

Fetuin-A (AHSG) and its usefulness in clinical practice. Review of the literature

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Background. Fetuin-A, also called Alpha 2-Heremans Schmid Glycoprotein, is a multifunctional plasma agent what has been proven in animal and human studies. It plays a role as a physiological inhibitor of insulin receptor tyrosine kinase associated with insulin resistance and a negative acute phase reactant. It also regulates bone remodeling and calcium metabolism being an important inhibitor of calcium salt precipitation and vascular calcifications.

Methods. PubMed database was searched for articles from 2002 up to December 2014 to identify the role of fetuin-A in the pathogenesis of selected internal diseases.

Results. Due to secretion of fetuin-A mainly by the liver, it may be a marker of liver function and predictor of mortality in patients with cirrhosis and hepatocellular cancer. The associations between high fetuin-A and metabolic syndrome as well as its hepatic manifestation- nonalcoholic fatty liver disease and atherogenic lipid profile have been well proven. However, fetuin-A relation with BMI is not so clear. Contrary to few reports, many authors suggest that fetuin-A may be an independent risk factor for type 2 diabetes and marker of diabetic complications. Close associations of high and low fetuin-A concentrations with cardiovascular diseases and mortality risk have been reported which is explained by differences in analyzed populations, stages of atherosclerosis and calcifications, coexistence of type 2 diabetes or kidney dysfunction and different main pathways of fetuin-A actions in various diseases.

Conclusions. Fetuin-A has a diagnostic potential as a biomarker for liver dysfunction, cardiovascular diseases and disorders associated with metabolic syndrome.

Key words: fetuin-A, diabetes, obesity, cardiovascular disease, atherosclerosis

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INTRODUCTION

Fetuin-A, also called Alpha 2-Heremans Schmid Glycoprotein (AHSG), is a multifunctional plasma agent with a molecular weight of approximately 60 kDa and half-life of several days^{1,2}. It was first discovered in 1944 by Kai O. Pedersen in calf serum³. Several years later, J.F. Heremans (in 1960) and K. Schmid with W. Burgi (in 1961), in the independent studies, isolated it in humans⁴. During fetal development fetuin-A is abundantly synthesized by multiple tissues. In adults it is secreted predominantly by the liver (>95%) (ref.^{1,5}). Fetuin-A is a physiological inhibitor of insulin receptor tyrosine kinase and thus associated with insulin resistance, metabolic syndrome (MetS) and an increased risk for type 2 diabetes^{1,6}. It also accumulates in bones and teeth as a major fraction of noncollagenous bone proteins and regulates bone remodeling and calcium metabolism being an important inhibitor of calcium salt precipitation and calcification in vivo^{2,7}. Fetuin-A has adipogenic properties, reduces expression of the atheroprotective adipokine- adiponectin and deteriorates free fatty acid uptake and storage in adipocytes^{3,8,10}. Therefore fat accumulation in the liver may be associated with higher levels of fetuin-A (ref.⁷). Further the glycoprotein plays a role as a negative acute phase reactant and its level is inversely correlated with CRP concentration in serum^{1,2,11}. The reference values of serum

fetuin-A in healthy human subjects range from 450 to 600 µg/mL (ref.^{6,7}). Data indicate that fetuin-A concentrations are independent of gender but basal serum fetuin-A level might be influenced genetically^{12,13}.

The role of fetuin-A in the pathogenesis of: type 2 diabetes mellitus, obesity, nonalcoholic fatty liver disease (NAFLD), dyslipidemia, atherosclerosis and cardiovascular disease (CVD) is still discussed. Whereas high fetuin-A concentrations are found to be associated with MetS and atherogenic lipid profile, low fetuin-A levels are connected to vascular calcifications and inflammation⁸.

