Association of Fetuin-A with Carotid Intima-Media Thickness and Vascular Diseases

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Abstract

Fetuin-A, also known as α2-Heremans–Schmid glycoprotein (AHSG), is a member of the cystatin superfamily of cysteine protease inhibitors and has different functions in human physiology and pathophysiology. Most studies suggest a biphasic effect of fetuin-A depending on the stages of atherosclerosis. Serum levels of fetuin-A are decreased in cases of acute inflammation. Therefore, it is known as a negative acute-phase protein. Fetuin-A inhibits insulin signaling and pathological calcification and has emerged as a diabetogenic agent. Fetuin-A levels are also found to be related to visceral obesity and dyslipidemia. Some authors have claimed that fetuin-A has a proatherogenic role, but it is still unclear whether high fetuin-A levels accelerate atherosclerosis except in the case of

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diabetes mellitus (DM). One of the most important reasons for this uncertainty is the fact that there is a very weak compatibility between fetuin-A measurements performed by two different commercial enzyme-linked immunosorbent assay (ELISA) kits. Because the early sign of atherosclerosis is carotid intima-media thickness (CIMT), attention has been drawn to the relationship between CIMT and fetuin-A, especially in patients with DM. A number of studies in the literature have demonstrated an inverse correlation between fetuin-A and CIMT in patients who had chronic inflammatory disease but not DM. In addition, there is no association between fetuin-A and CIMT in subjects without known clinical cardiovascular disease. However, it seems that high fetuin-A levels accelerate atherosclerosis in DM and that diabetic patients exhibit a positive correlation between fetuin-A and CIMT.

Keywords

Fetuin-A • Carotid intima-media thickness • Arterial stiffness • Atherosclerosis • Inflammation

Abbreviations

AHSG	Alpha-2-Heremans–Schmid glycoprotein
AMI	Acute myocardial infarction
CAD	Coronary artery disease
CIMT	Carotid intima-media thickness
CRP	C-reactive protein
CVD	Cardiovascular disease
DM	Diabetes mellitus
ELISA	Enzyme-linked immunosorbent assay
HMGB1	High-mobility group protein-1
IFN	Interferon
IL	Interleukin
MMP	Matrix metalloproteinase
mRNA	Messenger ribonucleic acid
PAD	Peripheral artery disease
TGF-β	Transforming growth factor β
TNF	Tumor necrotizing factor

Key Facts About Fetuin-A

- Fetuin was obtained from bovine fetal serum for the first time in 1944. It is also called α2-Heremans–Schmid glycoprotein (AHSG).
- Fetuin-A is a glycoprotein synthesized from the liver and is found abundantly in the circulation. It is the main carrier protein in the fetal circulation and is found at a higher rate compared to albumin.
- Fetuin-A belongs to the cystatin superfamily. This family is known to comprise cysteine protease inhibitors, which are responsible for bone resorption.

- Fetuin-A is a glycoprotein synthesized predominantly from the liver and represents a great part of the α2 band of serum electrophoresis, with a molecular mass of approximately 60 KDa.

Key Facts About Fetuin-A Functionality

- Fetuin-A is known as a negative acute-phase protein. Proinflammatory cytokines (TNF, IL-1, IL-6, and IFN-γ) decrease the release of fetuin-A.
- Fetuin-A inhibits insulin receptor tyrosine kinase by binding to insulin receptor. Elevated fetuin-A levels lead to insulin resistance in muscle and adipose tissue and are associated with hypertriglyceridemia.
- Sialic acid residues of fetuin-A have the ability to bind to Ca++ ions. Fetuin-A binds to excessive calcium in the circulation and calciprotein particles are formed. Thus, it prevents calcification of soft tissues.

Key Facts About the Effects of Fetuin-A on CIMT

- Fetuin-A binds to excessive calcium in the circulation and calciprotein particles are formed. Thus, calcification of the soft tissues is prevented. Vascular calcification may be manifested through intimal or medial involvement.
- Intimal calcification generally occurs in atherosclerosis-related plaques and as a result of an inflammatory process related with cardiovascular risk factors including DM, hypertension, smoking, and dyslipidemia.
- Medial calcification usually occurs in patients with DM or patients receiving dialysis and generally progresses asymptomatically to a process called arteriosclerosis which leads to increased vessel stiffness.
- Carotid stiffness and CIMT are useful for determining the presence of atherosclerosis.
- All studies suggest a biphasic effect of fetuin-A depending on the stages of atherosclerosis.
- A number of studies in the literature have demonstrated an inverse correlation between fetuin-A and CIMT in patients with chronic inflammatory disease but not DM.
- There is no association between fetuin-A and CIMT in subjects without known CVD. However, it seems that high fetuin-A levels accelerate atherosclerosis in patients with DM who exhibit a positive correlation between fetuin-A and CIMT.

Definitions

Arterial stiffness Reduced capability of an artery to expand and contract in response to pressure changes due to loss of elastic fibers within the arterial wall

Atherosclerosis A condition where the arteries become narrowed due to plaque

Endotoxemia The presence of endotoxins in the blood

Fetuin-A A protein released by the liver and secreted into the bloodstream

A protective response of host cells, blood vessels, and proteins and other mediators to pathogens, damaged cells or irritants

Intima-media thickness A measurement of the thickness of the tunica intima and tunica media

N-linked glycosylation The attachment of glycan, a sugar molecule, to a nitrogen atom and amino acid residue in a protein

O-linked glycosylation The attachment of glycan, a sugar molecule, to an oxygen atom and amino acid residue in a protein

Phosphorylation The addition of a phosphate group to a protein

Sepsis A potentially fatal whole-body inflammation

Transforming growth factor β A protein that controls proliferation, cellular differentiation, and other functions in most cells

Introduction

Fetuin was first obtained from bovine fetal serum in 1944. It is also called α2-Heremans–Schmid glycoprotein (AHSG). Fetuin-A belongs to the cystatin superfamily. This family is known to comprise cysteine protease inhibitors, which are responsible for bone resorption. Fetuin-A is a glycoprotein that is synthesized predominantly from the liver; it is found abundantly in the circulation, with serum levels in the range of 0.4–1.0 g/L, and represents a great part of the α2-band of serum electrophoresis, with a molecular mass of approximately 60 KDa. Extrahepatic fetuin-A synthesis may occur in the kidney and the choroid plexus.

