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REVIEW

Fetuin-A and angiopoietins in obesity and type 2 diabetes mellitus

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Abstract Although type 2 diabetes mellitus (DM) is a chronic metabolic disorder with multiple etiologies, obesity has been constantly linked with insulin resistance and manifestation of type 2 DM. In addition, obesity is associated with hypertension, dyslipidemia, and fatty liver disease and is regarded as a subclinical inflammatory condition characterized by release of pro-inflammatory mediators such as cytokines from adipose tissue. Both, type 2 DM and obesity are considered as major risks for developing micro- and macrovascular diseases. Recent studies showed that impaired circulating levels of fetuin-A, which is involved in propagating insulin resistance as well as circulating levels of angiopoietins, which are growth factors promoting angiogenesis, were observed in patients with obesity, metabolic syndrome, and type 2 DM. However, independent of type 2 DM and obesity, defective regulation of fetuin-A and angiopoietin are playing a critical role in predisposing to coronary and peripheral vascular diseases. Therefore, mechanisms linking type 2 DM and obesity with fetuin-A and angiopoietins seem to be complex and are in need of further exploration. In this review, we aimed to present a summary concerning associations of type 2 diabetes, obesity, and vascular diseases with circulating levels of angiopoietins and fetuin-A. Furthermore, we aimed to focus on roles of fetuin-A and angiopoietins and to highlight the most plausible mechanisms that might explain their associations with type 2 DM and obesity.

Keywords Type 2 diabetes · Obesity · Fetuin-A · Angiopoietins · Vascular diseases

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Introduction

Diabetes mellitus (DM) defines chronic metabolic disorders characterized by increased blood glucose levels due to absolute or relative deficiency of insulin. Type 2 DM is the most common type of diabetes, representing about 90–95 % of all diabetic cases around the world [1]. In addition to the genetic background, other factors such as age, overweight, physical inactivity, gestational diabetes [2], hypertension, dyslipidemia, polycystic ovary syndrome [3], history of vascular diseases, and race/ethnicity seem to have an important role in predisposing an individual to type 2 DM. In the future the prevalence of type 2 DM is expected to rise more rapidly because of increasing obesity and reduced physical activity.

Obesity, especially visceral or central obesity is very common in type 2 DM. Adipose tissue is a rich source of pro-inflammatory mediators that may directly contribute to inflammatory vascular injuries, insulin resistance, and atherogenesis [4]. Obesity increases the incidence of fatty liver diseases that alters the secretion pattern of many proteins produced by the liver [5] and is associated with peripheral as well as hepatic insulin resistance [6, 7]. Moreover, the long-standing metabolic derangement in diabetes and obesity is frequently associated with permanent and irreversible functional and structural changes in the cells of all tissues. Cells of the vascular system,

however, are particularly susceptible to these changes with subsequent development of micro- and macro-vascular complications. In addition, both type 2 DM and obesity, via increased systemic levels of reactive oxygen species (ROS) and oxidative stress further promote the occurrence of micro- as well as macrovascular diseases [8]. Furthermore, increased intracellular glucose in DM leads to the formation of advanced glycation end products (AGEs), which results from the interaction of glucose with amino groups on proteins with subsequent non-enzymatic glycosylation of intra- and extracellular proteins. Interactions of AGEs with their specific receptors induce activation of pro-inflammatory genes that favor the progression of diabetic micro- and macrovascular complications [9].

During the last decades, numerous cellular and clinical studies have been performed to elucidate mechanisms linking type 2 DM and obesity with the disease-related complications, particularly vascular complications. Plenty of mediators, cytokines, and factors have been identified and were assumed to play crucial roles in the pathophysiology of type 2 DM and obesity and their subsequent complications. Recently, elevated levels of fetuin-A, a protein secreted predominantly by the liver that promotes bone mineralization, and angiopoietins, growth factors produced by endothelial cells and various tissues which induce angiogenesis, have also been shown to represent independent modulating factors for type 2 DM and obesity. In addition, independent of type 2 DM and obesity, both fetuin-A and angiopoietins have previously been shown to be associated with vascular diseases. Low serum levels of fetuin-A were associated with vascular calcifications [10], whereas high levels of circulating angiopoietins were shown to be associated with endothelial damage and inflammations [11]. In this review, we aimed to concentrate on studies associating type 2 DM, obesity, and vascular diseases with two biomarkers namely angiopoietins and fetuin-A. In addition, we aimed to highlight the mechanisms that might explain these associations.

Obesity and adipose tissue

Obesity is a term which refers to excessive fat in the body. Obesity is usually determined by measuring the body mass index (BMI). Accordingly, individuals are defined to be obese when their BMI is equal or greater than 30 kg/m^2. Obesity is a multi-factorial chronic disease which is influenced by several genetic, endocrine, and psychological factors. Based on the latest Would Health Organization report, obesity, worldwide, has more than doubled since 1980. Solely in 2008, more than 1.4 billion adults were overweight. Among them over 200 million men and nearly 300 million women were obese [12]. The distribution but not the amount of the fat seems to be important in predisposing to metabolic disorders and insulin resistance. Patients with visceral/central obesity are more susceptible to cardiovascular as well as metabolic disorders than patients with peripheral (subcutaneous) obesity [13]. Sex hormones play an important role in determining the body fat distribution of an individual [14].

It is well established that visceral adipose tissue is an active endocrine and paracrine organ that secretes and produces a large number of cytokines and biological active mediators known as adipocytokines or adipokines. These include leptin, chemerin, angiotensinogen, apelin, resistin, visfatin, adiponectin, tumor necrosis factor-α (TNF-α), etc. These adipokines play a role in inflammation, coagulation, insulin resistance, diabetes, and atherosclerosis [15] that have recently been better identified using proteomics technology [16]. Therefore, adipokines are considered as significant therapeutic targets in several vascular, inflammatory, and metabolic diseases including obesity and type 2 DM [17, 18]. In addition, adipose tissue is a storage place of pro-inflammatory mediators such as interleukin-6 (IL-6), plasminogen activator inhibitor-1 (PAI-1), and C-reactive protein (CRP) that are participating directly in the pathogenesis of vascular damage, insulin resistance, and obesity-related atherogenesis. In a prospective, nested case–control study, Pradhan et al. showed that elevated levels of CRP and IL-6 predict the development of type 2 DM [19]. In contrary, adiponectin is the only adipocyte-derived adipokine that may play an important protective role against inflammation, atherosclerosis [20], and obesity-linked insulin resistance (Fig. 1). Circulating levels of adiponectin are significantly higher in lean than in obese individuals [21].

Moreover, obesity, particularly visceral obesity, alters the hepatic fat and protein metabolism. Presence of expanded adipose tissue facilitates infiltration of many pro-inflammatory mediators such as macrophages and cytokines into the adipose tissue, where they disturb the response mechanism of adipose tissue to insulin action [22]. This leads to insulin resistance and impairment of insulin-mediated antilipolysis with consequent increased release of FFA from adipose tissue and excessive FFA uptake by the liver [23], and hypertriglyceridemia (Fig. 2). Therefore, obesity is strongly associated with accumulation of fat in the liver that might progress to non-alcoholic fatty liver disease [24], one of the most common types of chronic liver diseases. Fatty liver disease is characterized by hepatic insulin resistance [25] and dyslipidemia and is related to vascular endothelial dysfunction, vascular diseases [26, 27], and the metabolic syndrome [28]. Presence of liver fat rather than total body and visceral fat is the best determinant of pre-diabetic categories in individuals at risk of type 2 DM [29]. Due to the close association of fatty

