Endocrinol. Invest. 40 (12) (2017) 1279–1288, https://doi.org/10.1007/s40618-017-0708-9.

[56] D.M. Selva, K.N. Hogeveen, S.M. Innis, G.L. Hammond, Monosaccharide-induced lipogenesis regulates the human hepatic sex hormone–binding globulin gene, J. Clin. Invest. (2007), JCI32249, https://doi.org/10.1172/JCI32249 (Published online November 8).

[57] X. Qu, R. Donnelly, Sex hormone-binding globulin (SHBG) as an early biomarker and therapeutic target in polycystic ovary syndrome, IJMS 21 (21) (2020) 8191, https://doi.org/10.3390/ijms21218191.
[58] R.J. Smith, R.G. Bryant, Metal substitutions incarbonic anhydrase: a halide ion probe study, Biochem. Biophys. Res. Commun. 66 (4) (1975) 1281–1286, https://doi.org/10.1016/0006-291x(75)90498-2.
[59] A.M. Xydakis, C.C. Case, P.H. Jones, et al., Adiponectin, inflammation, and the expression of the metabolic syndrome in obese individuals: the impact of rapid weight loss through caloric restriction, J. Clin. Endocrinol. Metab. 89 (6) (2004) 2697–2703, https://doi.org/10.1210/jc.2003-031826.
[60] K. Kornicka-Garbowska, L. Bourebaba, M. Röcken, K. Marycz, Sex hormone binding globulin (SHBG) mitigates ER stress in hepatocytes in vitro and ex vivo, Cells 10 (4) (2021) 755, https://doi.org/10.3390/cells10040755.
[61] C. Saez-Lopez, J.A. Villena, R. Simó, D.M. Selva, Sex hormone-binding globulin overexpression protects against high-fat diet-induced obesity in transgenic male mice, J. Nutr. Biochem. 85 (2020), 108480, https://doi.org/10.1016/j.jnutbio.2020.108480.