Fetuin-A expression is evident in all major organs during fetal development. It is the main carrier of protein in the fetal circulation and is found with a higher rate compared to albumin. This glycoprotein is cleared through binding to hepatocytes' asialo-glycoprotein receptor (Tolleshaug 1984) and through the formation of the protein–mineral complex, including fetuin, matrix gla protein, and calcium phosphate compounds (Price et al. 2002). This protein has several functions in human physiology and pathophysiology, including in bone metabolism, insulin resistance and diabetes mellitus (DM), ischemic stroke, and neurodegenerative diseases (Fig. 1). Although fetuin-A plays a role as a negative acute-phase reactant in

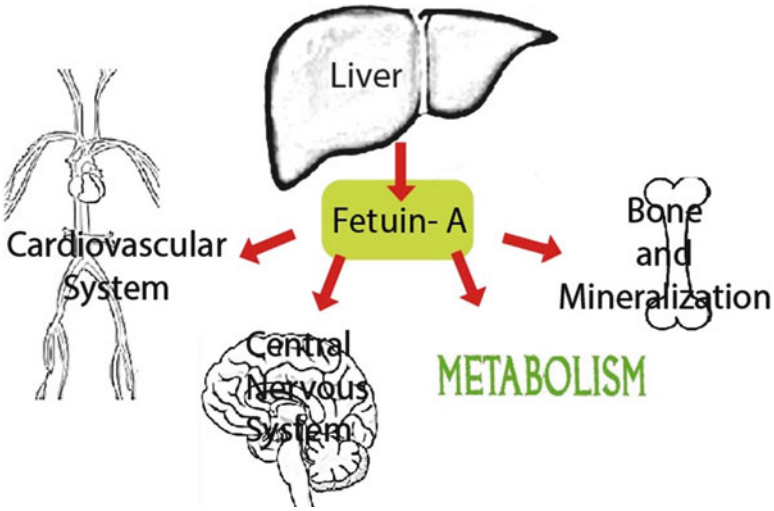

Fig. 1 Fetuin-A is secreted from the liver and has roles in bone mineralization, the cardiovascular and central nervous systems, and metabolism

subclinical atherosclerosis (Gangneux et al. 2003; Lebreton et al. 1979), its role in atherosclerosis is complex.

Fetuin-A exhibits protective effects against atherosclerosis through the inhibition of vascular calcification. However, it is also implicated in adipocyte dysfunction due to its inhibition of the insulin receptor, thereby seemingly promoting atherosclerosis (Ix and Sharma 2010). Elevated serum fetuin-A concentrations have been related to metabolic syndrome, obesity, type 2 DM, nonalcoholic fatty liver disease (Dogru et al. 2013), and events related to ischemic stroke (Tuttolomondo et al. 2010) and myocardial ischemia (Weikert et al. 2008). The potential functionality and prognostic value of fetuin-A in atherosclerosis are discussed in this review, especially in terms of its relationship with carotid intima-media thickness (CIMT), because of contradictory findings in the literature.

The Functionality of Fetuin-A

The serum levels of fetuin-A are decreased in cases of acute inflammation. Therefore, it is known as a negative acute-phase protein. Proinflammatory cytokines (tumor necrosis factor [TNF], interleukin 1 [IL]-1, IL-6, and interferon γ [IFN]-γ) decrease the release of fetuin-A (Lebreton et al. 1979; Gangneux et al. 2003; Daveau et al. 1988). However, Hennige et al. (2008) showed that fetuin-A strongly induced cytokine release in human monocytes in vitro and in mice in vivo; moreover, it had proinflammatory effects and suppressed atheroprotective adipokine adiponectin production. The other proinflammatory cytokine which determines this property is high-mobility group protein-1 (HMGB1). HMGB1 has been defined as a novel

proinflammatory cytokine and is released in the late phase in cases of endotoxemia and sepsis; moreover, in these disorders, fetuin-A is observed with a low level in the early phase and a high level in the late phase in blood (Li et al. 2011). A low level of fetuin-A has been found in conditions such as pancreatitis (Kusnierz-Cabala et al. 2010) and rheumatoid arthritis (Sato et al. 2007). Therefore, it has been defined as a negative acute-phase reactant.

Paradoxically, fetuin-A levels increase in cerebral ischemic injuries (stroke) (Weikert et al. 2008; Tuttolomondo et al. 2010). In traumatic injuries, it also increases, probably due to the release of HMGB1 protein (Zhu et al. 2010). In indirect injuries, it acts as a positive acute-phase protein. Therefore, it has a dual response to inflammation. Schure et al. reported that matrix metalloproteinases (MMPs), which are increased in inflammatory diseases such as periodontitis, bind and degrade fetuin and alter its ability to inhibit calcification in vitro; the increase in MMPs could affect the regulation of mineralization and potentially enhance the risk of the formation of calcified atheroma (Schure et al. 2013). In addition, fetuin-A binds to type 2 transforming growth factor β (TGF-β) receptors and competes with TGF-β (Demetriou et al. 1996). Fetuin-A carries two N-linked and three O-linked oligosaccharide chains ending with sialic acid residues. These sialic acid residues have the ability to bind to Ca++ ions (Fig. 2). Fetuin-A binds to excessive calcium in the circulation, resulting in the formation of calciprotein particles. Thus, the calcification of soft tissues is prevented (Jahnen-Dechent et al. 2011) (Fig. 3). Moreover, fetuin-A has been shown to prevent vascular calcium deposition, especially in animal models (Schafer et al. 2003). In addition, it mediates remodeling in bone formation by way of its inhibitory effect on TGF-β. This glycoprotein accumulates in the skeleton during mineralization and inhibits apatite formation due to its high binding affinity to hydroxyapatite (Schinke et al. 1996).

Fetuin-A is also involved in the release of insulin. Only two proteins can bind to the extracellular part of insulin receptors, namely, insulin and fetuin-A. In experimental models, it has been shown that fetuin-A inhibits insulin receptor tyrosine kinase by binding to insulin receptors (Auberger et al. 1989). Thus, increased fetuin-A levels lead to insulin resistance in muscle and adipose tissue (Rauth et al. 1992) and are associated with hypertriglyceridemia (Roos et al. 2010). Decreased plasma fetuin-A levels inhibit vascular calcification, while increased

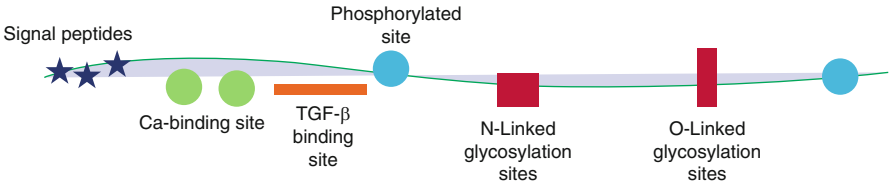

Fig. 2 The protein structure of fetuin-A has three carbohydrate units, which are present on a peptide chain linked with threonine and serine residues. Fetuin-A (AHSG) is a circulating serum glycoprotein with a molecular mass of approximately 60 KDa. There are N- and O-linked complexes in the structure, which may be responsible for the diverse functions of fetuin-A

