ACTA ABP BIOCHIMICA POLONICA

Hepatokines and non-alcoholic fatty liver disease

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Nowadays non-alcoholic fatty liver disease (NAFLD) is becoming the most common chronic liver pathology both in adults and children. NAFLD manifestation ranges from a simple liver steatosis to steatohepatitis (non-alcoholic steatohepatitis – NASH), which may progress to advanced fibrosis, cirrhosis and end-stage liver disease. Due to the coexistence of visceral obesity, insulin resistance and dyslipidemia, NAFLD is considered to be the hepatic manifestation of metabolic syndrome. In recent years, in the pathogenesis of metabolic syndrome, type 2 diabetes mellitus, cardiovascular disease and also NAFLD, more and more attention has been paid to the so-called organokines, proteins with both paracrine or/ and endocrine activities. These include most known adipokines (mainly produced by adipose tissue), myokines (mainly produced by skeletal muscles) and hepatokines exclusively or predominantly produced by the liver. It was shown that the liver may affect the lipids and glucose metabolism by hepatokines released into the blood and NAFLD seems to be associated with altered hepatokines production. Fetuin-A, fibroblast growth factor-21 (FGF-21), selenoprotein P, sex hormone-binding globulin (SHBG), angiopoietin-related growth factor (also known as angiopoietin-related protein 6) and leukocyte derived chemotaxin 2 (LECT2) are considered as the most important hepatokines. In this review, we provide an overview of the main hepatokines and we summarize the association of liver-derived proteins with the development and progression of NAFLD.

Key words: non-alcoholic fatty liver disease, fetuin-A, fibroblast growth factor-21, selenoprotein P, sex hormone-binding globulin, angiopoietin-related growth factor, leukocyte derived chemotaxin 2

Received: 19 January, 2016; revised: 10 May, 2016; accepted: 18 May, 2016; available on-line: 6 June, 2016

INTRODUCTION:

In recent years, more and more attention has been paid to the so-called organokines: proteins presenting both paracrine and/or endocrine activities. The most known and well-examined of them are adipokines: e.g. adiponectin, leptin, resistin, retinol-binding protein 4, visfatin, chemerin, omentine (fat-derived), myokines: e.g. irisin, interleukin 6, brain-derived neurotrophic factor, follistatin-related protein 1 (skeletal muscle-derived) and hepatokines, which are mainly produced by the liver. The latter include fetuin-A, fibroblast growth factor-21 (FGF-21), selenoprotein P, sex hormone-binding globulin (SHBG), angiopoietin-related growth factor (AGF), and leukocyte derived chemotaxin 2 (LECT2) (Parola &

Mara, 2011; Stefan & Haring, 2013; Reschke & Eckel, 2013; Lan et al., 2014).

Nonalcoholic fatty liver disease (NAFLD) is currently the most common chronic liver disorder both in adults and children. This is not a homogeneous disease entity; depending on the severity of the pathological process, it may present as an isolated hepatic steatosis (simple steatosis), nonalcoholic steatohepatitis (NASH), or may lead to liver cirrhosis and hepatocellular carcinoma (HCC) (Caldwell & Argo, 2010; Vernon et al., 2011; Ertle et al., 2011). In some cases, patients with NAFLD and advanced liver cirrhosis require liver transplantation (Burke et al., 2004; Heuer et al., 2012). Recently, genetic studies demonstrated that single nucleotide polymorphisms (SNPs) in genes involved in lipid metabolism (Lipin 1, patatin-like phospholipase domain containing-3-PNPLA3), oxidative stress (superoxide dismutase 2), insulin signaling (insulin receptor substrate-1), and fibrinogenesis (Kruppel-like factor 6) are associated with a risk for development and progression of NAFLD (Dongiovanni et al., 2013). It should be mentioned that NAFLD related to variation in PNPLA3 gene (SNP rs738409) is not associated with insulin resistance (Romeo et al., 2008; Kantartzis et al., 2009).