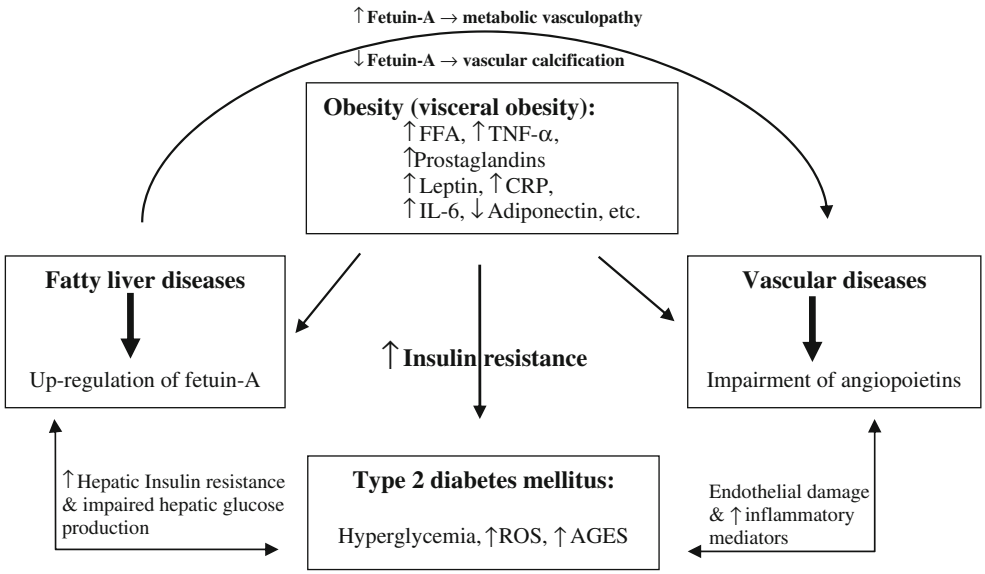

Fig. 1 Associations of obesity, type 2 diabetes mellitus, and vascular diseases with levels of circulating fetuin-A and angiopoietins. *TNF* tumor necrosis factor, *IL* interleukin, *CRP* C-reactive protein, *FFA* free fatty acid, *ROS* reactive oxygen species, *AGES* advanced glycation end products

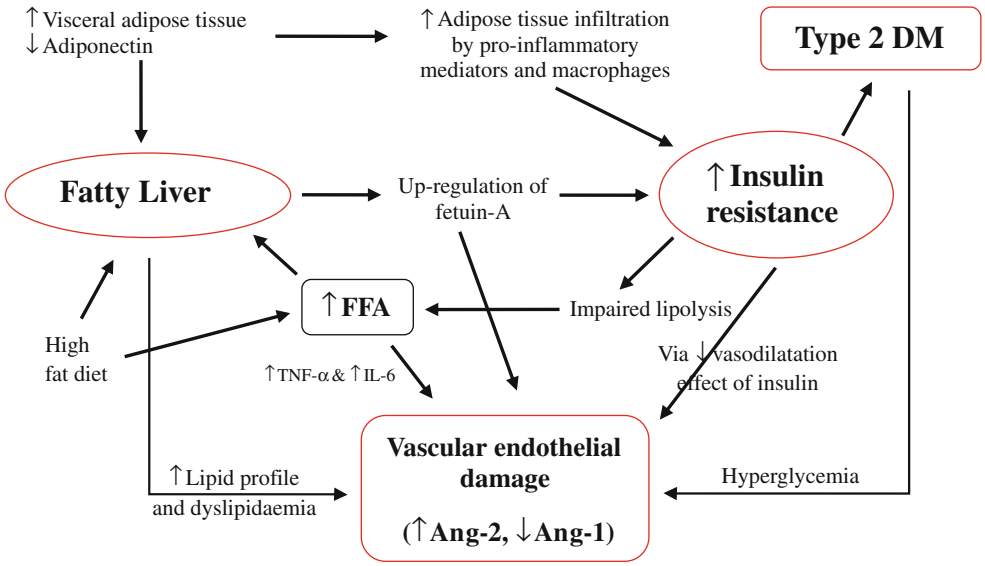

Fig. 2 Possible mechanisms connecting obesity and type 2 diabetes mellitus with fetuin-A and angiopoietins (Ang-1 and Ang-2). *FFA* free fatty acid, *TNF* tumor necrosis factor, *IL* interleukin

liver disease with insulin resistance, obesity, dyslipidemia, and metabolic syndrome, fatty liver is regarded as the hepatic sign of the metabolic syndrome [30].

Fetuin-A, type 2 diabetes mellitus, and obesity

Reports from previous studies showed that circulating levels of fetuin-A, which is also known as alpha2-Heremans-Schmid glycoprotein (AHSG), are elevated in obese and overweight individuals. Fetuin-A is an endogenous natural inhibitor of the insulin-stimulated insulin receptor tyrosine kinase [31]. It inhibits phosphorylation of the insulin receptors in the liver and muscles with the result of reduced insulin signaling and insulin resistance [32]. Thus, increased fetuin-A levels are an independent risk factor for insulin resistance and type 2 DM [33]. In a previous animal study, aged fetuin-A-null mice were protected against insulin resistance [34] and showed higher insulin receptor auto-phosphorylation and tyrosine kinase activity in skeletal

muscle and liver compared with wild-type mice. In human studies, high circulating fetuin-A levels were associated with increased risk of incidence of type 2 diabetes. Ix et al. observed in an observational study with 3,075 elderly individuals (aged between 70 and 79 years) an independent association of high circulating fetuin-A levels with incidence of diabetes [35]. Very recently, these results were confirmed in an even larger cohort study [36]. However, despite strong associations of fetuin-A with levels of insulin resistance in non-diabetic subjects [37], we [38] and Mori et al. [39] could not find any association of fetuin-A with insulin resistance among established type 2 diabetic patients.

Moreover, Lin et al. [40] provided the first evidence of association of fetuin-A with obesity. In rats overfed to induce obesity, they observed a significant increase in fetuin-A gene expression, indicating an important role of fetuin-A in predisposing to obesity. Moreover, Mathews et al. [41] showed that fetuin-A knockout mice, despite normal food intake, are more resistant to diet-induced obesity and have a lower body weight and adiposity than the wild-type control. In humans circulating fetuin-A levels were found positively associated with BMI, visceral fat and waist circumference, as demonstrated by two cross-sectional studies [35, 42]. In addition, fetuin-A correlates positively with the BMI of women with history of previous gestational DM [43]. In agreement with these results, Brix et al. [44] showed in a longitudinal-designed study that fetuin-A levels are significantly higher in patients with morbid obesity than in normal controls. Moreover, the study demonstrated reduction of fetuin-A levels in these patients following gastric bypass surgery. Also, in a 1 year longitudinal follow-up study, loss of body weight, based on exercise and diet therapies, was associated with a significant decrease of fetuin-A level in children [45]. In another cross-sectional study, fetuin-A levels were significantly higher in young- and older highly active men in comparison with young and older low-active men [46]. In this aspect, it is important also to note that mice revealed a marked decrease in Adipoq mRNA expression and lower circulating adiponectin levels after treatment with fetuin-A [47].

In addition, high circulating fetuin-A levels are strongly associated with fatty liver disease. Independent of BMI, the non-alcoholic fatty liver disease is a significant predictor of elevated fetuin-A levels [48]. As a result of fat accumulation, over-expression of fetuin-A mRNA was detected in the liver of rats after inducing obesity [40]. Furthermore, Stefan et al. showed in a human study that fat accumulation in the liver is associated with elevation of plasma fetuin-A levels [49], this has been further proven by other studies [50, 51]. Furthermore, Stefan et al. observed that reduction of hepatic fat was associated with reduced circulating fetuin-A levels. Indeed, both fatty liver and increased fetuin-A levels are associated with the metabolic syndrome [42, 52], are negatively related to the levels of adiponectin, and both are positively associated with levels of CRP [47, 53, 54]. Based on these results, the link of fetuin-A with obesity could find its reason in a common association of obesity with accumulation of fat in the liver [55], which might cause up-regulation of fetuin-A secretion with subsequent increase of insulin resistance and type 2 diabetes. Thus, fetuin-A might play an essential role in combining obesity and fatty liver with insulin resistance, type 2 diabetes, and the related vascular complications (Fig. 1). Genetic studies showed that the chromosomal locus 3q27 at which site the fetuin-A gene is found is linked with the metabolic syndrome and type 2 diabetes [56].