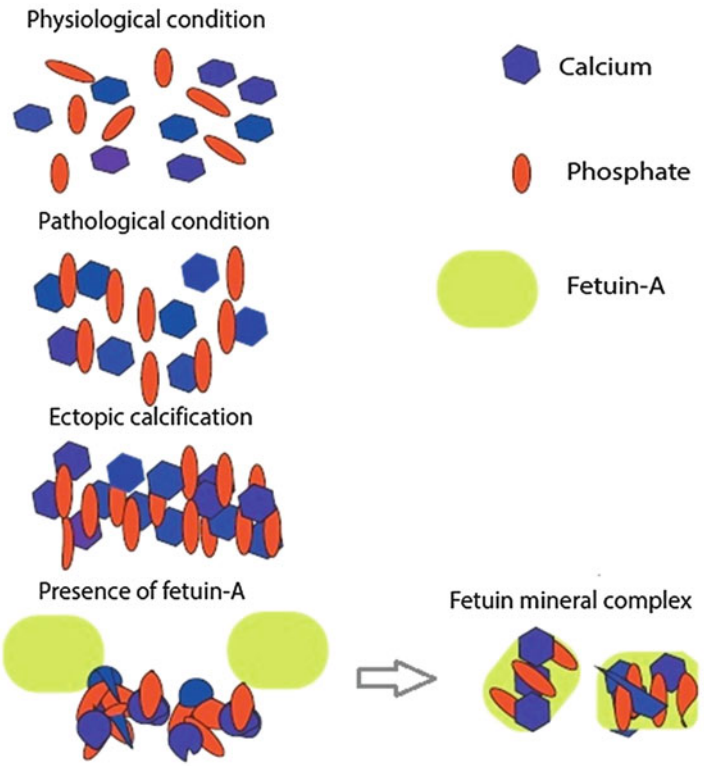

Fig. 3 Fetuin-A plays a role in calcium homeostasis and has an inhibitory effect on ectopic calcification. It is a potent inhibitor of spontaneous hydroxyapatite formation in supersaturated calcium- and phosphate-containing solutions. Low serum fetuin-A concentrations are associated with arterial calcification

serum fetuin-A levels may lead to insulin resistance and metabolic disorders (Ix et al. 2008; Stefan et al. 2008). Therefore, fetuin-A exhibits dual pathophysiological action. In addition, it has been reported that increased serum fetuin-A levels accelerate atherosclerosis by leading to insulin resistance (Fig. 4). Fetuin-A has been shown to be associated with acute myocardial infarction (AMI) and ischemic stroke (Weikert et al. 2008). Fetuin-A blood levels have been shown to be decreased, and vascular calcification has been observed at a high rate in patients with chronic renal failure on dialysis (Ketteler et al. 2003). In addition, low levels of fetuin-A in patients receiving dialysis have been shown to be related with increased mortality (Hermans et al. 2007a).

The presence of DM (Stefan et al. 2008), the level of renal functions (Mehrotra et al. 2005), obesity (Brix et al. 2010), the presence of obstructive sleep apnea (Akyuz et al. 2013), and blood levels of inflammatory cytokines (Gangneux et al. 2003; Lebreton et al. 1979) are the main confounding factors which determine

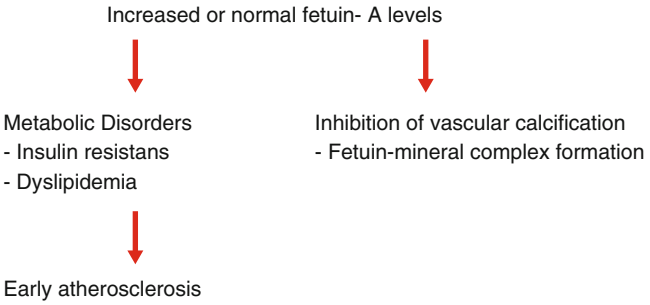

Fig. 4 Increased serum fetuin-A levels accelerate atherosclerosis by leading to insulin resistance, because it is a natural inhibitor of the insulin-stimulated insulin receptor tyrosine kinase. Increased fetuin-A levels are associated with obesity, metabolic syndrome, type 2 DM, and nonalcoholic fatty liver disease. Hyperglycemia and insulin resistance impair endothelium-derived nitric oxide production and promote early atherosclerosis. Fetuin-A inhibits hydroxyapatite formation

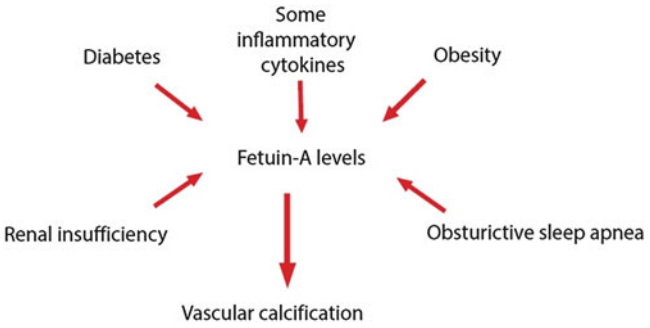

Fig. 5 The main confounding factors such as diabetes, obesity, renal insufficiency, obstructive sleep apnea, malnutrition, and some inflammatory cytokines can alter the serum levels of fetuin-A

the serum levels of fetuin-A (Figs. 5 and 6). Vascular calcification may be manifested as intimal or medial involvement (Ketteler et al. 2006). Intimal calcification generally occurs in atherosclerosis-related plaques and as a result of an inflammatory process related with cardiovascular risk factors, including DM, hypertension, smoking, and dyslipidemia. Medial calcification usually occurs in patients with DM or in patients receiving dialysis and generally progresses asymptomatically to a process called arteriosclerosis, which leads to increased vessel stiffness (Fig. 7). Although the publications in the literature have shown that medial calcification is related with fetuin-A deficiency, it is technically difficult to differentiate intimal and medial calcification, especially in patients with DM. In addition, it has recently been shown that other serum proteins, including matrix G1a protein, osteoprotegerin, and osteopontin, have an important role in the acceleration of vascular tissue calcification (Schlieper et al. 2007). Therefore, the effects of fetuin-A on inhibition of vascular calcification are also related with

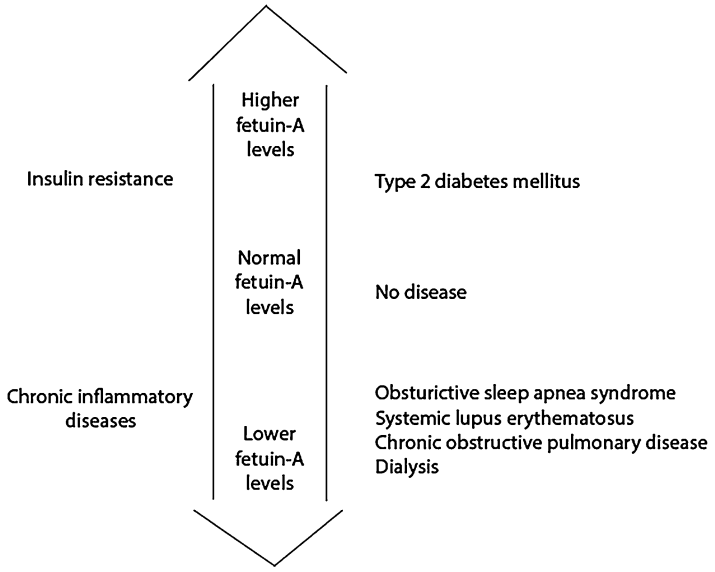

Fig. 6 Fetuin-A levels in relation to diseases

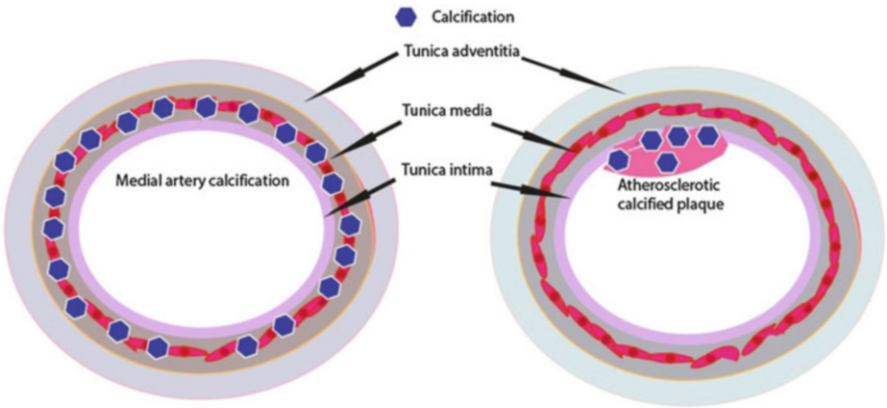

Fig. 7 There are two types of vascular calcification, namely, medial artery calcification and calcified intima atherosclerotic plaque (All figures were drawn by the author)

the serum levels of the serum proteins including matrix G1a protein, osteoprotegerin, and osteopontin (Kim et al. 2013; Schlieper et al. 2007). In addition, the measurement of serum fetuin–mineral complex rather than fetuin-A alone has been suggested a better marker of degree of extra-osseous calcification (Matsui et al. 2009).