NAFLD affects mainly patients with visceral obesity, dyslipidemia, insulin resistance or impaired glucose tolerance. These features are also included in the definition of the metabolic syndrome. Therefore, NAFLD is considered as a specific manifestation of the metabolic syndrome (Targher et al., 2010; Pacifico et al., 2011; Gaudio et al., 2012). It was shown that both disorders are characterized by an increased tendency to develop an earlier atherosclerotic process than in the general population, and the results of the recent studies indicate that NAFLD is an independent factor associated with the progression of atherosclerosis (Targher et al., 2010). In comparison with the general population, the NAFLD patients also exhibited an increased risk of mortality mainly from cardiovascular disease (CVD) (Adams & Angulo, 2005; Targher et al., 2005).

This paper is the comprehensive review outlining the most recent updates on association of hepatokines with NAFLD.

As it was mentioned above NAFLD affects mainly patients with insulin resistance. There are multiple organs/ tissues involved in the pathogenesis of insulin resistance

Table 1. The characteristics of hepatokines

Hepatokine	Source of hepatokine	Targeting organs	Mechanism of actions	Functions	Serum concentration in insulin resistant states
Fetuin-A (Mori et al., 2006; Stefan et al., 2006; Dogru et al., 2013)	Mainly liver	Liver, skeletal muscles	Inhibits autophosphorylation of insulin receptor in liver and skeletal muscles Promotes activation of TLR 4, which induce inflammatory signaling, by saturated fatty acids	Biomarker of NAFLD, insulin resistance, cardio-vascular risk, neuro-degenerative disorders, subclinical inflammation	Increased
FGF-21 (Zhang et al., 2015)	Mainly liver, also adipose tissue, pancreas	Liver, Adipose tissue, Brain, Kidney	Liver- FGF-21 directly regulates lipid metabolism and reduces hepatic lipid accumulation in an insulin-independent manner; Adipose tissue- increases glucose uptake via GLUT1, regulates lipolysis, induces synthesis and secretion of adiponectin; stimulates uncoupling of protein 1 (UCP-1)	Biomarker of NAFLD Treatment with recombinant FGF-21 improves insulin sensitivity	Increased
Seleno-protein P (Choi et al., 2013)	Liver	Liver	Impairs insulin signaling in hepatocytes	Biomarker of NAFLD	Increased
SHBG (Simo et al., 2015)	Liver	Targeting organs for androgens and estrogens	Binding of sex steroids (androgens and estrogens) and regulation of their bioavailability	Sex – hormones transporter Biomarker of insulin resistance	Decreased
AGF (Kadomatsu et al., 2011)	Liver	Liver, Skeletal muscles, Endothelial cells	Regulates lipids, glucose (inhibition of gluconeogenesis) and increases systemic energy metabolism; induces angiogenesis and arteriogenesis by ERK1/2–eNOS–NO pathway in endothelial cells	Biomarker of obesity, type 2 diabetes mellitus	Increased
LECT2 (Lan et al., 2014)	Liver	Liver, skeletal muscles	Impairs insulin signaling in skeletal muscles	Biomarker of NAFLD, obesity???	Increased

with the skeletal muscles, adipose tissue and liver playing a significant role.

Liver is a very important organ of glucose metabolism responsible for the glucose storage (hepatic glycogenogenesis) and glucose production (gluconeogenesis). Additionally, liver plays a role in the lipoprotein metabolism. Insulin inhibits glucose release by the liver; it influences the glycogenogenesis, glycogenolysis and gluconeogenesis by controlling the activity of the key enzymes of these processes (Roden, 2006; Stefan et al., 2008).