Angiogenesis and angiopoietins

Angiogenesis, which is a process of new blood vessel formation, is stimulated or inhibited by several factors such as vascular growth factors and nitric oxide as well as inflammatory mediators and cytokines. It is a pivotal feature in pathogenesis of cancer and many inflammatory diseases [57, 58] and plays an essential role in determining adipose tissue mass in obesity [59]. However, angiogenesis is not pathologic if its stimulating factors are in balance with its inhibiting factors. Angiopoietins (Ang) are growth factors that together with vascular endothelial growth factor (VEGF) promote angiogenesis. Among all identified Ang (1–4), only Ang-1 and Ang-2 have been well defined to have an essential role in inflammation and modulation of angiogenesis [60]. Binding of Ang-1 and Ang-2 to their endothelial-specific receptor tyrosine kinase 2 (Tie-2) is the key regulator of vascular remodeling [61]. Whereas binding of Ang-1 to Tie-2 leads to phosphorylation and activation of the receptor with subsequent vascular maturation and stabilization, and promotion of angiogenic remodeling [62], binding of Ang-2 to Tie-2 receptor prevents its activation and enhances destabilization, apoptosis, and disruption of new vessels. Thus, Ang-2 is a natural inhibitor of the Tie-2 receptor that antagonizes the Ang-1-specific vascular protective function. This is strongly expressed at sites of vascular remodeling [63]. In db/db mice subjected to myocardial ischemia, Chen and Stinnett observed a significant reduction of Tie-2 expression, disruption of Ang-1/Tie-2 signaling, and elevation of angiopoietin-2 levels [64]. Therefore, high levels of Ang-2 have been observed in diseases characterized by increased rate of vascular proliferation and endothelial damages/injuries such as tumor progression, hypertension, coronary artery diseases, chronic kidney diseases, and proliferative diabetic retinopathy [65–68]. Concurrently, in a cross-sectional study, we recently showed that levels of circulating Ang-2 but not Tie-2 are higher in type 2 diabetic patients with

than without history of cardiovascular diseases [69]. Levels of Ang-2 are negatively associated with the glomerular filtration rate [11] and are higher among female than male individuals [70, 71], which might contribute to the sexual dimorphism in impaired endothelial function observed among type 2 diabetic patients [72]. However, in our study there were no gender differences in levels of Ang-2 and Tie-2 in patients with established type 2 DM [69].

Angiopoietins, type 2 diabetes mellitus, and obesity

Vascular endothelial damage represents the most critical pathological process that links obesity, through release of pro-inflammatory mediators from adipose tissue, and type 2 DM, through hyperglycemia, with increased incidence of micro- and macrovascular diseases. Hyperglycemia impairs endothelial function even before the diagnosis of type 2 DM has been clinically established. Mechanisms underlying vascular endothelial damage by hyperglycemia are: (1) Formation of AGEs through the non-enzymatic glycosylation of intra- and extracellular proteins. Studies have associated high expression of AGEs with an increased incidence of diabetic vascular complications [73]. AGEs up-regulate expression of VEGF and Ang-2 in endothelial cell [74]. (2) Elevated sorbitol concentration that increases cellular osmolarity, and generates ROS, which leads to endothelial cell apoptosis and dysfunction and induced oxidative stress. (3) Hyperglycemia leads to the increase of intracellular glycolytic metabolites that promote synthesis of diacylglycerol which has been linked through the activation of protein kinase C to various vascular abnormalities [75]. (4) Hyperglycemia increases the flux through the hexosamine pathway, which generates fructose-6-phosphate. The hexosamine pathway might alter glycosylation of proteins such as endothelial nitric oxide synthase or activates the gene expression for transforming growth factor β (TGF-β) or PAI-1 that play pivotal roles in pathogenesis of diabetic vascular complications [76]. Furthermore, clinical studies showed that circulating Ang-2 but not Ang-1 are elevated in type 2 diabetic patients [70, 77]. Although the exact mechanism linking type 2 DM with increased levels of Ang-2 is still not clear, results from previous experimental studies revealed an increased Ang-2 expression in endothelial cells of pancreas, kidney, heart, brain, and retina exposed to the effects of hyperglycemia. This was accompanied by vascular damage, endothelial apoptosis, and decreased vascular density in these tissues [78–82]. Increased Ang-2 expression mediates expression of intracellular adhesion molecules that sensitize endothelial cells to the pro-inflammatory effects of TNF-α [83]. In mice model studies, hyperglycemia was associated with impaired wound healing due to the increased Ang-2 and decreased Tie-2 expression [84, 85]. Moreover, Singh et al. showed in an in vitro study that increased glucose enhances suppression of vascular protection by Ang-1 and predisposes to endothelia dysfunction and vascular damage [86]. In agreement with these results, blockade of Ang-2 action and enhancement of Ang-1 and VEGF production were associated with improvement of hyperglycemia-induced cardiac and pancreatic-vasculopathy in animal studies [82, 87–89]. Briefly, these studies concluded that hyperglycemia is the main cause of down-regulation of Ang-1 production and up-regulation of Ang-2 expression that result in destabilization of endothelial cells and vascular damage in type 2 DM.

On the other hand, obesity is strongly associated with increased vascular dysfunctions and vascular diseases. Mechanisms contributing to this association could be due to hypertension, dyslipidemia, fatty liver, inflammation, and insulin resistance [90]. Adipose tissue is an active and well-vascularized organ that has the ability to change and grow throughout human life span [91]. It contains angiogenic stimulators and inhibitors and its mass closely depends on the rate of angiogenesis [59]. Obesity is characterized by elevated pre-inflammatory mediators (indicated above) and reduced adiponectin levels [92]. Adiponectin is an adipokine that is highly related to insulin resistance and inversely associated with TNF-α [93]. It is an important natural angiogenic inhibitor that prevents growth of adipose tissue and induces apoptosis of endothelial cells [94]. Therefore, levels of adiponectin are higher in lean than in obese individuals and are highly associated with reduced incidence of vascular diseases [95] and negatively related to fatty liver in diabetic [96] and non-diabetic subjects [97]. Moreover, adipose tissue is rich of FFA that might directly contribute to the obesity-related endothelial dysfunction. In vitro, FFA were shown to disturb endothelial function by stimulating ROS formation and reducing the vasodilator factor nitric oxide [98]. In addition, FFA activate through multiple pathways production of inflammatory cytokines such as TNF-α and IL-6 [99]. Elevated levels of theses cytokine have shown to be associated with different types of inflammatory and vascular diseases, and atherosclerosis [100] (Fig. 2). In consequence, hepatic synthesis of CRP is stimulated by the effect of IL-6 [101]. CRP is a marker of chronic inflammation that is highly expressed in adipose tissue and it's negatively correlated with levels of adiponectin [102]. Levels of CRP predict vascular mortality and death from cancer [103] and are associated with the severity of coronary atherosclerosis [104]. Thus, obesity induces endothelial dysfunction and local inflammatory vascular injuries. Interestingly, previous evidence showed that Ang-2 facilitates vascular response to inflammation and is up-regulated at the sites of vascular remodeling. It sensitizes endothelial cells for TNF-α effect, thereby induces expression of endothelial cell adhesion molecules [105].

Consistent with these results, levels of Ang-2 are positively correlated with levels of the inflammatory marker CRP [106]. Correspondingly, previous studies demonstrated impaired vascular components toward elevation of Ang-2 levels in overweight and obese patients, which was independent of presence of hyperglycemia [71, 107]. Incubation of endothelial cells with high concentration of FFA, as it occurs in obesity, significantly suppresses signaling of the protective Ang-1 in these cells [86]. Increased Ang-2 and reduced Ang-1 is associated with reduced vascular density and increased vascular disruptions and damages, these are pathological settings commonly found in obesity-related vascular diseases [108].

Other pathophysiological impacts of fetuin-A

In addition to its potential impact on incidence of type 2 DM and obesity, fetuin-A plays critical roles in mechanisms of bone mineralization and vascular calcification. Fetuin-A acts as a carrier protein for calcium and phosphate, thereby inhibiting their precipitation in the serum [109] and enhancing calcification and mineralization of fibril within the bone [110]. In clinical studies, fetuin-A levels are positively associated with the degree of bone mineral density [111] and negatively related to the levels of bone resorption biomarkers [38, 112]. Feeding a mineral and vitamin D-rich diet to fetuin-A-deficient mice resulted in a widespread calcification outside the skeletal system. Therefore, fetuin-A potentially prevents undesirable or ectopic mineralization [113, 114]. Deficiency of fetuin-A was associated with soft tissue and artery calcification in rat model studies [115]. However, in vivo studies have reported conflicting results regarding association of fetuin-A with vascular diseases. Several studies, which involved patients with end stage renal failure [116–118] as well as patients with intact kidney function [119, 120], have observed reverse association of fetuin-A levels with incidence of vascular calcification. Involvement of fetuin-A in the pathogenesis of cardiovascular diseases has been confirmed in a genetic study with 2,500 patients [121]. Other studies have reported no association of fetuin-A level with peripheral as well as cardiovascular events [122]. Very recently, Lorant et al. [123] showed in a cross-sectional study that high fetuin-A levels, independent of diabetes, are associated with increased incidence of peripheral vascular diseases. However, they observed an inverse association of fetuin-A with degree of vascular calcification in type 2 diabetic patients with peripheral vascular diseases. In fact, fetuin-A is a multifunctional protein that might be involved in pathophysiological mechanisms of a variety of metabolic and atherosclerotic diseases. Based on the results of these studies, contribution of fetuin-A to the pathophysiology of vascular diseases could be either due to its potential relationship with obesity, type 2 DM, metabolic syndrome, and fatty liver diseases, which detrimentally affect the vascular system, or due to its crucial role in promoting bone mineralization and preventing ectopic calcification.