Structure of fetuin-A

In humans, the gene for fetuin-A is localized on chromosome 3q27, which has been mapped as a susceptibility locus for MetS and type 2 diabetes mellitus^{6,14,15}. What is more, several single nucleotide polymorphisms (SNPs) of the fetuin-A gene were found to be associated with adipocyte insulin action, dyslipidemia and type 2 diabetes as well¹⁶⁻¹⁸. The precursor protein of fetuin-A is made up of three chains, but only A and B chains, which are encoded by a single mRNA transcript, form the active protein. The longer A chain is composed of two cystatin-like domains D1 and D2, consists of 282 amino acid residues and contains 24% β-pleated sheet, 29% α-helix and 26% reverse turns. Domain D1 mediates the most studied function of fetuin-A but it has not been known yet which domain

interacts with the insulin receptor^{7,19}. The B chain is made up of 27 amino acid residues which are distributed unequally into the neutral and charged portion⁷.

The relationship between fetuin-A and insulin resistance

Insulin resistance is the main outcome caused by nutrient overload, lipids, infections and sepsis-induced inflammation that affects insulin-sensitive tissues such as: the liver, muscles, adipose tissue, hypothalamus and which also promotes defects in cell signaling pathways and homeostasis²⁰. It is well known that fetuin-A belongs to the cystatin family of the proteinase inhibitors and natural inhibitors of insulin receptor^{4,7}. Stefan et al. reported that fetuin-A levels were positively associated with fasting and stimulated insulin levels and negatively with insulin sensitivity⁵. Animal studies in rats following acute injection of human recombinant fetuin-A, have shown that it suppresses tyrosine kinase activity in muscles and in the liver by inhibiting the autophosphorylation of this enzyme and insulin receptor substrate proteins (IRS-1) as well^{14,17}. The function of insulin receptor and the role of fetuin-A in its action have been shown in Fig. 1 (ref.⁷).

Moreover fetuin-A knockout mice demonstrated increased basal and insulin-stimulated phosphorylation of insulin receptor, increased glucose clearance and improved insulin sensitivity^{5,14}. Additionally fetuin-null mice have been protected to weight gain when they have been challenging with a high-fat diet^{14,21}. Furthermore in those animal model lower serum free fatty acid and triglyceride levels have been observed^{15,22}. Mathews et al., based on mice study, suggested that the absence of fetuin-A may contribute to the improvement of insulin sensitivity associated with aging²³. Thus fetuin-A is found to be involved in the development of insulin resistance and is

an independent determinant of it measured by the index of homeostasis model (HOMA-IR) (ref.^{3,12,13}). Brix et al. reported that fetuin-A levels and HOMA-IR decreased significantly after bariatric surgery-induced weight loss¹⁸. Study conducted in pregnant women indicates that fetuin-A concentrations have been positively associated with maternal insulin resistance¹⁴. Moreover *in vitro* and *in vivo* fetuin-A suppresses adiponectin expression which improves insulin resistance and therefore plays a role as an independent determinant of circulating adiponectin in humans^{3,12,24}. Higher fetuin-A and lower adiponectin levels may contribute to obesity-induced insulin resistance and development of diabetes^{13,24}.

Fetuin-A and inflammation

Fetuin-A is a negative acute phase reactant and its level is inversely correlated with CRP concentration in the serum^{1,2,11}. The glycoprotein is downregulated by interleukin-1 beta (IL-1 β) which is over-expressed in adipose tissue from obese individuals and is reported to regulate lipid metabolism *in vivo* and to control adipocyte differentiation^{5,19}. What is more, fetuin-A may induce low grade inflammation which is associated with MetS and atherogenic lipid profile¹³. In addition, tumour necrosis factor- α (TNF- α) decreases fetuin-A expression *in vitro*^{5,12}. On the other hand, fetuin-A increases the expression of TNF- α and IL-6 *in vitro* and in rats as well¹². It has been also suggested that fetuin-A increases the risk of infection by promoting the phagocytic activity of macrophages⁷.