Epidemiological studies revealed that the NAFLD is connected mostly to the visceral obesity and impaired glucose tolerance and/or type 2 diabetes mellitus. Another important observation is high prevalence of NAFLD in non-obese men from Asia, with polymorphism of apolipoprotein C3 (APOC3), which increases plasma apolipoprotein C3 concentrations. The consequence of this is the inhibition of lipoprotein lipase activity and impaired chylomicrons clearance (Petersen et al., 2010). Moreover, APOC3 mRNA which is increased in fatty liver is regulated by single nucleotide polymorphisms in APOC3 and its influence depends on visceral obesity (Peter et al., 2012). Additionally, NAFLD is a typical finding in lipodystrophic patients. The lipodystrophic syndromes, characterized by the lack of subcutaneous adipose tissue are associated with the marked insulin resistance in the skeletal muscles and the liver, increased plasma triglycerides (TG) concentrations and hepatic steatosis (Oral et al., 2002). These data suggest that the inability of adipose tissue to store lipids in the form of TG results in lipid overflow and ectopic fat accumulation in other organs. This can be caused by an excess of nutrients which leads to obesity with an inflammation in adipose tissue or dysfunctional adipose tissue which is seen in lipodystrophic syndromes. In these situations lipid overflow is crucial for the induction of insulin resistance in the liver. The important question is how intracellular hepatic fat accumulation influences insulin signaling and induces hepatic insulin resistance. An excessive free fatty acids (FFA) flux from the adipose tissue increases the availability of long chain fatty acids to the hepatocytes. The imbalance between fatty acids oxidation and storage in hepatocytes results in the synthesis of the intermediates of lipid metabolism like diacylglycerol (DAG) and ceramides. Data from the experimental studies revealed that lipid infusion results in an increase of DAG content in skeletal muscles in rodents and activation of the theta form of protein kinase C (PKCθ), so-called DAG-novel protein kinase C (nPKC). PKCθ inhibits insulin signaling by activation of serine phosphorylation in insulin receptor substrate-1 (IRS-1) (Yu et al., 2002). In experimental model of NAFLD, induced by high-fat diet, the impairment of insulin action was also related to another nPKC activity – epsilon PKC (PKCε) (Samuel et al., 2004). The data obtained by Kumashiro et al. (2011) in humans confirmed the role of DAG accumulation in the liver and activation of PKCε in pathogenesis of hepatic insulin resistance. Moreover, DAG content was the strongest

predictor of hepatic insulin resistance. Data from the mouse model with the deletion of the gene encoding PKCε confirmed the crucial role of this kinase in impairment of insulin action in the liver (Raddatz *et al.*, 2011). Review of the data indicates that accumulation of DAG in hepatocytes and subsequent activation of PKCε in the liver are responsible for the hepatic insulin resistance (Perry *et al.*, 2014, Bierkenfeld *et al.*, 2014). It gives the possibility for novel therapeutic approaches to diminish the epidemic of NAFLD.

An impaired insulin signaling in the liver results in increased endogenous glucose production and altered lipoprotein metabolism which leads to hyperglycemia, type 2 diabetes and atherogenic dyslipidemia. However, it is claimed that glucose and lipid metabolism might be also influenced by the liver-derived proteins (Bierkenfeld *et al.*, 2014).

FETUIN-A

Fetuin-A, also known as alpha-2-Heremans-Schmid glycoprotein (64 kDa), is produced mainly in the liver. The placenta and also tongue are mentioned as minor sources of this hepatokine (Denecke *et al.*, 2003). This glycoprotein was discovered in 1944 in bovine calves (Pedersen, 1944). In humans, fetuin-A gene is located on chromosome 3q27, which was identified as susceptibility locus for type 2 diabetes and metabolic syndrome (Siddiq *et al.*, 2005). This multipotential glycoprotein is involved in the regulation of bone metabolism, migration of keratinocytes, and control of proteolytic activity. It is also considered as a biomarker for neurodegenerative diseases as well as indicator of insulin resistance, cardiovascular risk, endothelial dysfunction and subclinical atherosclerosis (Stefan *et al.*, 2006; Roos *et al.*, 2010; Mori *et al.*, 2011; Mori *et al.*, 2012; Dogru *et al.*, 2013).