Conclusion

Obesity and type 2 DM are two chronic metabolic disorders strongly connected with increased incidence of peripheral and cardiovascular diseases. Whereas increased levels of fetuin-A protein is highly related to the pathophysiological mechanisms of type 2 DM, obesity, and fatty liver and their related vascular complications, low fetuin-A levels are associated with increased vascular calcifications. Hyperglycemia and release of pro-inflammatory mediators from adipose tissue might contribute to the mechanisms of elevated Ang-2 in type 2 DM and obesity, respectively. Elevated Ang-2 levels solely but not Ang-1 enhances endothelial cell apoptosis, vascular disruption, and endothelial dysfunctions, pathological events closely linked to obesity- and type 2 diabetes-related vascular complications.

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Conflict of interest The authors declare that they have no conflicts of interest regarding the content of this review.

References

1. American Diabetes Association, Diagnosis and classification of diabetes mellitus. Diabetes Care **33**(Suppl 1), S62–S69 (2010). doi:10.2337/dc10-S062
2. L. Bellamy, J.P. Casas, A.D. Hingorani, D. Williams, Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. Lancet **373**(9677), 1773–1779 (2009). doi:10.1016/S0140-6736(09)60731-5
3. R.J. Chang, R.M. Nakamura, H.L. Judd, S.A. Kaplan, Insulin resistance in nonobese patients with polycystic ovarian disease. J. Clin. Endocrinol. Metab. **57**(2), 356–359 (1983)
4. G.R. Hajer, T.W. van Haeften, F.L. Visseren, Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. Eur. Heart J. **29**(24), 2959–2971 (2008). doi:10.1093/eurheartj/ehn387
5. N. Stefan, A.M. Hennige, H. Staiger, J. Machann, F. Schick, E. Schleicher, A. Fritsche, H.U. Haring, High circulating retinol-binding protein 4 is associated with elevated liver fat but not with total, subcutaneous, visceral, or intramyocellular fat in humans. Diabetes Care **30**(5), 1173–1178 (2007). doi:10.2337/dc06-2342
6. A. Seppala-Lindroos, S. Vehkavaara, A.M. Hakkinen, T. Goto, J. Westerbacka, A. Sovijarvi, J. Halavaara, H. Yki-Jarvinen, Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. J. Clin. Endocrinol. Metab. **87**(7), 3023–3028 (2002)

7. J.H. Hwang, D.T. Stein, N. Barzilai, M.H. Cui, J. Tonelli, P. Kishore, M. Hawkins, Increased intrahepatic triglyceride is associated with peripheral insulin resistance: in vivo MR imaging and spectroscopy studies. Am. J. Physiol. Endocrinol. Metab. **293**(6), E1663–E1669 (2007). doi:10.1152/ajpendo.00590.2006
8. E.J. de Belin Chantemele, D.W. Stepp, Influence of obesity and metabolic dysfunction on the endothelial control in the coronary circulation. J. Mol. Cell. Cardiol. **52**(4), 840–847 (2012). doi:10.1016/j.yjmcc.2011.08.018
9. J.B. Lindsey, F. Cipollone, S.M. Abdullah, D.K. McGuire, Receptor for advanced glycation end-products (RAGE) and soluble RAGE (sRAGE): cardiovascular implications. Diabetes Vasc. Dis. Res. **6**(1), 7–14 (2009). doi:10.3132/dvdr.2009.002
10. R. Westenfeld, C. Schafer, T. Kruger, C. Haarmann, L.J. Schurgers, C. Reutelingsperger, O. Ivanovski, T. Drueke, Z.A. Massy, M. Ketteler, J. Floege, W. Jahnen-Dechent, Fetuin-A protects against atherosclerotic calcification in CKD. J. Am. Soc. Nephrol. **20**(6), 1264–1274 (2009). doi:10.1681/ASN.2008060572
11. S. David, P. Kumpers, A. Lukasz, D. Fliser, J. Martens-Lobenhoffer, S.M. Bode-Boger, V. Kliem, H. Haller, J.T. Kielstein, Circulating angiopoietin-2 levels increase with progress of chronic kidney disease. Nephrol. Dial. Transplant. **25**(8), 2571–2576 (2010). doi:10.1093/ndt/gfq060
12. Obesity and Overweight. World Health Organization Fact sheet No. 311 (May 2012)
13. A. Rodriguez, V. Catalan, J. Gomez-Ambrosi, G. Fruhbeck, Visceral and subcutaneous adiposity: are both potential therapeutic targets for tackling the metabolic syndrome? Curr. Pharm. Des. **13**(21), 2169–2175 (2007)
14. E.B. Geer, W. Shen, Gender differences in insulin resistance, body composition, and energy balance. Gend. Med. **6**(Suppl 1), 60–75 (2009). doi:10.1016/j.genm.2009.02.002
15. V. Mohamed-Ali, J.H. Pinkney, S.W. Coppack, Adipose tissue as an endocrine and paracrine organ. Int. J. Obes. Rel. Metab. Disord. **22**(12), 1145–1158 (1998)
16. M. Pardo, A. Roca-Rivada, L.M. Seoane, F.F. Casanueva, Obesidomics: contribution of adipose tissue secretome analysis to obesity research. Endocrine **41**(3), 374–383 (2012). doi:10.1007/s12020-012-9617-z
17. I. Castan-Laurell, C. Dray, C. Attane, T. Duparc, C. Knauf, P. Valet, Apelin, diabetes, and obesity. Endocrine **40**(1), 1–9 (2011). doi:10.1007/s12020-011-9507-9
18. K. Brochu-Gaudreau, C. Rehfeldt, R. Blouin, V. Bordignon, B.D. Murphy, M.F. Palin, Adiponectin action from head to toe. Endocrine **37**(1), 11–32 (2010). doi:10.1007/s12020-009-9278-8