The role of fetuin-A in metabolic syndrome and its components

Metabolic syndrome, currently affecting 10-30% of the world population, is a clustering of increased waist circumference, dyslipidemia, impaired glucose metabolism and hypertension^{14,25}. A close association between fetuin-A concentration and MetS has been shown in cross sectional studies^{8,14,26}. Moreover in longitudinal analyses fetuin-A has been correlated to many components of MetS such as high blood pressure, waist circumference, cholesterol levels^{3,13,14}. The Heart and Soul Study indicated that in non-diabetic patients with coronary artery disease higher fetuin-A level was associated with MetS and atherogenic lipid profile^{3,23}. Hypothesis that fetuin-A may promote the MetS phenotype in humans is supported by few arguments. Firstly, the human fetuin-A gene residues on chromosome 3q27 which has been mapped as MetS quantitative trait locus^{6,14,16}. Secondly, fetuin-A interferes with insulin effect at peripheral tissues through interaction with the insulin receptor and enhancement insulin resistance which is thought to be the mechanism leading to the MetS phenotype⁸. Thirdly, fetuin-A induces suppression of adiponectin production which is the insulin-sensitizing adipocytokine representing an important determinant of the whole body sensitivity and cardiovascular disease^{27,28}. Fourthly, fetuin-A induces low-grade inflammation also associated with MetS and atherogenic lipid profile^{13,14}. Finally, fetuin-A knockout mice are resistant to weight gain on high-fat diet²².

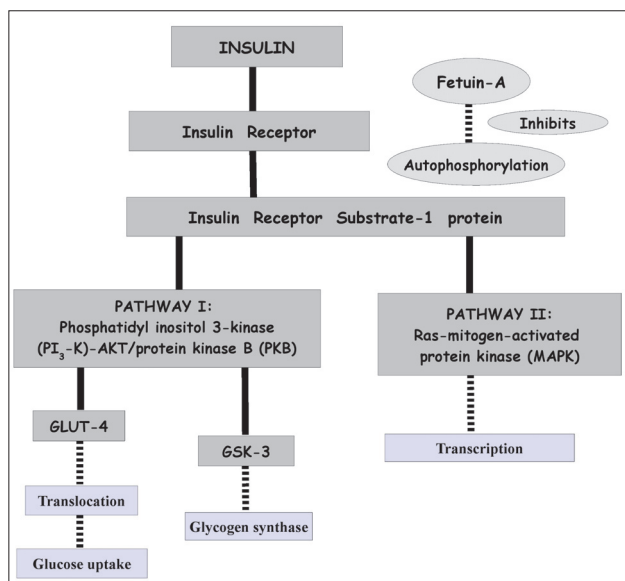


Fig. 1. The action of insulin receptor. Two main pathways: the phosphatidylinositol 3-kinase (PI3-K)-AKT/protein kinase B (PKB) and the Ras-mitogen-activated protein kinase (MAPK) pathway. GLUT4- glucose transporter-4; GSK-3- glycogen synthase kinase-3.

Although the association between fetuin-A and metabolic syndrome (understood as a disease entity) has been well proven, the relationship between BMI and fetuin-A is not so clear. On the one hand, Stefan et al. reported no association between fetuin-A and the percentage of body fat as well as Kaushik et al. found no correlation between BMI and fetuin-A in individuals with metabolic syndrome^{5,6}. On the other hand, data from the Heart and Soul Study as well as reports by Ismail et al. and Singh et al. demonstrated that elevated fetuin-A concentrations were connected with BMI and waist circumference^{7,8,13}. In turn, Erdmann et al. suggested that fetuin-A level may have not a similar connection to BMI when the entire range of obesity and morbid obesity is considered and the link between these parameters is rather non-linear²⁶. Brix et al. reported that fetuin-A was markedly increased in patients with morbid obesity and the reduction of its level after weight loss could play an important role in the beneficial effects of gastric bypass surgery¹⁸. Obese children showed elevated fetuin-A which decreased significantly during exercise- and diet-induced weight loss^{14,18}. Chen et al. supported the thesis that high fetuin-A concentration is independently predictive of truncal obesity and its complications such as type 2 diabetes and cardiovascular diseases¹¹. Lavebratt et al. proved that a common variant of fetuin-A gene (homozygosity for the *rs2593813:G-230:Met-238:Ser* haplotype of the fetuin-A gene), associated with a lower fetuin-A level, was more common among lean than obese and overweight Swedish men¹⁹. In another study, conducted by Lavebratt et al. the authors suggested that a common variation, i.e. single nucleotide polymorphism *rs4917 (Thr230Met)* in the fetuin-A gene is associated with a marked increase in β 2-adrenoceptor sensitivity (affecting on sensitivity for lipolysis) in subcutaneous fat cells which may be of importance in body weight regulation¹⁶.