Fetuin-A, Cardiovascular Disease, and CIMT

Many studies have shown that greater coronary artery calcification is associated with a greater risk of cardiovascular diseases (CVDs) such as angina pectoris, AMI, and stroke (Westenfeld et al. 2007; Mori et al. 2007; Folsom et al. 2008). There are disagreements about the role of fetuin-A in cardiovascular diseases, except in terms of the condition of vascular calcification in hemodialysis patients and in the early stage of atherosclerosis in subjects with normal renal function. Fetuin-A levels were significantly found to be decreased in patients with advanced three-vessel disease compared with those without stenosis and inversely correlated with advanced calcified coronary artery disease (CAD) in patients with normal renal function (Mori et al. 2010). However, it has also been reported that serum fetuin-A correlates positively with coronary artery calcification in nondialyzed diabetic patients with nephropathy (Mehrotra et al. 2005).

Another study (Mikami et al. 2008) suggested that there is no relationship between coronary artery calcification and serum fetuin-A levels. In addition, an inverse relationship has been found between mitral annular calcification and fetuin-A levels in patients with CAD but without uremia (Ziyrek et al. 2013). These data potentially demonstrate contradictory findings related to fetuin-A and CVD in the presence of DM and uremia. Decreased serum concentrations of the calcification inhibitor fetuin-A are related to increased cardiovascular mortality in dialysis patients. Although fetuin-A-deficient rats have various soft tissue calcifications rather than vasculature due to the protection of the intact endothelium without atherosclerosis, fetuin-A deficiency accelerates intimal rather than medial calcification of atherosclerotic plaques.

Fetuin-A inhibits pathological calcification in both the soft tissue and vasculature, even in the setting of atherosclerosis (Westenfeld et al. 2009). Arterial calcification is evident in the medial or intimal vascular layer. Intimal layer involvement is the main characteristic of atherosclerosis. Calcification caused by fetuin-A deficiency usually occurs in the medium- and large-sized arteries, myocardium, or heart valves. Medial layer calcifications usually occur within the lamina elastica interna and smooth muscle cell layer. However, one study put forward that fetuin-A levels are increased in patients with type 2 DM and peripheral arterial disease (PAD) (Lorant et al. 2011), and it is inversely associated with medial sclerosis; meanwhile, another study demonstrated that lower circulating fetuin-A is associated with PAD in type 2 DM (Eraso et al. 2010). Szeberin et al. showed that fetuin-A levels are negatively associated with the severity of atherosclerosis in nonuremic patients with PAD due to its putative protective role in the progression of vessel calcification (Szeberin et al. 2011). In other words, there are contradictory results concerning the relationship between fetuin-A and PAD.

Interestingly, PAD patients with medial sclerosis have lower serum fetuin-A concentrations compared to those without medial sclerosis. According to these findings, fetuin-A has dual effects on vascular atherosclerosis, both as an atherogenic factor and a calcification inhibiting factor. It is possible that fetuin-A levels might change according to the balance between the severity of arterial wall calcification

and atherosclerosis progression. In addition, fetuin-A might increase the collagen content of the arterial wall by blocking TGF-β signaling, thereby accelerating arterial stiffness.

Fetuin-A-deficient rats have normal phenotype but exhibit severe calcification of various organs (Westenfeld et al. 2009). Increased organ calcification in the heart or kidney may accelerate myocardial or renal dysfunction (Schafer et al. 2003). Intramyocardial calcification with fibrotic tissue is associated with diastolic dysfunction, less ischemic tolerance, and decreased sympathetic response. Both the inverse relationship between fetuin-A levels and coronary artery calcification in patients with renal disease (Westenfeld et al. 2007) and the positive relationship between fetuin-A levels and peripheral artery calcification in nondialyzed diabetic patients with renal dysfunction (Mehrotra et al. 2005) potentially suggest that fetuin-A counteracts vascular calcification in the early stages of DM and atherosclerosis. Some studies showed that dyslipidemia and hyperinsulinemia increase secretion of hepatic fetuin-A (Ix et al. 2006; Stefan et al. 2006). One study reported that serum fetuin-A levels are positively related to the degree of atherosclerosis (Rittig et al. 2009). Another showed that high serum fetuin-A levels are correlated with increased cardiovascular risk, irrespective of the presence of DM (Weikert et al. 2008). Increased fetuin-A levels might facilitate both atherosclerotic progression and insulin resistance (Rittig et al. 2009).

Some studies have shown that there is an inverse correlation between fetuin-A and adiponectin levels in patients with increased cardiovascular risk (Hennige et al. 2008; Ix and Sharma 2010). Decreased serum adiponectin levels might increase serum-free fatty acid levels, thereby causing atherosclerosis or accelerating atherosclerotic progression. Given these convincing findings, the modulation of adiponectin caused by fetuin-A appears to be an important factor in atherosclerosis progression (Hennige et al. 2008; Ix and Sharma 2010). Mori et al. found (2007) a positive relationship between fetuin-A and arterial stiffness, an important marker of atherosclerosis, in healthy subjects. Moreover, Fiore et al. reported that fetuin-A levels are positively correlated with arterial intima-media thickness, an indicator of remodeling of the arterial wall (Fiore et al. 2007). A study by Merx et al. was the first to show the functional role of isolated myocardial calcification independent of arterial stiffness in fetuin-A-deficient mice and found impaired left ventricle relaxation due to dystrophic cardiac calcification. Remarkably, these researchers also identified an association with the profound induction of profibrotic TGF-β and downstream collagen and fibronectin mRNA in these mice (Merx et al. 2005). Merx et al. also suggested that higher serum fetuin-A levels might be protective for some cardiovascular diseases, because of fetuin-A's ability to prevent calcium/phosphate precipitation and ectopic mineralization in the arterial wall (Merx et al. 2005).

Lower fetuin-A levels are related to higher inflammatory response and cause the release of some cardiotoxic cytokines (e.g., TNF) (Ombrellino et al. 2001), and the inverse relationship between cardiotoxic cytokines and cardiac contractility has been well documented (Kelly and Smith 1997). In addition, a negative relationship between serum C-reactive protein (CRP) and fetuin-A levels was found to be decreased in patients with CAD (Bilgir et al. 2010), as well as in dialysis patients

(Hermans et al. 2007a). CRP is an important acute-phase inflammatory protein caused by IL-6 secretion from macrophages.