19. A.D. Pradhan, J.E. Manson, N. Rifai, J.E. Buring, P.M. Ridker, C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA **286**(3), 327–334 (2001)
20. N. Kubota, Y. Terauchi, T. Yamauchi, T. Kubota, M. Moroi, J. Matsui, K. Eto, T. Yamashita, J. Kamon, H. Satoh, W. Yano, P. Froguel, R. Nagai, S. Kimura, T. Kadowaki, T. Noda, Disruption of adiponectin causes insulin resistance and neointimal formation. J. Biol. Chem. **277**(29), 25863–25866 (2002). doi:10.1074/jbc.C200251200
21. J.V. Silha, M. Krsek, J.V. Skrha, P. Sucharda, B.L. Nyomba, L.J. Murphy, Plasma resistin, adiponectin and leptin levels in lean and obese subjects: correlations with insulin resistance. Eur. J. Endocrinol. **149**(4), 331–335 (2003)
22. H. Xu, G.T. Barnes, Q. Yang, G. Tan, D. Yang, C.J. Chou, J. Sole, A. Nichols, J.S. Ross, L.A. Tartaglia, H. Chen, Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J. Clin. Investig. **112**(12), 1821–1830 (2003). doi:10.1172/JCI19451
23. M. Roden, Mechanisms of disease: hepatic steatosis in type 2 diabetes-pathogenesis and clinical relevance. Nat. Clin. Pract. Endocrinol. Metab. **2**(6), 335–348 (2006). doi:10.1038/ncpendmet0190
24. S.R. Weston, W. Leyden, R. Murphy, N.M. Bass, B.P. Bell, M.M. Manos, N.A. Terrault, Racial and ethnic distribution of nonalcoholic fatty liver in persons with newly diagnosed chronic liver disease. Hepatology **41**(2), 372–379 (2005). doi:10.1002/hep.20554
25. D.M. Ferreira, R.E. Castro, M.V. Machado, T. Evangelista, A. Silvestre, A. Costa, J. Coutinho, F. Carepa, H. Cortez-Pinto, C.M. Rodrigues, Apoptosis and insulin resistance in liver and peripheral tissues of morbidly obese patients is associated with different stages of non-alcoholic fatty liver disease. Diabetologia **54**(7), 1788–1798 (2011). doi:10.1007/s00125-011-2130-8
26. N. Villanova, S. Moscatiello, S. Ramilli, E. Bugianesi, D. Magalotti, E. Vanni, M. Zoli, G. Marchesini, Endothelial dysfunction and cardiovascular risk profile in nonalcoholic fatty liver disease. Hepatology **42**(2), 473–480 (2005). doi:10.1002/hep.20781
27. E. Scorletti, P.C. Calder, C.D. Byrne, Non-alcoholic fatty liver disease and cardiovascular risk: metabolic aspects and novel treatments. Endocrine **40**(3), 332–343 (2011). doi:10.1007/s12020-011-9530-x
28. G. Marchesini, M. Brizi, G. Bianchi, S. Tomassetti, E. Bugianesi, M. Lenzi, A.J. McCullough, S. Natale, G. Forlani, N. Melchionda, Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. Diabetes **50**(8), 1844–1850 (2001)
29. K. Kantartzis, J. Machann, F. Schick, A. Fritsche, H.U. Haring, N. Stefan, The impact of liver fat vs visceral fat in determining categories of prediabetes. Diabetologia **53**(5), 882–889 (2010). doi:10.1007/s00125-010-1663-6
30. G. Marchesini, E. Bugianesi, G. Forlani, F. Cerrelli, M. Lenzi, R. Manini, S. Natale, E. Vanni, N. Villanova, N. Melchionda, M. Rizzetto, Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology **37**(4), 917–923 (2003). doi:10.1053/jhep.2003.50161
31. G. Rauth, O. Poschke, E. Fink, M. Eulitz, S. Tippmer, M. Kellerer, H.U. Haring, P. Nawratil, M. Haasemann, W. Jahnen-Dechent 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. **204**(2), 523–529 (1992)
32. P. Auberger, L. Falquerho, J.O. Contreres, G. Pages, G. Le Cam, B. Rossi, A. Le Cam, Characterization of a natural inhibitor of the insulin receptor tyrosine kinase: cDNA cloning, purification, and anti-mitogenic activity. Cell **58**(4), 631–640 (1989)
33. N. Stefan, A. Fritsche, C. Weikert, H. Boeing, H.G. Joost, H.U. Haring, M.B. Schulze, Plasma fetuin-A levels and the risk of type 2 diabetes. Diabetes **57**(10), 2762–2767 (2008). doi:10.2337/db08-0538
34. S.T. Mathews, S. Rakhade, X. Zhou, G.C. Parker, D.V. Coscina, G. Grunberger, Fetuin-null mice are protected against obesity and insulin resistance associated with aging. Biochem. Biophys. Res. Commun. **350**(2), 437–443 (2006). doi:10.1016/j.bbrc.2006.09.071
35. J.H. Ix, C.L. Wassel, A.M. Kanaya, E. Vittinghoff, K.C. Johnson, A. Koster, J.A. Cauley, T.B. Harris, S.R. Cummings, M.G. Shlipak, Health ABC Study, Fetuin-A and incident diabetes mellitus in older persons. JAMA **300**(2), 182–188 (2008). doi:10.1001/jama.300.2.182
36. J.H. Ix, M.L. Biggs, K.J. Mukamal, J.R. Kizer, S.J. Zieman, D.S. Siscovick, D. Mozzaffarian, M.K. Jensen, L. Nelson, N. Ruderman, L. Djousse, Association of fetuin-a with incident diabetes mellitus in community-living older adults: the cardiovascular health study. Circulation **125**(19), 2316–2322 (2012). doi:10.1161/CIRCULATIONAHA.111.072751
37. A. Ishibashi, Y. Ikeda, T. Ohguro, Y. Kumon, S. Yamanaka, H. Takata, M. Inoue, T. Suehiro, Y. Terada, Serum fetuin-A is an independent marker of insulin resistance in Japanese men. J. Atheroscler. Thromb. **17**(9), 925–933 (2010)