We speculate that the discrepancies of the results may be associated with the differences between the analyzed group of patients that vary in populations, number of participants, gender, age, comorbidities and BMI size as well.

Some authors supported the idea that elevated serum fetuin-A concentration is connected with atherogenic lipid profile and may contribute to the development of atherosclerosis^{7,12,29}. Chen et al. found a positive association between fetuin-A and dyslipidemia in non-diabetic hemodialysis patients¹¹. They thought that fetuin-A may predict visceral adiposity and dyslipidemia, especially hypertriglyceridemia in those subjects¹¹. It has been suggested that also in non-diabetic coronary artery disease patients without chronic kidney disease (CKD), fetuin-A correlates with dyslipidemia, especially with HDL-cholesterol, LDL-cholesterol and triglycerides levels⁸. A positive association between fetuin-A and LDL-cholesterol and triglycerides levels and an inverse correlation between fetuin-A and HDL-cholesterol concentration have been explained by the inhibitory effect of fetuin-A on adipose tissue (through suppression the insulin receptor tyrosine kinase), which leads to increased lipolysis and free fatty acid efflux⁸. In turn, efflux free fatty acids from adipose tissue leads to increased production of apolipoprotein B,

the component of VLDL- and LDL-cholesterol. It may promote the atherogenic lipid profile⁸.

Moreover the role of fetuin-A gene variations in regulation of lipolysis and lipid disturbances have been postulated, for instance two fetuin-A SNPs (*-469T>G* and *IVS6+98C>T*) were connected to dyslipidemia and *-469T>G* SNP which is located in the 5' region of fetuin-A gene was associated with insulin-mediated inhibition of lipolysis and stimulation of lipogenesis. What is more, three fetuin-A gene SNPs (*-843A>T*, *-469T>G* and *6670T>C*) have been linked to circulating levels of cholesterol^{15,30}. Furthermore, fetuin-A knockout mice have a lower triglyceride and free fatty acid level as compared with wild-type controls⁷.

The role of fetuin-A in blood pressure regulation is discussed^{6,14,19}. As well the insulin resistance- regulated partially by fetuin-A activity as vascular calcifications- inhibited by fetuin-A, are risk factors for hypertension¹⁹. Reinehr and Roth described that in obese children with MetS fetuin-A has been correlated significantly to the systolic and diastolic blood pressure¹⁴. Similar results in adult obese patients have been reported by many authors^{3,6,13}. In contrast to them, Brix et al. and Lavebratt et al. did not find any connection of fetuin-A to blood pressure^{18,19}. We postulate that the discrepancies of the results may be associated with differences between analyzed group of patients, e.g. number of participants or used drugs.

Fetuin-A as a marker of liver function

In adults about 95% of fetuin-A is secreted by the liver¹. Studies in humans with severe liver damage such as cirrhosis (alcoholic liver cirrhosis, primary biliary cirrhosis), chronic hepatitis and cancer indicate that serum fetuin-A levels are decreased in such patients^{5,31,32}. It may be the consequence of reduced fetuin-A synthesis by hepatocytes. This hypothesis is supported by the fact that subjects with advanced stages of liver failure have lower fetuin-A concentrations compared to those in less advanced stages³¹. However, not only the severity of the liver disease may influence on fetuin-A levels but also proinflammatory cytokines may play the additional but not main role³¹. Daveau et al. reported that IL-1, IL-6 and TNF- α inhibit synthesis of fetuin-A in rat liver and human HepG2 cells and elevated levels of these cytokines have been found in some diseases mentioned above^{33,34}. On the other hand, Kalabay et al. postulated that acute phase response does not play the crucial role of fetuin-A concentrations in subjects with liver diseases because fetuin-A is not markedly reduced during febrile states or episodes of infections (sepsis or pneumonias) and its serum level is strongly correlated with prothrombin activity- the best indicator of protein synthetic liver function³². What is more, Kalabay et al. proved that decreased serum fetuin-A level is a predictor of short-term mortality in patients with liver cirrhosis and hepatocellular cancer³².