At present, increased serum CRP levels are used for determining cardiovascular risk (Danesh et al. 2004). Interestingly, Kadaglou et al. showed that statin therapy reduces fetuin-A levels, as well as serum total cholesterol, low-density lipoprotein cholesterol, and CRP levels (Kadoglou et al. 2014). Zhao et al. documented that serum fetuin-A levels are related to the presence and severity of CAD in DM patients and put forward that fetuin-A might be used as a marker for the progression of CAD in patients with DM (Zhao et al. 2013). Elevated fetuin-A levels were a negative predictor of CAD and an independent predictor of nonalcoholic fatty liver disease (Ballestri et al. 2013). Afsar et al. found lower serum fetuin-A levels in patients with acute coronary syndrome, independent of heart valve calcification, and defined fetuin-A as a negative acute-phase protein after AMI (Afsar et al. 2012). In addition, a fetuin-A level lower than 140 mg/L was shown to be a predictor of death at 6 months after ST-elevation AMI (Lim et al. 2007). Plasma fetuin-A levels usually decrease within a few hours after the onset of AMI and reach normal serum levels in 5–7 days (Mathews et al. 2002). Roos et al. demonstrated that serum fetuin-A levels did not predict cardiovascular events during 6 years of follow-up in 1,049 patients (Roos et al. 2010).

CIMT and Atherosclerosis

Age, hyperlipidemia, hypertension, DM, smoking, and sedentary lifestyle are the factors which increase CIMT. Measurement of CIMT by ultrasonography is an inexpensive, simple, reliable, and reproducible noninvasive method. CIMT can also be shown by magnetic resonance imaging, but its measurement by this method is not recommended. The thickness of the tunica intima and tunica media, which constitute the inner layer of the arterial wall, is measured by ultrasonography. In addition, ultrasonography is also useful for determining the presence of atherosclerosis (Baldassarre et al. 2012) and the efficiency of lipid lowering (Hodis et al. 1996) or antihypertensive drug usage (Pitt et al. 2000). However, in recent meta-analyses, it has been recommended only as an assistive method in determining cardiovascular risk, not for direct risk assessment (Costanza et al. 2010; Lorenz et al. 2012; Den Ruijter et al. 2012). Nevertheless, the American Heart Society and American College of Cardiology recommended measuring CIMT to obtain a better risk assessment in asymptomatic patients with moderate cardiovascular risk (Goff et al. 2014). Measurement of CIMT is not recommended for patients with low or high risk or for patients with known cardiovascular disease. CIMT measurements are performed on the posterior carotid artery just above the bulbus from the area which does not contain plaque (Montauban van Swijndregt et al. 1999). Normal CIMT is approximately 0.4–0.5 mm at the age of about 10 years, while it is approximately 0.7–0.8 mm in adulthood. In adults, a value $\geq$0.9 mm is considered high. Localized thickenings of at least 1.5 mm and above are considered plaque.

Fetuin-A and CIMT

In the literature, it is still unclear whether the relation between fetuin-A and carotid stiffness and CIMT is positive or negative. However, there are studies showing that fetuin-A is a negative inflammatory marker (Gangneux et al. 2003) and inversely correlated with aortic (Roos et al. 2009) and carotid stiffness (Akyuz et al. 2013) in patients with chronic inflammatory diseases in contrast to the publication's relation with diabetic patients. Guarneri et al. (2013) found that CIMT was inversely correlated with fetuin-A in patients with essential hypertension. In a study we performed (Akyuz et al. 2013), an inverse correlation was found between fetuin-A and CIMT in normotensive patients with obstructive sleep apnea. Mori et al. demonstrated that fetuin-A levels are significantly associated with carotid artery stiffness in healthy subjects (Mori et al. 2007), but they did not study the associate fetuin-A with CIMT. The above mentioned studies suggest a biphasic effect of fetuin-A depending on the stages of atherosclerosis.

The Positive Correlation Between Fetuin-A and CIMT in Patients with DM

It is thought that fetuin-A is metabolically related with the initiation and progression of atherosclerosis, like DM, by triggering insulin resistance in the muscle and adipose tissue. Studies have shown a positive correlation between fetuin-A levels and increased CIMT and carotid stiffness (Mori et al. 2007), especially in patients with type 2 DM (Dogru et al. 2013; Fiore et al. 2007; Rittig et al. 2009; Yin et al. 2014; Koluman et al. 2013) or insulin resistance (Dogru et al. 2013) (Table 1). In addition, serum fetuin-A levels have been reversely correlated with carotid and femoral artery calcifications in patients with type 2 DM with preserved renal function (Emoto et al. 2010). In these studies, fetuin-A has been reported to lead to increased CIMT or increased stiffness because of its diabetogenic effect and proinflammatory properties. These studies mostly suggest that fetuin-A levels might represent a surrogate marker for the severity of the atherosclerosis in patients with type 2 DM and increased CIMT.

The Negative Correlation Between Fetuin-A and CIMT in Patients with Chronic Inflammatory Disease

A number of studies in the literature have demonstrated an inverse correlation between fetuin-A and CIMT in patients with chronic inflammatory disease but not DM. According to the findings of these studies, since fetuin-A is a negative acute-phase reactant, low serum fetuin-A concentrations could be a consequence of the chronic inflammatory state in conditions such as chronic obstructive pulmonary disease (COPD) (Alpsoy et al. 2014), obstructive sleep apnea (Akyuz et al. 2013), uremia (Hermans et al. 2007b; Wang et al. 2007), systemic lupus erythematosus (Abdel-Wahab et al. 2013) or subclinical vascular inflammation caused by essential hypertension (Guarneri et al. 2013).

Table 1 Studies demonstrating whether there is a positive, negative or no correlation between fetuin-A and CIMT

Patients/subjects	The correlation between fetuin-A and CIMT	Fetuin-A analysis	References
New-onset type 2 DM ($n = 100$)	Positively	ELISA kit (R&D Systems, Minneapolis, MN, USA)	Yin et al. (2014)
Type 2 DM ($n = 120$)	Positively (only in normoalbumineric diabetic patients)	ELISA kit (BioVendor Human Elisa kit, Brno, Czech Republic)	Koluman et al. (2013)
The subjects at risk for type 2 DM ($n = 315$)	Positively	–	Rittig et al. (2009)
With NAFLD and insulin resistance ($n = 115$)	Positively	ELISA kit (Epitope Diagnostics, Inc., San Diego, USA)	Dogru et al. (2013)
Peripheral artery disease with low bone mass ($n = 90$)	Positively (51 % patients with DM)	ELISA (Human Fetuin ELISA Kit, Epitope Diagnostics Inc., San Diego, CA, USA)	Fiore et al. (2007)
Dialysis patients ($n = 134$)	Negatively	Nephelometry method	Hermans et al. 2007b
Dialysis patients ($n = 147$)	Negatively	ELISA kit (Epitope Diagnostics, Inc., San Diego, USA)	Wang et al. (2007)
Systemic lupus erythematosus ($n = 40$)	Negatively	ELISA kit (Epitope Diagnostics, Inc., San Diego, USA)	Abdel-Wahab et al. (2013)
Without cardiovascular disease ($n = 1374$)	No	ELISA kit (Epitope Diagnostics, Inc., San Diego, USA)	Ix et al. (2011)
Normotensive chronic obstructive pulmonary disease ($n = 65$)	Negatively	ELISA kit (BioVendor Human Elisa kit, Brno, Czech Republic)	Alpsoy et al. (2014)
Normotensive obstructive sleep apnea syndrome ($n = 50$)	Negatively	ELISA kit (BioVendor Human Elisa kit, Brno, Czech Republic)	Akyuz et al. (2013)