38. S. Rasul, A. Ilhan, M.H. Reiter, J. Todoric, S. Farhan, H. Esterbauer, A. Kautzky-Willer, Levels of fetuin-A relate to the levels of bone turnover biomarkers in male and female patients with type 2 diabetes. Clin. Endocrinol. **76**(4), 499–505 (2012). doi:10.1111/j.1365-2265.2011.04246.x
39. K. Mori, M. Emoto, H. Yokoyama, T. Araki, M. Teramura, H. Koyama, T. Shoji, M. Inaba, Y. Nishizawa, Association of serum fetuin-A with insulin resistance in type 2 diabetic and nondiabetic subjects. Diabetes Care **29**(2), 468 (2006)
40. X. Lin, H.D. Braymer, G.A. Bray, D.A. York, Differential expression of insulin receptor tyrosine kinase inhibitor (fetuin) gene in a model of diet-induced obesity. Life Sci. **63**(2), 145–153 (1998)
41. S.T. Mathews, G.P. Singh, M. Ranalletta, V.J. Cintron, X. Qiang, A.S. Goustin, K.L. Jen, M.J. Charron, W. Jahnen-Dechent, G. Grunberger, Improved insulin sensitivity and resistance to weight gain in mice null for the Ahsg gene. Diabetes **51**(8), 2450–2458 (2002)
42. J.H. Ix, M.G. Shlipak, V.M. Brandenburg, S. Ali, M. Ketteler, M.A. Whooley, Association between human fetuin-A and the metabolic syndrome: data from the heart and soul study. Circulation **113**(14), 1760–1767 (2006). doi:10.1161/CIRCULATIONAHA.105.588723
43. S. Farhan, A. Handisurya, J. Todoric, A. Tura, G. Pacini, O. Wagner, K. Klein, R. Jarai, K. Huber, A. Kautzky-Willer, Fetuin-A characteristics during and after pregnancy: result from a case control pilot study. Int. J. Endocrinol. **2012**, 896736 (2012). doi:10.1155/2012/896736
44. J.M. Brix, H. Stingl, F. Hollerl, G.H. Schernthaner, H.P. Kopp, G. Schernthaner, Elevated Fetuin-A concentrations in morbid obesity decrease after dramatic weight loss. J. Clin. Endocrinol. Metab. **95**(11), 4877–4881 (2010). doi:10.1210/jc.2010-0148
45. T. Reinehr, C.L. Roth, Fetuin-A and its relation to metabolic syndrome and fatty liver disease in obese children before and after weight loss. J. Clin. Endocrinol. Metab. **93**(11), 4479–4485 (2008). doi:10.1210/jc.2008-1505
46. N.T. Jenkins, J.A. McKenzie, J.M. Hagberg, S. Witkowski, Plasma fetuin-A concentrations in young and older high- and low-active men. Metab. Clin. Exp. **60**(2), 265–271 (2011). doi:10.1016/j.metabol.2010.01.026
47. A.M. Hennige, H. Staiger, C. Wicke, F. Machicao, A. Fritsche, H.U. Haring, N. Stefan, Fetuin-A induces cytokine expression and suppresses adiponectin production. PLoS One **3**(3), e1765 (2008). doi:10.1371/journal.pone.0001765
48. J.W. Haukeland, T.B. Dahl, A. Yndestad, I.P. Gladhaug, E.M. Loberg, T. Haaland, Z. Konopski, C. Wium, E.T. Aasheim, O.E. Johansen, P. Aukrust, B. Halvorsen, K.I. Birkeland, Fetuin A in nonalcoholic fatty liver disease: in vivo and in vitro studies. Eur. J. Endocrinol. **166**(3), 503–510 (2012). doi:10.1530/EJE-11-0864
49. N. Stefan, A.M. Hennige, H. Staiger, J. Machann, F. Schick, S.M. Krober, F. Machicao, A. Fritsche, H.U. Haring, Alpha2-Heremans-Schmid glycoprotein/fetuin-A is associated with insulin resistance and fat accumulation in the liver in humans. Diabetes Care **29**(4), 853–857 (2006)
50. K. Mussig, H. Staiger, F. Machicao, J. Machann, A.M. Hennige, F. Schick, C.D. Claussen, A. Fritsche, H.U. Haring, N. Stefan, AHSG gene variation is not associated with regional body fat distribution—a magnetic resonance study. Exp. Clin. Endocrinol. Diabetes **117**(8), 432–437 (2009). doi:10.1055/s-0028-1103299
51. Y. Yilmaz, O. Yonal, R. Kurt, F. Ari, A.Y. Oral, C.A. Celikel, S. Korkmaz, E. Ulukaya, O. Ozdogan, N. Imeryuz, E. Avsar, C. Kalayci, Serum fetuin A/alpha2HS-glycoprotein levels in patients with non-alcoholic fatty liver disease: relation with liver fibrosis. Ann. Clin. Biochem. **47**(Pt 6), 549–553 (2010). doi:10.1258/acb.2010.010169
52. A. Kotronen, H. Yki-Jarvinen, Fatty liver: a novel component of the metabolic syndrome. Arterioscler. Thromb. Vasc. Biol. **28**(1), 27–38 (2008). doi:10.1161/ATVBAHA.107.147538
53. S.H. Park, B.I. Kim, J.W. Yun, J.W. Kim, D.I. Park, Y.K. Cho, I.K. Sung, C.Y. Park, C.I. Sohn, W.K. Jeon, H. Kim, E.J. Rhee, W.Y. Lee, S.W. Kim, Insulin resistance and C-reactive protein as independent risk factors for non-alcoholic fatty liver disease in non-obese Asian men. J. Gastroenterol. Hepatol. **19**(6), 694–698 (2004). doi:10.1111/j.1440-1746.2004.03362.x
54. J.M. Hui, A. Hodge, G.C. Farrell, J.G. Kench, A. Kriketos, J. George, Beyond insulin resistance in NASH: tNF-alpha or adiponectin? Hepatology **40**(1), 46–54 (2004). doi:10.1002/hep.20280
55. S. Bellentani, G. Saccoccio, F. Masutti, L.S. Croce, G. Brandi, F. Sasso, G. Cristanini, C. Tiribelli, Prevalence of and risk factors for hepatic steatosis in Northern Italy. Ann. Intern. Med. **132**(2), 112–117 (2000)
56. N. Vionnet, E.H. Hani, S. Dupont, S. Gallina, S. Francke, S. Dotte, F. De Matos, E. Durand, F. Lepretre, C. Lecoeur, P. Gallina, L. Zekiri, C. Dina, P. Froguel, Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21–q24. Am. J. Hum. Genet. **67**(6), 1470–1480 (2000). doi:10.1086/316887
57. J. Folkman, Angiogenesis-dependent diseases. Semin. Oncol. **28**(6), 536–542 (2001)
58. D.A. Walsh, C.I. Pearson, Angiogenesis in the pathogenesis of inflammatory joint and lung diseases. Arthr. Res. **3**(3), 147–153 (2001)
59. M.A. Rupnick, D. Panigrahy, C.Y. Zhang, S.M. Dallabrida, B.B. Lowell, R. Langer, M.J. Folkman, Adipose tissue mass can be regulated through the vasculature. Proc. Natl. Acad. Sci. U.S.A. **99**(16), 10730–10735 (2002). doi:10.1073/pnas.162349799
60. N.P. Fam, S. Verma, M. Kutryk, D.J. Stewart, Clinician guide to angiogenesis. Circulation **108**(21), 2613–2618 (2003). doi:10.1161/01.CIR.0000102939.04279.75
61. U. Fiedler, H.G. Augustin, Angiopoietins: a link between angiogenesis and inflammation. Trends Immunol. **27**(12), 552–558 (2006). doi:10.1016/j.it.2006.10.004
62. F. Shalaby, J. Rossant, T.P. Yamaguchi, M. Gertsenstein, X.F. Wu, M.L. Breitman, A.C. Schuh, Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. Nature **376**(6535), 62–66 (1995). doi:10.1038/376062a0
63. P.C. Maisonpierre, C. Suri, P.F. Jones, S. Bartunkova, S.J. Wiegand, C. Radziejewski, D. Compton, J. McClain, T.H. Aldrich, N. Papadopoulos, T.J. Daly, S. Davis, T.N. Sato, G.D. Yancopoulos, Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. Science **277**(5322), 55–60 (1997)
64. J.X. Chen, A. Stinnett, Disruption of Ang-1/Tie-2 signaling contributes to the impaired myocardial vascular maturation and angiogenesis in type II diabetic mice. Arterioscler. Thromb. Vasc. Biol. **28**(9), 1606–1613 (2008). doi:10.1161/ATVBAHA.108.169235
65. D. Watanabe, K. Suzuma, I. Suzuma, H. Ohashi, T. Ojima, M. Kurimoto, T. Murakami, T. Kimura, H. Takagi, Vitreous levels of angiopoietin 2 and vascular endothelial growth factor in patients with proliferative diabetic retinopathy. Am. J. Ophthalmol. **139**(3), 476–481 (2005). doi:10.1016/j.ajo.2004.10.004
66. A.Y. Chong, G.J. Caine, B. Freestone, A.D. Blann, G.Y. Lip, Plasma angiopoietin-1, angiopoietin-2, and angiopoietin receptor tie-2 levels in congestive heart failure. J. Am. Coll. Cardiol. **43**(3), 423–428 (2004). doi:10.1016/j.jacc.2003.08.042
67. S. Anuradha, V. Mohan, K. Gokulakrishnan, M. Dixit, Angiopoietin-2 levels in glucose intolerance, hypertension, and metabolic syndrome in Asian Indians (Chennai Urban Rural Epidemiology Study-74). Metab. Clin. Exp. **59**(6), 774–779 (2010). doi:10.1016/j.metabol.2009.09.022
68. Y.C. Chung, Y.C. Hou, C.N. Chang, T.H. Hseu, Expression and prognostic significance of angiopoietin in colorectal carcinoma. J. Surg. Oncol. **94**(7), 631–638 (2006). doi:10.1002/jso.20423