The situation is different in the case of diseases with fat accumulation in the liver, i.e. hepatic manifestation of the metabolic syndrome^{35,36}. In human serum fetuin-A levels were positively correlated with liver fat measured

by H magnetic resonance spectroscopy⁵. Fetuin-A concentration has been also linked to non-alcoholic fatty liver disease (NAFLD), which is frequently associated with insulin resistance and MetS (ref.^{5,14}). Reinehr and Roth showed that obese children with NAFLD demonstrated higher fetuin-A levels than obese children without liver disease and healthy controls¹⁴. Similar results were obtained by Lebensztejn et al. in the larger group of children³⁷. In longitudinal analyses, under weight loss due to short-term diet or exercise, improvement in NAFLD, measured by a decrease in liver fat, was accompanied by a reduction of fetuin-A concentration^{3,5,24}. Therefore Stefan et al. proposed the hypothesis that fetuin-A expression is upregulated in fatty liver⁵. It has been supported by two arguments. Firstly, it is known that fetuin-A concentration is associated with insulin resistance, a liver fat is an important regulator of hepatic insulin sensitivity and fatty liver plays a crucial role in the development of type 2 diabetes. Secondly, in a rat model of fatty liver, an increase in fetuin-A mRNA expression has been observed⁵.

The link between fetuin-A and type 2 diabetes mellitus

The increasing worldwide prevalence of type 2 diabetes mellitus and obesity has projected concerns for increasing burden of cardiovascular morbidity and mortality. More than 25 million US adults have type 2 diabetes mellitus, and this figure will likely reach 50 million by 2050 given the current demographic trends and continued progression of obesity³⁸.

In cross-sectional studies, genetic analyses revealed that SNPs in the fetuin-A gene (located at a susceptibility locus for type 2 diabetes) are linked to type 2 diabetes^{15,17}. For instance, Siddiq et al. based on the cohort of French Caucasians, found that the major allele of fetuin-A SNP *rs1071592* is associated with type 2 diabetes. On the other hand, the minor allele of SNP *rs1071592* has been protective against the disease¹⁷. In turn, Andersen et al. showed a significant relationship of fetuin-A variants: *-469T>G* and *IVS6+98C>T* and type 2 diabetes in Danish subjects¹⁵.

Many authors suggest that fetuin-A may be an independent risk factor for type 2 diabetes^{9,21}. Both The Health, Aging and Body Composition Study (Health ABC) and European Prospective Investigation into Cancer and Nutrition (EPIC)- Potsdam Study indicate that participants with high fetuin-A levels have an increased risk of incident diabetes¹⁸. Similar results have been also described in other significant studies, including The Cardiovascular Health Study, The Nurses' Health Study (NHS) and The Tübingen Lifestyle Intervention Program (TULIP) (ref.^{39,40}). Some clinical reports confirm that patients with impaired glucose tolerance but not impaired fasting glucose have higher fetuin-A levels than normal subjects²⁶. A positive association between fetuin-A and type 2 diabetes has been also observed among participants with elevated plasma glucose levels within the nondiabetic range, whereas fetuin-A has been not associated with diabetes risk among individuals with normal glucose levels^{9,24}. What is more, the connection between serum fetuin-A concentration and the development of cardiovascular complications in diabetics has been reported¹. It is emphasized that an

elevated serum fetuin-A level is a sensitive marker of macrovascular complications in diabetics such as coronary atherosclerosis^{3,7}. Moreover, Mehrotra et al. observed higher serum concentration of fetuin-A among patients with advanced diabetic nephropathy (stages III and IV based on GFR) than in diabetics without kidney disease⁴¹. Lorant et al. found that serum fetuin-A levels were higher in patients without renal disease and with type 2 diabetes and peripheral arterial disease (PAD) than those in individuals with type 2 diabetes and without PAD (ref.⁴²).