CIMT carotid intima-media thickness, *DM* diabetes mellitus, *NAFLD* nonalcoholic fatty liver disease

The Lack of Correlation Between Fetuin-A and CIMT in Subjects Without Known CVD

Ix et al. demonstrated that there was no association between fetuin-A and CIMT in a large population ($n = 1,375$) without known clinical CVD; here, fetuin-A was only inversely correlated with severity of carotid artery calcification (Ix et al. 2011). In addition, a correlation was found between fetuin-A and carotid stiffness, while no correlation was found between fetuin-A and CIMT in a study involving healthy subjects performed by Mori et al. (2007).

The Reasons for the Uncertain Fetuin-A Results in the Literature

It is still unclear whether high fetuin-A levels accelerate atherosclerosis, except in the case of DM. One of the most important reasons for this uncertainty is the fact that there is a very weak compatibility between fetuin-A measurements performed by two different commercial enzyme-linked immunosorbent assay (ELISA) kits (BioVendor Research and Diagnostic Products vs. Epitope Diagnostics, Inc.) (Smith et al. 2010). In the ELISA tests, specific antibody responses to different glycosylated forms of fetuin may be variable. Nephelometry is also used for measurement of fetuin-A. Therefore, fetuin-A measurements should be standardized. In addition, the companies which manufacture these kits still have not reported the normal values in healthy individuals. The other reason is that some threonine and serine residues of fetuin-A are modified with N-linked and O-linked glycosylation and phosphorylation. In this case, fetuin-A may have different functional properties (Gejyo et al. 1983; Yoshioka et al. 1986). Therefore, the levels of modified fetuin-A should also be determined in clinical studies. Thus, more studies are needed to determine the role of fetuin-A in determining CIMT and carotid artery stiffness.

Potential Applications to Prognosis and Other Diseases or Conditions

Fetuin-A has roles in bone metabolism, insulin resistance and DM, ischemic stroke, and neurodegenerative diseases. Some data suggest a link between high plasma fetuin-A levels and increased AMI and ischemic stroke. Low levels of fetuin-A, a systemic calcification inhibitor, are linked to mortality in patients on dialysis. One study suggested that a fetuin-A level lower than 140 mg/L is a predictor of death at 6 months after ST-elevation AMI (Lim et al. 2007). However, there are no exact data concerning fetuin-A for potential applications to prognosis, except in the case of chronic renal disease.

Summary Points

- This chapter focuses on the relationship between fetuin-A and CIMT.
- Fetuin-A has several functions in human physiology and pathophysiology, including in bone metabolism, insulin resistance and DM, ischemic stroke, and neurodegenerative diseases. The serum levels of fetuin-A are decreased in cases of acute inflammation. Therefore, it is known as a negative acute-phase protein. Fetuin-A also prevents calcification of soft tissues, especially in the vascular system.
- The companies which manufacture fetuin-A kits have still not reported the normal values in healthy individuals.

- There are no exact data concerning fetuin-A for potential applications to prognosis, except for chronic renal disease. Low fetuin-A is associated with increased mortality in dialyzed patients.
- Age, hyperlipidemia, hypertension, DM, smoking, and sedentary lifestyle are the factors which increase CIMT.
- Although increased CIMT is accepted as an early marker of atherosclerosis, its measurement is only recommended for a better risk assessment in asymptomatic patients with a moderate cardiovascular risk.
- A number of studies in the literature have demonstrated an inverse correlation between fetuin-A and CIMT in patients with chronic inflammatory disease and without DM. There is no association between fetuin-A and CIMT in subjects without known clinical cardiovascular disease. However, it seems that high fetuin-A levels accelerate atherosclerosis in DM and diabetic patients exhibit a positive correlation between fetuin-A and CIMT.
- It is still unclear whether high fetuin-A levels accelerate atherosclerosis, except in the case of DM. One of the most important reasons for this uncertainty is the fact that there is very weak compatibility between fetuin-A measurements performed by two different commercial ELISA kits. In addition, nephelometry is used for the measurement of fetuin-A. Therefore, fetuin-A measurements should be standardized. Some threonine and serine residues of fetuin-A are modified with N-linked and O-linked glycosylation and phosphorylation. In this case, fetuin-A may have different functional properties.

References

Abdel-Wahab AF, Fathy O, Al-Harizy R. Negative correlation between fetuin-A and indices of vascular disease in systemic lupus erythematosus patients with and without lupus nephritis. Arab J Nephrol Transpl. 2013;6:11–20.

Afsar CU, Uzun H, Yurdakul S, et al. Association of serum fetuin-A levels with heart valve calcification and other biomarkers of inflammation among persons with acute coronary syndrome. Clin Invest Med. 2012;35:E206–15.

Akyuz A, Oran M, Alpsoy S, et al. Association between serum fetuin-a levels, carotid artery stiffness, and intima-media thickness in patients with normotensive obstructive sleep apnea syndrome. Angiology. 2013;65:607–13.

Alpsoy S, Akyuz A, Mutlu LC, et al. Serum fetuin-A levels are associated with carotid intima-media thickness in patients with normotensive chronic obstructive pulmonary disease. Cardiol J. 2014;21:191–7.

Auberger P, Falquerho L, Contreres JO, et al. Characterization of a natural inhibitor of the insulin receptor tyrosine kinase: cDNA cloning, purification, and anti-mitogenic activity. Cell. 1989;58:631–40.

Baldassarre D, Hamsten A, Veglia F, et al. Measurements of carotid intima-media thickness and of interadventitia common carotid diameter improve prediction of cardiovascular events: results of the IMPROVE (Carotid Intima Media Thickness [IMT] and IMT-Progression as Predictors of Vascular Events in a High Risk European Population) study. J Am Coll Cardiol. 2012;60:1489–99.