69. S. Rasul, M.H. Reiter, A. Ilhan, K. Lampichler, L. Wagner, A. Kautzky-Willer, Circulating angiopoietin-2 and soluble Tie-2 in type 2 diabetes mellitus: a cross-sectional study. Cardiovasc. Diabetol. **10**, 55 (2011). doi:10.1186/1475-2840-10-55
70. W. Lieb, J.P. Zachariah, V. Xanthakis, R. Safa, M.H. Chen, L.M. Sullivan, M.G. Larson, H.M. Smith, Q. Yang, G.F. Mitchell, J.A. Vita, D.B. Sawyer, R.S. Vasan, Clinical and genetic correlates of circulating angiopoietin-2 and soluble Tie-2 in the community. Circ. Cardiovasc. Genet. **3**(3), 300–306 (2010). doi:10.1161/CIRCGENETICS.109.914556
71. J.V. Silha, M. Krsek, P. Sucharda, L.J. Murphy, Angiogenic factors are elevated in overweight and obese individuals. Int. J. Obes. **29**(11), 1308–1314 (2005). doi:10.1038/sj.ijo.0802987
72. H.O. Steinberg, G. Paradisi, J. Cronin, K. Crowde, A. Hempfling, G. Hook, A.D. Baron, Type II diabetes abrogates sex differences in endothelial function in premenopausal women. Circulation **101**(17), 2040–2046 (2000)
73. Y. Yamamoto, I. Kato, T. Doi, H. Yonekura, S. Ohashi, M. Takeuchi, T. Watanabe, S. Yamagishi, S. Sakurai, S. Takasawa, H. Okamoto, H. Yamamoto, Development and prevention of advanced diabetic nephropathy in RAGE-overexpressing mice. J. Clin. Investig. **108**(2), 261–268 (2001). doi:10.1172/JCI11771
74. T. Okamoto, S. Yamagishi, Y. Inagaki, S. Amano, K. Koga, R. Abe, M. Takeuchi, S. Ohno, A. Yoshimura, Z. Makita, Angiogenesis induced by advanced glycation end products and its prevention by cerivastatin. FASEB J. **16**(14), 1928–1930 (2002). doi:10.1096/fj.02-0030fje
75. B. Williams, B. Gallacher, H. Patel, C. Orme, Glucose-induced protein kinase C activation regulates vascular permeability factor mRNA expression and peptide production by human vascular smooth muscle cells in vitro. Diabetes **46**(9), 1497–1503 (1997)
76. X.L. Du, D. Edelstein, L. Rossetti, I.G. Fantus, H. Goldberg, F. Ziyadeh, J. Wu, M. Brownlee, Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation. Proc. Natl. Acad. Sci. U.S.A. **97**(22), 12222–12226 (2000). doi:10.1073/pnas.97.22.12222
77. H.S. Lim, G.Y. Lip, A.D. Blann, Angiopoietin-1 and angiopoietin-2 in diabetes mellitus: relationship to VEGF, glycaemic control, endothelial damage/dysfunction and atherosclerosis. Atherosclerosis **180**(1), 113–118 (2005). doi:10.1016/j.atherosclerosis.2004.11.004
78. J.X. Chen, H. Zeng, J. Reese, J.L. Aschner, B. Meyrick, Overexpression of angiopoietin-2 impairs myocardial angiogenesis and exacerbates cardiac fibrosis in the diabetic db/db mouse model. Am. J. Physiol. Heart Circ. Physiol. **302**(4), H1003–H1012 (2012). doi:10.1152/ajpheart.00866.2011
79. Q.H. Tuo, H. Zeng, A. Stinnett, H. Yu, J.L. Aschner, D.F. Liao, J.X. Chen, Critical role of angiopoietins/Tie-2 in hyperglycemic exacerbation of myocardial infarction and impaired angiogenesis. Am. J. Physiol. Heart Circ. Physiol. **294**(6), H2547–H2557 (2008). doi:10.1152/ajpheart.01250.2007
80. H.P. Hammes, J. Lin, P. Wagner, Y. Feng, F. Vom Hagen, T. Krzizok, O. Renner, G. Breier, M. Brownlee, U. Deutsch, Angiopoietin-2 causes pericyte dropout in the normal retina: evidence for involvement in diabetic retinopathy. Diabetes **53**(4), 1104–1110 (2004)
81. X. Cui, M. Chopp, A. Zacharek, X. Ye, C. Roberts, J. Chen, Angiopoietin/Tie2 pathway mediates type 2 diabetes induced vascular damage after cerebral stroke. Neurobiol. Dis. **43**(1), 285–292 (2011). doi:10.1016/j.nbd.2011.04.005
82. S. Calderari, C. Chougnet, M. Clemessy, H. Kempf, P. Corvol, E. Larger, Angiopoietin 2 alters pancreatic vascularization in diabetic conditions. PLoS One **7**(1), e29438 (2012). doi:10.1371/journal.pone.0029438
83. D. Yao, T. Taguchi, T. Matsumura, R. Pestell, D. Edelstein, I. Giardino, G. Suske, N. Rabbani, P.J. Thornalley, V.P. Sarthy, H.P. Hammes, M. Brownlee, High glucose increases angiopoietin-2 transcription in microvascular endothelial cells through methylglyoxal modification of mSin3A. J. Biol. Chem. **282**(42), 31038–31045 (2007). doi:10.1074/jbc.M704703200
84. H. Kampfer, J. Pfeilschifter, S. Frank, Expressional regulation of angiopoietin-1 and -2 and the tie-1 and -2 receptor tyrosine kinases during cutaneous wound healing: a comparative study of normal and impaired repair. Lab. Investig. **81**(3), 361–373 (2001)
85. L. Qiao, S.L. Lu, J.Y. Dong, F. Song, Abnormal regulation of neo-vascularisation in deep partial thickness scalds in rats with diabetes mellitus. Burns **37**(6), 1015–1022 (2011). doi:10.1016/j.burns.2011.03.020
86. H. Singh, N.P. Brindle, V.A. Zammit, High glucose and elevated fatty acids suppress signaling by the endothelium protective ligand angiopoietin-1. Microvasc. Res. **79**(2), 121–127 (2010). doi:10.1016/j.mvr.2010.01.005
87. M. Brissova, A. Shostak, M. Shiota, P.O. Wiebe, G. Poffenberger, J. Kantz, Z. Chen, C. Carr, W.G. Jerome, J. Chen, H.S. Baldwin, W. Nicholson, D.M. Bader, T. Jetton, M. Gannon, A.C. Powers, Pancreatic islet production of vascular endothelial growth factor—a is essential for islet vascularization, revascularization, and function. Diabetes **55**(11), 2974–2985 (2006). doi:10.2337/db06-0690
88. Q.H. Tuo, G.Z. Xiong, H. Zeng, H.D. Yu, S.W. Sun, H.Y. Ling, B.Y. Zhu, D.F. Liao, J.X. Chen, Angiopoietin-1 protects myocardial endothelial cell function blunted by angiopoietin-2 and high glucose condition. Acta Pharmacol. Sin. **32**(1), 45–51 (2011). doi:10.1038/aps.2010.183
89. D. Su, N. Zhang, J. He, S. Qu, S. Slusher, R. Bottino, S. Bertera, J. Bromberg, H.H. Dong, Angiopoietin-1 production in islets improves islet engraftment and protects islets from cytokine-induced apoptosis. Diabetes **56**(9), 2274–2283 (2007). doi:10.2337/db07-0371
90. P. Mathieu, P. Pibarot, E. Larose, P. Poirier, A. Marette, J.P. Despres, Visceral obesity and the heart. Int. J. Biochem. Cell Biol. **40**(5), 821–836 (2008). doi:10.1016/j.biocel.2007.12.001
91. D.L. Crandall, G.J. Hausman, J.G. Kral, A review of the microcirculation of adipose tissue: anatomic, metabolic, and angiogenic perspectives. Microcirculation **4**(2), 211–232 (1997)
92. P.A. Kern, G.B. Di Gregorio, T. Lu, N. Rassouli, G. Ranganathan, Adiponectin expression from human adipose tissue: relation to obesity, insulin resistance, and tumor necrosis factor-alpha expression. Diabetes **52**(7), 1779–1785 (2003)
93. N. Maeda, I. Shimomura, K. Kishida, H. Nishizawa, M. Matsuda, H. Nagaretani, N. Furuyama, H. Kondo, M. Takahashi, Y. Arita, R. Komuro, N. Ouchi, S. Kihara, Y. Tochino, K. Okutomi, M. Horie, S. Takeda, T. Aoyama, T. Funahashi, Y. Matsuzawa, Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nat. Med. **8**(7), 731–737 (2002). doi:10.1038/nm724
94. E. Brakenhielm, N. Veitonmaki, R. Cao, S. Kihara, Y. Matsuzawa, B. Zhivotovsky, T. Funahashi, Y. Cao, Adiponectin-induced antiangiogenesis and antitumor activity involve caspase-mediated endothelial cell apoptosis. Proc. Natl. Acad. Sci. U.S.A. **101**(8), 2476–2481 (2004)
95. M. Kumada, S. Kihara, S. Sumitsuji, T. Kawamoto, S. Matsumoto, N. Ouchi, Y. Arita, Y. Okamoto, I. Shimomura, H. Hiraoka, T. Nakamura, T. Funahashi, Y. Matsuzawa, Osaka CAD Study Group. Coronary Artery Disease, Association of hypoadiponectinemia with coronary artery disease in men. Arterioscler. Thromb. Vasc. Biol. **23**(1), 85–89 (2003)
96. S. Rasul, A. Ilhan, M.H. Reiter, S. Baumgartner-Parzer, A. Kautzky-Willer, Relations of adiponectin to levels of metabolic parameters and sexual hormones in elderly type 2 diabetic