There is a suggestion that fetuin-A constitutes a link between obesity, insulin resistance and NAFLD (Mori *et al.*, 2006; Stefan *et al.*, 2006; Dogru *et al.*, 2013). Fetuin-A was shown to be an inhibitor of insulin receptor tyrosine kinase in the liver and skeletal muscles, causing inhibition of the autophosphorylation of tyrosine kinase in insulin receptor and insulin receptor substrate 1 (IRS-1) which leads to insulin resistance in rodents (Auberger *et al.*, 1989). This observation was also confirmed in humans (Srinivas *et al.*, 1993; Mori *et al.*, 2011). Several years later, further studies showed that mice lacking *Ahsg* gene, which encodes fetuin-A, had improved signaling of insulin (Mathews *et al.*, 2002). This finding suggested that this hepatokine might play a crucial role in regulation of insulin sensitivity. It was also shown that fetuin-A plays an important role in lipid-induced insulin resistance in mice (Pal *et al.*, 2012) as well as in humans (Stefan & Haring, 2013; Stefan *et al.*, 2014). The high concentration of saturated fatty acids (via NF-kB activation) and increased blood glucose (via ERK 1 or 2 signaling pathways activation) induce the hepatic synthesis of this hepatokine (Takata *et al.*, 2009; Dasgupta *et al.*, 2010). Hepatocytes secrete fetuin-A into the blood and the protein binds the insulin receptor in tissues, inhibiting insulin signaling and therefore inducing insulin resistance. In addition, this protein acts as an adapter for saturated fatty acids which by activating Toll-like receptors (TLR) 4 induce inflammatory signaling and insulin resistance (Pal *et al.*, 2012; Heinrichsdorff *et al.*, 2012).

Many authors (Stefan *et al.*, 2006; Reinekr *et al.*, 2008; Haukeland *et al.*, 2012) found an association of fetuin-A and fat accumulation in the liver measured in proton magnetic resonance spectroscopy in obese adults. It therefore appears that the increase in the production of fetuin-A in patients with hepatic steatosis is closely associated with ectopic fat accumulation in the liver, which is observed in NAFLD.

A number of publications demonstrated significantly higher serum fetuin A concentration in a population of adults with morphologically confirmed NAFLD (Yilmaz *et al.*, 2010; Haukeland *et al.*, 2012; Dogru *et al.*, 2013) and also in children (Reinehr *et al.*, 2008; Lebensztejn *et al.*, 2014) compared to the control group.

In our study (Lebensztejn *et al.*, 2014) the correlation of this glycoprotein with HOMA-IR and lipid profile parameters in patients with NAFLD was not confirmed. Haukeland *et al.* (2012), like in the above-mentioned analysis, did not find any relationship of this biomarker with insulin resistance (HOMA-IR) and lipid metabolism. However, they confirmed the correlation of hepatic expression of fetuin-A with carbohydrate and lipid metabolism key enzymes (SREBP1c, CPT1, PEPCK1, Glu-6-P) but there was no correlation of serum fetuin-A with mRNA of this glycoprotein in the liver, which may explain the lack of correlation in serum. In other study (Ou *et al.*, 2012), positive fetuin-A correlations with HOMA-IR and serum triglycerides concentrations were described. In humans, in the study by Stefan *et al.* (2006) the negative correlation between plasma fetuin A and insulin sensitivity assessed by the euglycemic clamp technique was observed. Importantly, plasma fetuin A concentration was also related to the fat accumulation in the liver, thus suggesting that this protein can serve as a potential link between fatty liver and insulin resistance. Accordingly, the strong relationship between plasma fetuin A and insulin resistance (Matsuda index) in patients with NAFLD was reported (Stefan *et al.,* 2014). Additionally, fetuin A was claimed to be a predictor of type 2 diabetes in humans (Stefan *et al.,* 2014).

Divergent results were observed regarding fetuin-A associations with the severity of liver fibrosis. Yilmaz *et al.* (2010) showed a positive correlation of this hepatokine and fibrosis score in patients with NAFLD, Sato *et al.* (2015) confirmed the fetuin-A negative correlation with NAFLD fibrosis score, whereas others did not confirm the association of fetuin-A with the result of the morphological study of liver bioptates (Haukeland *et al.*, 2012; Dogru *et al.*, 2013; Kahraman *et al.*, 2013; Rametta *et al.*, 2014). It was demonstrated that fetuin-A mRNA expression was higher in NASH compared to patients with simple fatty liver (Kahraman *et al.*, 2013). Moreover, the administration of metformin in patients with NAFLD (randomized placebo control trial), although did not cause histological improvement (Haukeland *et al.*, 2009), resulted in the reduction of the serum concentration of fetuin-A (Haukeland *et al.*, 2012). Similarly, the reduction of body weight in obese NAFLD patients leading to a reduction in hepatic fat content resulted in lowering its serum concentration (Reinehr *et al.*, 2008). It was also shown that even short–term, 7-day lasting physical exercise, brought a positive response in lowering the serum concentration of this hepatokine despite the fact, that there was no effect on body weight or hepatic triglyceride content in proton MRI (Malin *et al.*, 2013).