However, in contrast to some authors findings, there are researchers who suggest that low serum fetuin-A levels are found in diabetics^{1,6}. Wojtysiak-Duma et al. in their study demonstrated that in subjects with type 2 diabetes serum concentration of fetuin-A was significantly lower as compared with healthy participants¹. Contrary to Lorant et al., Eraso et al. reported that decrease in fetuin-A level was associated with PAD in type 2 diabetes, in the absence of advanced kidney disease, beyond traditional cardiovascular risk factors^{42,43}. Some data indicate that fetuin-A concentrations are lower among individuals (in that diabetics) with end-stage renal disease (ESRD), on dialysis².

The association between elevated levels of fetuin-A and high risk of type 2 diabetes development is explained by mechanisms of insulin and fetuin-A actions. It is well known that insulin resistance has been identified as the major pathophysiologic determinant of type 2 diabetes^{6,7}. Fetuin-A playing the role as a inhibitor of the insulin receptor tyrosine kinase activity in the muscle and in the liver, inhibits insulin signaling and introduces insulin resistance which leads to deterioration of insulin secretion and decompensation of glucose homeostasis^{18,24}. What is more, the direct correlation of fetuin-A with visceral adiposity, observed in many diabetics, may lie on causal pathway between fetuin-A and incident diabetes²¹. It should not be forgotten that fetuin-A impacts on the regulation of subclinical inflammation but whether fetuin-A induces a chronic pro-inflammatory process in the human islets needs to be investigated in future studies⁴⁰. The reason of differences of fetuin-A effects on macroangiopathies and microangiopathies is not clear but the discrepancy may be due to the differences of the study populations and different distributions of severity of complications in the participants⁴⁴. Moreover, the discrepancy of fetuin-A concentration is interpreted that fetuin-A secretion may be a feedback defense mechanism against vascular calcification in early stages of diabetic and atherosclerotic disease, whereas lipid disturbances and hyperinsulinaemia could serve as triggers for hepatic fetuin-A release¹. In addition, the differences in fetuin-A levels in patients with chronic kidney disease (CKD) may be caused by stage of CKD and progressive loss of GFR- fetuin-A levels probably decline to subnormal concentrations only late during the course of progression of diabetic CKD, i.e. after approaching ESRD (ref.⁴¹). Finally, some drugs - insulin-sensitizing medications such as metformin, pioglitazone and niacin may affect on the level of fetuin-A in serum^{6,26}. Treatment with pioglitazone and metformin results in a decline in fetuin-A levels^{6,24}. Niacin treatment lowers

serum fetuin-A concentration as well and these change correlates with the beneficial changes in serum lipids^{6,26}. Furthermore, a short-term diet and exercise interventions result in reduction of serum fetuin-A concentrations²⁴. Other possible explanations have been also discussed in section below.

Fetuin-A as a marker of cardiovascular disease

Cardiovascular disease (CVD) is the leading cause of death in the world; the estimated number of deaths from it in 2008 was 17.3 million, comprising almost 30% of all deaths. Further, global CVD deaths are projected to increase to 23.4 million and it will be roughly 35% of all deaths in 2030 (ref.⁴⁵).

Previous studies indicate a close relationship between fetuin-A and CVD but the opposite correlations of this glycoprotein and CVD have been reported.

In a case-cohort study, Weikert et al. showed that patients with high fetuin-A concentrations had 4-fold increased risk for myocardial infarction and ischaemic stroke compared to subjects with low fetuin-A levels. What is more, higher fetuin-A levels were more strongly associated with a higher CVD risk in women than in men⁴⁶. Similarly, Vörös et al. observed that individuals with previous myocardial infarction had significantly higher concentrations of fetuin-A than healthy controls¹². Furthermore, a positive correlations of fetuin-A with arterial stiffness and increased *intima-media* thickness have been observed in healthy subjects and in patients with normal renal function^{12,18,47}.

Both the MetS and type 2 diabetes increase the risk of CVD (ref.⁷). Many authors found that high serum fetuin-A level may be a sensitive marker of macrovascular complications in diabetics^{3,7}. Singh et al. suggested that the high concentrations of fetuin-A have been linked to cardiovascular complications in the non-renal patients⁷. In turn, Zhao et al. reported that serum fetuin-A levels were independently associated with the presence and severity of coronary artery disease in participants with type 2 diabetes and Mehrotra et al. described that fetuin-A concentrations were positively correlated with coronary artery calcifications in nondialyzed subjects with diabetic nephropathy^{41,48}. On the other hand, some clinical studies have indicated the opposite trends- a decrease in fetuin-A levels have been associated with the presence of PAD in diabetics, an increased arterial stiffness, atherosclerotic plaque calcification and progression of cardiovascular disease^{14,48}.