Ballestri S, Meschiari E, Baldelli E, et al. Relationship of serum fetuin-A levels with coronary atherosclerotic burden and NAFLD in patients undergoing elective coronary angiography. Metab Syndr Relat Disord. 2013;11:289–95.
Bilgir O, Kebapcilar L, Bilgir F, et al. Decreased serum fetuin-A levels are associated with coronary artery diseases. Intern Med. 2010;49:1281–5.
Brix JM, Stingl H, Hollerl F, et al. Elevated fetuin-A concentrations in morbid obesity decrease after dramatic weight loss. J Clin Endocrinol Metab. 2010;95:4877–81.
Costanzo P, Perrone-Filardi P, Vassallo E, et al. Does carotid intima-media thickness regression predict reduction of cardiovascular events? A meta-analysis of 41 randomized trials. J Am Coll Cardiol. 2010;56:2006–20.
Danesh J, Wheeler JG, Hirschfield GM, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med. 2004;350:1387–97.
Daveau M, Christian D, Julen N, et al. The synthesis of human alpha-2-HS glycoprotein is down-regulated by cytokines in hepatoma HepG2 cells. FEBS Lett. 1988;241:191–4.
Demetriou M, Binkert C, Sukhu B, et al. Fetuin/alpha2-HS glycoprotein is a transforming growth factor-beta type II receptor mimic and cytokine antagonist. J Biol Chem. 1996;271:12755–61.
Den Ruijter HM, Peters SA, Anderson TJ, et al. Common carotid intima-media thickness measurements in cardiovascular risk prediction: a meta-analysis. JAMA. 2012;308:796–803.
Dogru T, Genc H, Tapan S, et al. Plasma fetuin-A is associated with endothelial dysfunction and subclinical atherosclerosis in subjects with nonalcoholic fatty liver disease. Clin Endocrinol (Oxf). 2013;78:712–7.
Emoto M, Mori K, Lee E, et al. Fetuin-A and atherosclerotic calcified plaque in patients with type 2 diabetes mellitus. Metabolism. 2010;59:873–8.
Eraso LH, Ginwala N, Qasim AN, et al. Association of lower plasma fetuin-a levels with peripheral arterial disease in type 2 diabetes. Diabetes Care. 2010;33:408–10.
Fiore CE, Celotta G, Politi GG, et al. Association of high alpha2-Heremans-Schmid glycoprotein/fetuin concentration in serum and intima-media thickness in patients with atherosclerotic vascular disease and low bone mass. Atherosclerosis. 2007;195:110–5.
Folsom AR, Kronmal RA, Detrano RC, et al. Coronary artery calcification compared with carotid intima-media thickness in the prediction of cardiovascular disease incidence: the Multi-Ethnic Study of Atherosclerosis (MESA). Arch Intern Med. 2008;168:1333–9.
Gangneux C, Daveau M, Hiron M, et al. The inflammation-induced down-regulation of plasma fetuin- (alpha2HS-glycoprotein) in liver results from the loss of interaction between long C/EBP isoforms at two neighbouring binding sites. Nucleic Acids Res. 2003;31:5957–70.
Gejyo F, Chang JL, Burgi W, et al. Characterization of the B-chain of human plasma alpha 2HS-glycoprotein. The complete amino acid sequence and primary structure of its heteroglycan. J Biol Chem. 1983;258:4966–71.
Goff Jr DC, Lloyd-Jones DM, Bennett G, et al. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. 2014;63:2935–59.
Guarneri M, Geraci C, Incalcaterra F, et al. Subclinical atherosclerosis and fetuin-A plasma levels in essential hypertensive patients. Hypertens Res. 2013;36:129–33.
Hennige AM, Staiger H, Wicke C, et al. Fetuin-A induces cytokine expression and suppresses adiponectin production. PLoS One. 2008;3:e1765.
Hermans MM, Brandenburg V, Ketteler M, et al. Association of serum fetuin-A levels with mortality in dialysis patients. Kidney Int. 2007a;72:202–7.
Hermans MM, Kooman JP, Brandenburg V, et al. Spatial inhomogeneity of common carotid artery intima-media is increased in dialysis patients. Nephrol Dial Transplant. 2007b;22:1205–12.
Hodis HN, Mack WJ, LaBree L, et al. Reduction in carotid arterial wall thickness using lovastatin and dietary therapy: a randomized controlled clinical trial. Ann Intern Med. 1996;124:548–56.
Ix JH, Sharma K. Mechanisms linking obesity, chronic kidney disease, and fatty liver disease: the roles of fetuin-A, adiponectin, and AMPK. J Am Soc Nephrol. 2010;21:406–12.

Ix JH, Shlipak MG, Brandenburg VM, et al. Association between human fetuin-A and the metabolic syndrome: data from the Heart and Soul Study. Circulation. 2006;113:1760–7.
Ix JH, Chertow GM, Shlipak MG, et al. Association of fetuin-A with mitral annular calcification and aortic stenosis among persons with coronary heart disease: data from the Heart and Soul Study. Circulation. 2007;115:2533–9.
Ix JH, Wassel CL, Kanaya AM, et al. Fetuin-A and incident diabetes mellitus in older persons. JAMA. 2008;300:182–8.
Ix JH, Barrett-Connor E, Wassel CL, et al. The associations of fetuin-A with subclinical cardiovascular disease in community-dwelling persons: the Rancho Bernardo Study. J Am Coll Cardiol. 2011;58:2372–9.
Jahnen-Dechent W, Heiss A, Schafer C, et al. Fetuin-A regulation of calcified matrix metabolism. Circ Res. 2011;108:1494–509.
Kadoglou NP, Kottas G, Lampropoulos S, et al. Serum levels of fetuin-A, osteoprotegerin and osteopontin in patients with coronary artery disease: effects of statin (HMGCoA-reductase inhibitor) therapy. Clin Drug Investig. 2014;34:165–71.
Kelly RA, Smith TW. Cytokines and cardiac contractile function. Circulation. 1997;95:778–81.
Ketteler M, Bongartz P, Westenfeld R, et al. Association of low fetuin-A (AHSG) concentrations in serum with cardiovascular mortality in patients on dialysis: a cross-sectional study. Lancet. 2003;361:827–33.
Ketteler M, Schlieper G, Floege J. Calcification and cardiovascular health: new insights into an old phenomenon. Hypertension. 2006;47:1027–34.
Kim HR, Kim SH, Han MJ, et al. The ratio of osteoprotegerin to fetuin-a is independently associated with vascular stiffness in hemodialysis patients. Nephron Clin Pract. 2013;123:165–72.
Koluman BU, Mutluay R, Derici UB, et al. Association between osteoprotegerin, fetuin-A, carotid intima media thickness, and urinary albumin excretion in Type 2 diabetes. Clin Nephrol. 2013;80:9–16.
Kusnierz-Cabala B, Gurda-Duda A, Panek J, et al. Serum fetuin A concentrations in patients with acute pancreatitis. Clin Lab. 2010;56:191–5.
Lebreton JP, Joisel F, Raoult JP, et al. Serum concentration of human alpha 2 HS glycoprotein during the inflammatory process: evidence that alpha 2 HS glycoprotein is a negative acute-phase reactant. J Clin Invest. 1979;64:1118–29.
Lorant DP, Grujicic M, Hoebaus C, et al. Fetuin-A levels are increased in patients with type 2 diabetes and peripheral arterial disease. Diabetes care. 2011;34:156–61.
Li W, Zhu S, Li J, et al. A hepatic protein, fetuin-A, occupies a protective role in lethal systemic inflammation. PLoS One. 2011;6:e16945.
Lim P, Collet JP, Moutereau S, et al. Fetuin-A is an independent predictor of death after ST-elevation myocardial infarction. Clin Chem. 2007;53:1835–40.
Lorenz MW, Polak JF, Kavousi M, et al. Carotid intima-media thickness progression to predict cardiovascular events in the general population (the PROG-IMT collaborative project): a meta-analysis of individual participant data. Lancet. 2012;379:2053–62.
Mathews ST, Deutsch DD, Iyer G, et al. Plasma alpha2-HS glycoprotein concentrations in patients with acute myocardial infarction quantified by a modified ELISA. Clin Chim Acta. 2002;319:27–34.
Matsui I, Hamano T, Mikami S, et al. Fully phosphorylated fetuin-A forms a mineral complex in the serum of rats with adenine-induced renal failure. Kidney Int. 2009;75:915–28.
Mehrotra R, Westenfeld R, Christenson P, et al. Serum fetuin-A in nondialyzed patients with diabetic nephropathy: relationship with coronary artery calcification. Kidney Int. 2005;67:1070–7.
Merx MW, Schafer C, Westenfeld R, et al. Myocardial stiffness, cardiac remodeling, and diastolic dysfunction in calcification-prone fetuin-A-deficient mice. J Am Soc Nephrol. 2005;16:3357–64.