Although it was demonstrated in the general population that fetuin-A is associated with an increased risk of myocardial infarction and ischemic stroke independently of standard risk factors (Weikert *et al.*, 2008); it has not yet been resolved, whether fetuin-A may be considered as a valuable marker of cardiovascular risk in patients with NAFLD. Only Dogru *et al.* (2013) showed a posi-

tive correlation between fetuin-A and carotid intima media thickness (cIMT) in adults with NAFLD and markers of serum endothelial dysfunction. The opposite results were obtained by Sato et al. (2015); they found a negative correlation of fetuin-A with average IMT values. Our study found that there was no correlation between hepatokine concentration and any atherosclerotic endothelial changes assessed by measuring IMT, which is a widely recognized marker of early biophysical atherosclerotic process. Additionally, no significant difference in the IMT of children with NAFLD compared to non-NAFLD children was observed (Lebensztejn et al., 2014). Probably, longer exposure to the disease in children is necessary to determine significant differences in IMT value. Yoo et al. (2015) suggested that a very probable mechanism wherein the fetuin-A may cause atherosclerosis is the induction of insulin resistance and inflammatory processes by an increased expression of cytokines on monocytes.

FIBROBLAST GROWTH FACTOR-21

FGF-21 is a 209 amino acid peptide produced mainly in the liver, but the expression of this protein in the lower extent was also found in the pancreas, testes, duodenum and adipose tissue; gene for FGF-21 is localized on chromosome 19 (Nishimura et al., 2000; Fon Tacer et al., 2010; Li et al., 2013). It is a hepatokine which regulates lipid and carbohydrate metabolism and it was demonstrated that it may play a significant role in the pathogenesis of NAFLD (Liu et al., 2015). FGF-21 directly regulates lipid metabolism and reduces hepatic lipid accumulation in an insulin-independent manner (Zhang et al., 2011; Li et al., 2012; Lee et al., 2014). It affects selected elements of the "multi-hits theory", such as oxidative stress, endoplasmic reticulum stress, mitochondrial dysfunction, and low-grade chronic inflammation (Nishimura et al., 2000; Jiang et al., 2014; Fisher et al., 2014; Kim et al., 2015).

A number of publications showed significantly higher serum concentrations of FGF-21 in a population of patients with NAFLD compared to the controls. It should be emphasized that NAFLD was diagnosed on the basis of different diagnostic criteria (ultrasound, MRI and liver biopsy) (Li et al., 2010; Yilmaz et al., 2010; Reinehr et al., 2012; Giannini et al., 2013). In our preliminary study serum FGF-21 level was significantly higher in children with NAFLD (diagnosed in ultrasound and ^{1}HMRS) compared to the controls and to the non-hepatopathic obese patients (Lebensztejn et al., 2016). Only Alisi et al. (2013) in the group of children with biopsy-proven NAFLD showed significantly lower serum concentrations of FGF-21 compared to the controls.

In the studies conducted on patients with NAFLD, it was confirmed that circulating FGF-21 was significantly positively correlated with the hepatic fat content assessed in proton magnetic resonance spectroscopy (Yan et al., 2011), MRI (HFF%) (Giannini et al., 2013); moreover, the correlation of this peptide with intrahepatic triglycerides assessed in liver biopsy was also confirmed (Li et al., 2010).