Contrary to some reports, many previous studies indicate that fetuin-A concentrations are decreased in patients with stable angina and acute myocardial infarction, this glycoprotein may be independent predictor of death after ST-elevation myocardial infarction and low levels of fetuin-A are correlated to the severity of coronary arterial calcification^{49,50,51}.

It should be mentioned that the renal function has influence on cardiovascular risk due to accelerated vascular calcifications aggravated by hyperphosphataemia and hypercalcaemia. In addition, a direct link between inflammation and calcification has been suggested in di-

alysis patients². Consequently, patients with ESRD and CKD have higher cardiovascular disease risk than individuals with normal kidney function. Although Ix et al. reported that among subjects with coronary artery disease there is no evidence that mild to moderate CKD is associated with lower concentrations of serum fetuin-A, it is well known that subjects with ESRD have decreased levels of fetuin-A compared to patients with mild and moderate CKD and healthy individuals²⁷. Ketteler et al. proved that low fetuin-A concentrations may predict increased cardiovascular and all-cause mortality in persons with ESRD (ref.²). These results were also confirmed in haemodialysis as well as peritoneal dialysis individuals²⁷. As a calcification inhibitor and a negative acute phase protein, fetuin-A seems to have a protective role against cardiovascular mortality in dialyzed patients with severe vascular calcifications^{2,46,52}.

The reasons for the discrepancies mentioned above have not been clearly identified but some explanations have been proposed.

Roos et al. implied a biphasic association of fetuin-A with vascular disease, depending on the stage of atherosclerosis. In rather healthy diabetic and nondiabetic subjects without prevalent vascular disease, higher level of fetuin-A is associated with vascular risks but the reverse relationship is observed in patients with established vasculopathy⁵³. It is suggested that in early stages of CVD fetuin-A exacerbates the disease due to its effects to promote insulin resistance and dyslipidemia but in later stages of CVD high concentrations of fetuin-A have positive results because of fetuin-A ability to prevent vascular calcium deposition⁴⁸.

A dual function of fetuin-A has been also suggested by Mori et al. who found that the existence of kidney dysfunction may affect the different results in various studies⁴⁷. Lower fetuin-A concentrations are associated with CVD events and mortality in ESRD subjects and high levels of fetuin-A are connected to atherosclerosis and CVD in non-renal patients⁴⁴.

In turn, Zhao et al. supported the hypothesis that higher serum fetuin-A promotes the development of atherosclerosis in patients with metabolic disorders, including type 2 diabetes, in which insulin resistance is important mechanism leading to coronary artery disease. Therefore, in diabetes-related atherosclerosis metabolic pathways of fetuin-A action (through insulin resistance and adipocyte dysfunction) are more likely to play the main role⁴⁸. Besides the induction of insulin resistance, fetuin-A promotes cytokine expression in human monocytes and reduces the expression of the atheroprotective adipokine- adiponectin in animals^{8,9,46}. Moreover fetuin-A deteriorates free fatty acid uptake and storage in adipocytes¹⁰. A decrease in adiponectin levels that usually enhances oxidation of free fatty acids, observed in patients with coronary artery disease or with at high risk for CVD, may increase free fatty acid levels and lead to worsening atherosclerosis^{12,24,28}. On the other hand, in patients without diabetes, fetuin-A plays potentially protective role against coronary artery disease and acute cardiovascular events (inflammatory states) and prevents spontaneous

mineral precipitation in the vasculature. In this case, the fetuin-A pathway through inflammation and calcification is supposed to play the main role^{12,48}. This thesis is also supported by Laughlin et al. in the Rancho Bernardo Study with older persons, in which higher fetuin-A levels were associated with CVD death only in individuals with type 2 diabetes, whereas fetuin-A was inversely associated with CVD death in subjects without diabetes⁵⁴. Similar conclusions emerged from studies in patients with aortic stenosis- only in nondiabetic individuals an inverse correlation between fetuin-A and the presence of stenosis has been observed^{27,55}. Moreover, a strong association between low fetuin-A and CVD has been found in lean and without insulin resistance (measures as HOMA-IR) persons⁵⁵.