Mikami S, Hamano T, Fujii N, et al. Serum osteoprotegerin as a screening tool for coronary artery calcification score in diabetic pre-dialysis patients. Hypertens Res. 2008;31:1163–70.
Montauban van Swijndregt AD, De Lange EE, De Groot E, et al. An in vivo evaluation of the reproducibility of intima-media thickness measurements of the carotid artery segments using B-mode ultrasound. Ultrasound Med Biol. 1999;25:323–30.
Mori K, Emoto M, Araki T, et al. Association of serum fetuin-A with carotid arterial stiffness. Clin Endocrinol (Oxf). 2007;66:246–50.
Mori K, Ikari Y, Jono S, et al. Fetuin-A is associated with calcified coronary artery disease. Coron Artery Dis. 2010;21:281–5.
Ombrellino M, Wang H, Yang H, et al. Fetuin, a negative acute phase protein, attenuates TNF synthesis and the innate inflammatory response to carrageenan. Shock. 2001;15:181–5.
Pitt B, Byington RP, Furberg CD, et al. Effect of amlodipine on the progression of atherosclerosis and the occurrence of clinical events. PREVENT Investigators. Circulation. 2000;102:1503–10.
Price PA, Thomas GR, Pardini AW, et al. Discovery of a high molecular weight complex of calcium, phosphate, fetuin, and matrix gamma-carboxyglutamic acid protein in the serum of etidronate-treated rats. J Biol Chem. 2002;277:3926–34.
Rauth G, Poschke O, Fink E, et al. The nucleotide and partial amino acid sequences of rat fetuin. Identity with the natural tyrosine kinase inhibitor of the rat insulin receptor. Eur J Biochem. 1992;204:523–9.
Rittig K, Thamer C, Haupt A, et al. High plasma fetuin-A is associated with increased carotid intima-media thickness in a middle-aged population. Atherosclerosis. 2009;207:341–2.
Roos M, Richart T, Kouznetsova T, et al. Fetuin-A and arterial stiffness in patients with normal kidney function. Regul Pept. 2009;154:39–43.
Roos M, von Eynatten M, Heemann U, et al. Serum fetuin-A, cardiovascular risk factors, and six-year follow-up outcome in patients with coronary heart disease. Am J Cardiol. 2010;105:1666–72.
Sato H, Kazama JJ, Wada Y, et al. Decreased levels of circulating alpha2-Heremans-Schmid glycoprotein/Fetuin-A (AHSG) in patients with rheumatoid arthritis. Intern Med. 2007;46:1685–91.
Schafer C, Heiss A, Schwarz A, et al. The serum protein alpha 2-Heremans-Schmid glycoprotein/fetuin-A is a systemically acting inhibitor of ectopic calcification. J Clin Invest. 2003;112:357–66.
Schinke T, Amendt C, Trindl A, et al. The serum protein alpha2-HS glycoprotein/fetuin inhibits apatite formation in vitro and in mineralizing calvaria cells. A possible role in mineralization and calcium homeostasis. J Biol Chem. 1996;271:20789–96.
Schlieper G, Westenfeld R, Brandenburg V, et al. Inhibitors of calcification in blood and urine. Semin Dial. 2007;20:113–21.
Schure R, Costa KD, Rezaei R, et al. Impact of matrix metalloproteinases on inhibition of mineralization by fetuin. J Periodontal Res. 2013;48:357–66.
Smith ER, Ford ML, Tomlinson LA, et al. Poor agreement between commercial ELISAs for plasma fetuin-A: an effect of protein glycosylation? Clin Chim Acta. 2010;411:1367–70.
Stefan N, Hennige AM, Staiger H, et al. Alpha2-Heremans-Schmid glycoprotein/fetuin-A is associated with insulin resistance and fat accumulation in the liver in humans. Diabetes Care. 2006;29:853–7.
Stefan N, Fritsche A, Weikert C, et al. Plasma fetuin-A levels and the risk of type 2 diabetes. Diabetes. 2008;57:2762–7.
Szeberin Z, Fehervari M, Krepuska M, et al. Serum fetuin-A levels inversely correlate with the severity of arterial calcification in patients with chronic lower extremity atherosclerosis without renal disease. Int Angiol. 2011;30:474–50.
Tolleshaug H. Intracellular segregation of asialo-transferrin and asialo-fetuin following uptake by the same receptor system in suspended hepatocytes. Biochim Biophys Acta. 1984;803: 182–90.

Tuttolomondo A, Di Raimondo D, Di Sciacca R, et al. Fetuin-A and CD40 L plasma levels in acute ischemic stroke: differences in relation to TOAST subtype and correlation with clinical and laboratory variables. Atherosclerosis. 2010;208:290–6.

Wang AY, Ho SS, Liu EK, et al. Differential associations of traditional and non-traditional risk factors with carotid intima-media thickening and plaque in peritoneal dialysis patients. Am J Nephrol. 2007;27:458–65.

Weikert C, Stefan N, Schulze MB, et al. Plasma fetuin-a levels and the risk of myocardial infarction and ischemic stroke. Circulation. 2008;118:2555–62.

Westenfeld R, Jahnen-Dechent W, Ketteler M. Vascular calcification and fetuin-A deficiency in chronic kidney disease. Trends Cardiovasc Med. 2007;17:124–8.

Westenfeld R, Schafer C, Kruger T, et al. Fetuin-A protects against atherosclerotic calcification in CKD. J Am Soc Nephrol. 2009;20:1264–74.

Yin L, Cai WJ, Chang XY, et al. Association between fetuin-A levels with insulin resistance and carotid intima-media thickness in patients with new-onset type 2 diabetes mellitus. Biomed Rep. 2014;2:839–42.

Yoshioka Y, Gejyo F, Marti T, et al. The complete amino acid sequence of the A-chain of human plasma alpha 2HS-glycoprotein. J Biol Chem. 1986;261:1665–76.

Zhao ZW, Lin CG, Wu LZ, et al. Serum fetuin-A levels are associated with the presence and severity of coronary artery disease in patients with type 2 diabetes. Biomarkers. 2013;18:160–4.

Zhu S, Li W, Ward MF, et al. High mobility group box 1 protein as a potential drug target for infection- and injury-elicited inflammation. Inflamm Allergy Drug Targets. 2010;9:60–72.

Ziyrek M, Tayyareci Y, Yurdakul S, et al. Association of mitral annular calcification with endothelial dysfunction, carotid intima-media thickness and serum fetuin-A: an observational study. Anadolu Kardiyol Derg. 2013;13:752–8.