It was also shown that hepatic FGF-21 mRNA was significantly elevated in NAFLD patients and it correlated with serum levels of this hepatokine (Li et al., 2010; Dushay et al., 2010); in addition it also correlated positively with the degree of liver steatosis (Li et al., 2010). However, Yan et al. (2011) demonstrated lower concentrations of FGF-21 in the patients with severe steatosis measured in ^{1}HMRS. The study of Li et al. (2013) suggested that high level of serum FGF-21 may be considered to be an independent predictor of NAFLD. Moreover, Jiang et al. (2014) confirmed that the single nucleotide polymorphism rs499765 is associated with FGF-21 and NAFLD in non-diabetic population.

There is some data regarding the association of FGF-21 and the stages of the severity of liver fibrosis. In the animal model, studies demonstrated that FGF-21 is induced in response to toxic lipid accumulation in the early stage of methionine- and choline-deficient NASH (Tanaka et al., 2015). In pediatric population, Alisi et al. (2013) found a strong inverse correlation between FGF-21 circulating level and NAFLD severity (fibrosis). This data was supported by animal studies which showed that tumor necrosis factor alpha and NFE2 (oxidative stress-activated transcription factors) may impair FGF-21 transcription and release (Chartoumekis et al., 2011; Diaz-Delfin et al., 2012). It was also shown that FGF-21 could be regarded as a non-invasive biomarker useful in differentiation of simple fatty liver and NASH. Shen et al. (2012) showed that the total rating of cytokeratin-18 and FGF-21 may be used in non-invasive diagnosis of NASH.

It is suggested that the increase in the hepatic expression of FGF-21 is a response to FGF-21 resistance, and just FGF-21 resistance may be involved in the pathogenesis of NAFLD/NASH. The increase of FGF-21 level may be a potential protective factor against lipid and carbohydrate metabolism disorders (Hui et al., 2013; Liu et al., 2015). In diet-induced obese mice, administration of FGF-21 caused a decrease in hepatic steatosis, reduced levels of triglycerides in serum and liver, and reversible fatty liver by inhibition of SREBP-1, a key transcription factor of lipogenesis (Badman et al., 2007; Xu et al., 2009).

In addition to the reversibility of hepatic steatosis, intravenous administration of FGF-21 improved insulin sensitivity and decreased fasting glucose in animal models of NAFLD (Coskun et al., 2008; Xu et al., 2009). It was also shown that adenovirus-mediated knockdown of hepatic FGF-21 leads to the development of hepatic steatosis and dyslipidemia due to impaired expression of genes involved in lipid metabolism in the liver (Hui et al., 2013). Fisher et al. (2014) conducted a study of FGF-21-knockout mice, stating that in the absence of FGF-21, accumulation of inactivated fatty acids results in lipotoxic damage and increased steatosis. It was demonstrated by studying OLEFT rats that lifestyle modification (chronic exercise and caloric restriction) reduces hepatic expression of FGF-21 mRNA and circulating FGF-21 concentrations, which may indicate that such therapy alleviate FGF-21 resistance (Fletcher et al., 2012).

At present, FGF-21 is considered a novel metabolic regulator, which is involved in the pathogenesis of NAFLD. Thus, it seems that it could be used in the treatment of NAFLD/NASH. Subcutaneously administered short-acting FGF-21 analog (LY2405319) improved insulin sensitivity and lipid profile in ob/ob and diet-induced obese mice (Kharitonenkov et al., 2013). Gaich et al. (2013) analyzed the effect of LY2405319 in a randomized, placebo controlled trial in a small group of obese patients with type 2 diabetes. They found the significant improvements in dyslipidemia and favorable effect on body weight, fasting insulin and adiponectin level. Mu et al. (2012) using pegylated human FGF-21 analog twice-weekly observed reduced blood glucose, plasma lipids, liver triglycerides, and plasma glucagon and enhanced pancreatic insulin content, islet num-

ber, and glucose-dependent insulin secretion in rodents (*db/db* mice). Further studies are needed to confirm the efficacy of this therapy in humans with NAFLD.