Similar explanation has been proposed by Jensen et al. They emphasized that the beneficial effects of fetuin-A to lower arterial calcification could be counterbalanced by insulin resistance, free fatty acid efflux from adipose tissue, and cytokine production in patients with type 2 diabetes or obesity, resulting in no association or even a direct association between fetuin-A and incident CVD in individuals with type 2 diabetes. In contrast, it is possible that the impact of worsened insulin resistance induced by fetuin-A may be more aptly tolerated among subjects without type 2 diabetes, obesity or insulin resistance. In this setting, low fetuin-A levels may predispose to greater arterial calcification, which may affect the risk of CVD events through mechanisms distinct from insulin resistance⁵⁵.

It should be also remembered about other reasons that may cause the discrepancies in discussed reports such as: different sizes of the analyzed groups, various mean age of participants and ELISA kits.

The possible explanations for the discrepancies in correlations between fetuin-A and CVD have been collected in Fig. 2.

Fetuin-A and atherosclerosis

Many factors have been identified to support the development of atherosclerosis including: insulin resistance, lipid abnormalities, hyperhomocysteinaemia, hyperuricaemia, age and sex⁶. Hyperglycemia and insulin resistance impair the normal function of endothelium and platelets which leads to inflammation, vasoconstriction and thrombosis. All these conditions increase the chances of atherosclerosis⁷. Ectopic calcifications can also contribute to atherosclerosis¹⁹. Recent studies indicate that fetuin-A concentration is inversely correlated with the severity of

atherosclerosis and the presence of atherosclerotic calcified plaques^{7,56}. Worsening of the atherogenic condition due to lower level of fetuin-A has been explained by the role of this glycoprotein as a vascular calcification inhibitor that keeps calcium and phosphorus solubilizes in serum and prevents hydroxyapatite deposition in vessel walls^{6,8,19}. Animal studies showed that fetuin-A knockout mice developed severe soft tissue and intravascular calcifications¹. As a major serum agent which prevents vascular calcification, fetuin-A is responsible for roughly 50% of the inhibition of calcium and phosphorus precipitation^{2,27}. Vörös et al. suggested that fetuin-A is an independent determinant of adiponectin levels which may indicate that the modulation of adiponectin by fetuin-A would be an important factor in the progression of coronary atherosclerosis¹². Fetuin-A deficiency has been proposed as a predictor of all-cause and cardiovascular mortality in haemodialysis patients due to accelerating vascular calcification^{7,19}. Moreover a relationship of fetuin-A with coronary artery calcifications in non-dialysed patients with diabetic nephropathy has been reported¹.

CONCLUSIONS

It seems that fetuin-A plays a crucial role in the pathogenesis of many disorders. However, further investigations should be performed to precisely establish the concentration range for fetuin-A in particular diseases and in specific group of patients. We conclude that fetuin-A may be a useful marker in clinical practice in the future.

ABBREVIATIONS

AHSG- Alpha 2-Heremans Schmid Glycoprotein, fetuin-A; HOMA-IR- homeostasis model assessment-insulin resistance; MetS- metabolic syndrome; PAD- peripheral arterial disease; CVD- Cardiovascular disease; NAFLD- nonalcoholic fatty liver disease; ESRD- end-stage renal disease; CKD- chronic kidney disease; TNF- α - tumour necrosis factor- α ; SNP- single nucleotide polymorphism.

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- Stage of atherosclerosis and different stages of arterial calcifications
- Presence / lack of type 2 diabetes
- Presence / lack of kidney dysfunction (ESRD, dialysis)
- Different main pathways of fetuin-A actions in various diseases
- Different analyzed populations (size, age)
- Different ELISA kits used to determination of serum fetuin-A levels

Fig. 2. The possible reasons for the discrepancies in correlations between fetuin-A and CVD.

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