SELENOPROTEIN P

Selenoprotein P (SEPP1) is a 60kDa glycoprotein produced primarily in the liver; it is considered a selenium-carrier protein transporting it to tissues such as the testes and brain (Burk & Hill, 2005). Human selenoprotein P gene was mapped to 5q31 (Hill *et al.*, 1996). In patients diagnosed with type 2 diabetes, it was shown that serum concentrations of this hepatokine positively correlated with the insulin hepatic overproduction of selenoproteins P causing insulin resistance (Misu *et al.*, 2010). Similarly, a positive correlation with the parameters associated with cardiovascular risk factors (circumference waist, hsCRP, triglycerides level, carotid intima media thickness) as well as aspartate aminotransferase activity was noted (Yang *et al.*, 2011). An inverse correlation of selenoproteins P level with the adiponectin was also observed (Misu *et al.*, 2012).

In NAFLD patients with visceral obesity, higher concentration of selenoprotein P were observed compared to the control group. In addition, patients in the highest selenoprotein P tertile showed a 7.5 times greater risk of NAFLD than those in the lowest tertile. Moreover, the correlation between serum selenoprotein P and LAI (liver attenuation index) assessed by computed tomography was also found, which can be considered as semi-quantitative indicator of hepatic fat accumulation. Therefore, this hepatokine may be regarded as a novel biomarker of NAFLD (Choi *et al.*, 2013). In patients with type 2 diabetes and the patients with NAFLD alike, there was the correlation of selenoprotein P with cardiovascular risk factors, i.e. subclinical parameters of inflammation and arterial stiffness (Choi *et al.*, 2013).

Misu *et al.* (2010) demonstrated that administration of selenoprotein P impaired insulin signaling in hepatocytes and selenoprotein P deficiency in mice leads to an improvement in insulin sensitivity due to inactivation of adenosine monophosphate-activated protein kinase. Therefore, they suggested that selenoprotein P may be the target for the treatment of insulin resistance-associated diseases. However, further experimental and clinical studies are necessary to confirm this hypothesis.

SEX HORMONE-BINDING GLOBULIN

Sex hormone-binding globulin (SHBG) is produced mainly in the liver (Khan *et al.*, 1981). The gene coding human SHBG is localized in the p12-p13 region on the short arm of chromosome 17 (Berube *et al.*, 1990). The main function of this hepatokine is the sex hormone transport, however, SHBG circulating concentration is also associated with glucose metabolism, adiposity and metabolic syndrome components (Sutton-Tyrrell *et al.*, 2005; Peter *et al.*, 2010; Lazo *et al.*, 2015).

In postmenopausal women with biopsy-proven NAFLD (Polyzos *et al.*, 2013), there was lower concentration of this hepatokine detected compared to the control group and it was found that the serum concentration of SHBG was associated with NAFLD regardless of age, BMI and waist circumference. Similarly, the association of low concentration of this hepatokine with NAFLD was also demonstrated in type 2 diabetic patients (Hua *et al.*, 2014).

In addition, it was also shown that serum concentrations of SHBG decreased with an increase of intrahepatic fat content and that introduction of lifestyle modification as a therapy for obesity caused an increase in circulating concentration of SHBG, which was associated with a decrease of liver fat content (Stefan *et al.*, 2009). An analogous relationship between SHBG and intrahepatic fat content was confirmed in different groups of patients where liver steatosis was assessed using ultrasound (Shin *et al.*, 2011; Fletcher-Mors *et al.*, 2014), MRI (Bonnet *et al.*, 2013) or computed tomography (Kavanagh *et al.*, 2013; Lazo *et al.*, 2015).

Recently, it was found that the production of SHBG is regulated by adiponectin. Simo *et al.* (2014) showed that adiponectin increases SHBG production by the activation of AMPK leading to a reduction in hepatic lipid content. Peter *et al.* (2010) demonstrated that circulating SHBG concentration was closely associated with hepatic insulin sensitivity independently of adiponectin and fetuin-A.

So far, the only one study regarding the simultaneous evaluation of several hepatokines in the group of men with the disturbances of glucose and lipid metabolism did not confirm an association between SHBG and neither fetuin-A nor FGF-21 concentrations (Bonnet *et al.*, 2013).

ANGIOPOIETIN-RELATED GROWTH FACTOR

Angiopoietin-related growth factor (AGF) (also known as angiopoietin-related protein 6) encoded by the gene *Angptl6,* secreted into the circulation predominantly from the liver, was identified by Oike *et al.* (2003). They also found that AGF-deficient mice developed obesity, insulin resistance and lipid accumulation in liver and skeletal muscle, and hepatic overexpression of AGF by adenoviral transduction was also connected with an increased level of circulating AGF. Therefore this hepatokine might have an important function in lipid and carbohydrate metabolism and might be regarded as a protein that antagonizes obesity and insulin resistance (Oike *et al.*, 2005). However, Ebert *et al.* (2009) showed that serum AGF concentration is significantly increased in diabetic compared with non-diabetic patients. In the further study conducted by these authors, in a large, well- metabolically characterized cohort (Ebert *et al.*, 2014), patients with type 2 diabetes had significantly higher serum AGF concentration in comparison to controls. In addition, AGF positively correlated with insulin resistance markers and negatively with HDL-cholesterol. Namkung *et al.* (2011) showed higher levels of AGF in patients with metabolic syndrome compared to the controls and the metabolic syndrome was a predictor of serum AGF level. Therefore, it was demonstrated, that the results for AGF concentrations in humans are different than those obtained in an experimental model. Namkung *et al.* (2011) suggested that the function of AGF may be different in humans than in animals. The patients with metabolic syndrome or type 2 diabetes may present decreased sensitivity to AGF (AGF resistance). Kitazawa *et al.* (2007) proposed a theory that the mechanism through which the AGF affects the glucose metabolism is due to the fact that the AGF suppresses glucose production in hepatocytes in a concentration-dependent manner by reduced expression of glucose-6-phosphatase at both transcriptional and translational levels. In addition, phosphatidylinositol 3-kinase- and protein kinase B-dependent nuclear export of forkhead box class O1 appears

to mediate this effect. So far, there are no publications on the role of the AGF in NAFLD available but the abovementioned data suggest that this hepatokine might potentially be involved in the pathogenesis of this disease. In addition, *in vitro* assays showed reduced hepatic gluconeogenesis after treatment with AGF (Kitazawa *et al.*, 2007), suggesting that AGF can be considered as a novel therapeutic target of diabetes, obesity, metabolic syndrome as well as NAFLD.

LEUKOCYTE DERIVED CHEMOTAXIN 2

Recently, a novel hepatokine, leukocyte derived chemotaxin 2 (LECT2), linking obesity with muscle insulin resistance was discovered (Lan *et al.*, 2014). Firstly, LECT2 was identified as a novel neutrophil chemotactic protein (Yamagoe *et al.*, 1996). In humans LECT2 is mainly expressed in hepatocytes (Yamogoe S *et al.*, 1998). Data from animal studies pointed out that LECT2 expression in the liver is higher after high-fat diet. The mice knockout for LECT2 exhibited improved insulin sensitivity in skeletal muscles thus suggesting LECT2 role in metabolic disorders. The treatment with recombinant LECT2 protein induced insulin resistance in skeletal muscles by impairing insulin signaling (Lan *et al.*, 2014). In experimental studies, *Lect2* expression in the liver was negatively regulated by energy-depletion sensing AMP kinase. Moreover, in human study serum LECT2 correlated positively with BMI, waist circumference, HOMA-IR and HbA1c (Lan *et al.*, 2014). Okumura *et al.* (2013) reported that serum LECT2 level was significantly higher in patients with obesity and NAFLD.

SUMMARY

The obesity epidemic has also led to the changes in the epidemiology of liver diseases. At present, NAFLD is the most common liver disorder. The pathogenesis of this disease is complex and has not been fully elucidated yet. The experimental and human studies point out the role of the exclusively or predominantly liver-derived hepatokines. It was shown that the liver may affect the metabolism of lipids and glucose by the hepatokines release into the blood and NAFLD seems to be associated with their altered production. Moreover, it appears that hepatokines may be regarded as biomarkers of an ectopic fat accumulation in the liver and markers of the disease progression. It was suggested that some of them may be the target for prevention and treatment of insulin resistance-associated diseases also including NAFLD. However, further experimental and clinical studies are necessary to confirm these hypotheses.

Acknowledgements

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