patients. Gend. Med. **8**(2), 93–102 (2011). doi:10.1016/j.genm.2011.01.004
97. A. Gastaldelli, M. Kozakova, K. Hojlund, A. Flyvbjerg, A. Favuzzi, A. Mitrakou, B. Balkau, RISC Investigators, Fatty liver is associated with insulin resistance, risk of coronary heart disease, and early atherosclerosis in a large European population. Hepatology **49**(5), 1537–1544 (2009). doi:10.1002/hep.22845
98. I. Edirisinghe, K. McCormick Hallam, C.T. Kappagoda, Effect of fatty acids on endothelium-dependent relaxation in the rabbit aorta. Clin. Sci. **111**(2), 145–151 (2006). doi:10.1042/CS20060001
99. M.T. Nguyen, S. Favelyukis, A.K. Nguyen, D. Reichart, P.A. Scott, A. Jenn, R. Liu-Bryan, C.K. Glass, J.G. Neels, J.M. Olefsky, A subpopulation of macrophages infiltrates hypertrophic adipose tissue and is activated by free fatty acids via Toll-like receptors 2 and 4 and JNK-dependent pathways. J. Biol. Chem. **282**(48), 35279–35292 (2007). doi:10.1074/jbc.M706762200
100. J.S. Yudkin, M. Kumari, S.E. Humphries, V. Mohamed-Ali, Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? Atherosclerosis **148**(2), 209–214 (2000)
101. M.Y. Abeywardena, W.R. Leifert, K.E. Warnes, J.N. Varghese, R.J. Head, Cardiovascular biology of interleukin-6. Curr. Pharm. Des. **15**(15), 1809–1821 (2009)
102. N. Ouchi, S. Kihara, T. Funahashi, T. Nakamura, M. Nishida, M. Kumada, Y. Okamoto, K. Ohashi, H. Nagaretani, K. Kishida, H. Nishizawa, N. Maeda, H. Kobayashi, H. Hiraoka, Y. Matsuzawa, Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. Circulation **107**(5), 671–674 (2003)
103. Emerging Risk Factors Collaboration, S. Kaptoge, E. Di Angelantonio, G. Lowe, M.B. Pepys, S.G. Thompson, R. Collins, J. Danesh, C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet **375**(9709), 132–140 (2010). doi:10.1016/S0140-6736(09)61717-7
104. Y. Momiyama, R. Ohmori, Z.A. Fayad, T. Kihara, N. Tanaka, R. Kato, H. Taniguchi, M. Nagata, H. Nakamura, F. Ohsuzu, Associations between plasma C-reactive protein levels and the severities of coronary and aortic atherosclerosis. J. Atheroscler. Thromb. **17**(5), 460–467 (2010)
105. U. Fiedler, Y. Reiss, M. Scharpfenecker, V. Grunow, S. Koidl, G. Thurston, N.W. Gale, M. Witzenrath, S. Rosseau, N. Suttorp, A. Sobke, M. Herrmann, K.T. Preissner, P. Vajkoczy, H.G. Augustin, Angiopoietin-2 sensitizes endothelial cells to TNF-alpha and has a crucial role in the induction of inflammation. Nat. Med. **12**(2), 235–239 (2006). doi:10.1038/nm1351
106. E. Volkova, J.A. Willis, J.E. Wells, B.A. Robinson, G.U. Dachs, M.J. Currie, Association of angiopoietin-2, C-reactive protein and markers of obesity and insulin resistance with survival outcome in colorectal cancer. Br. J. Cancer **104**(1), 51–59 (2011). doi:10.1038/sj.bjc.6606005
107. M. Skopkova, A. Penesova, H. Sell, Z. Radikova, M. Vlcek, R. Imrich, J. Koska, J. Ukropec, J. Eckel, I. Klimes, D. Gasperikova, Protein array reveals differentially expressed proteins in subcutaneous adipose tissue in obesity. Obesity **15**(10), 2396–2406 (2007). doi:10.1038/oby.2007.285
108. Z. Wang, T. Nakayama, Inflammation, a link between obesity and cardiovascular disease. Mediators Inflamm. **2010**, 535918 (2010). doi:10.1155/2010/535918
109. A. Heiss, A. DuChesne, B. Denecke, J. Grotzinger, K. Yamamoto, T. Renne, W. Jahnen-Dechent, Structural basis of calcification inhibition by alpha 2-HS glycoprotein/fetuin-A. Formation of colloidal calciprotein particles. J. Biol. Chem. **278**(15), 13333–13341 (2003). doi:10.1074/jbc.M210868200
110. D. Toroian, P.A. Price, The essential role of fetuin in the serum-induced calcification of collagen. Calcif. Tissue Int. **82**(2), 116–126 (2008). doi:10.1007/s00223-007-9085-2
111. J.H. Ix, C.L. Wassel, D.C. Bauer, D. Toroian, F.A. Tylavsky, J.A. Cauley, T.B. Harris, P.A. Price, S.R. Cummings, M.G. Shlipak, Health ABC Study, Fetuin-A and BMD in older persons: the Health Aging and Body Composition (Health ABC) study. J. Bone Miner. Res. **24**(3), 514–521 (2009). doi:10.1359/jbmr.081017
112. L. Chailurkit, A. Kruavit, R. Rajatanavin, B. Ongphiphadhanakul, The relationship of fetuin-A and lactoferrin with bone mass in elderly women. Osteoporosis Int. **22**(7), 2159–2164 (2011). doi:10.1007/s00198-010-1439-3
113. C. Schafer, A. Heiss, A. Schwarz, R. Westenfeld, M. Ketteler, J. Floege, W. Muller-Esterl, T. Schinke, W. Jahnen-Dechent, The serum protein alpha 2-Heremans-Schmid glycoprotein/fetuin-A is a systemically acting inhibitor of ectopic calcification. J. Clin. Investig. **112**(3), 357–366 (2003). doi:10.1172/JCI17202
114. T. Schinke, C. Amendt, A. Trindl, O. Poschke, W. Muller-Esterl, W. Jahnen-Dechent, 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. **271**(34), 20789–20796 (1996)
115. P.A. Price, J.E. Lim, The inhibition of calcium phosphate precipitation by fetuin is accompanied by the formation of a fetuin-mineral complex. J. Biol. Chem. **278**(24), 22144–22152 (2003). doi:10.1074/jbc.M300744200
116. R.C. Shroff, V. Shah, M.P. Hiorns, M. Schoppet, L.C. Hofbauer, G. Hawa, L.J. Schurgers, A. Singhal, I. Merryweather, P. Brogan, C. Shanahan, J. Deanfield, L. Rees, The circulating calcification inhibitors, fetuin-A and osteoprotegerin, but not matrix Gla protein, are associated with vascular stiffness and calcification in children on dialysis. Nephrol. Dial. Transplant. **23**(10), 3263–3271 (2008). doi:10.1093/ndt/gfn226
117. A. Kirkpantur, B. Altun, T. Hazirolan, D. Akata, M. Arici, S. Kirazli, C. Turgan, Association among serum fetuin-A level, coronary artery calcification, and bone mineral densitometry in maintenance hemodialysis patients. Artif. Organs **33**(10), 844–854 (2009). doi:10.1111/j.1525-1594.2009.00814.x
118. M. Wang, M. Wang, L.Y. Gan, S.J. Li, N. Hong, M. Zhang, Vascular calcification in maintenance hemodialysis patients. Blood Purif. **28**(1), 15–20 (2009). doi:10.1159/000210033
119. J.H. Ix, R. Katz, I.H. de Boer, B.R. Kestenbaum, C.A. Peralta, N.S. Jenny, M. Budoff, M.A. Allison, M.H. Criqui, D. Siscovick, M.G. Shlipak, Fetuin-A is inversely associated with coronary artery calcification in community-living persons: the Multi-Ethnic Study of Atherosclerosis. Clin. Chem. **58**(5), 887–895 (2012). doi:10.1373/clinchem.2011.177725
120. J.H. Ix, E. Barrett-Connor, C.L. Wassel, K. Cummins, J. Bergstrom, L.B. Daniels, G.A. Laughlin, The associations of fetuin-A with subclinical cardiovascular disease in community-dwelling persons: the Rancho Bernardo Study. J. Am. Coll. Cardiol. **58**(23), 2372–2379 (2011). doi:10.1016/j.jacc.2011.08.035
121. E. Fisher, N. Stefan, K. Saar, D. Drogan, M.B. Schulze, A. Fritsche, H.G. Joost, H.U. Haring, N. Hubner, H. Boeing, C. Weikert, Association of AHSG gene polymorphisms with fetuin-A plasma levels and cardiovascular diseases in the EPIC-Potsdam study. Circul. Cardiovasc. Genet. **2**(6), 607–613 (2009). doi:10.1161/CIRCGENETICS.109.870410
122. G. Cianciolo, G. La Manna, G. Donati, E. Persici, A. Dormi, M.L. Cappuccilli, S. Corsini, R. Fattori, V. Russo, V. Nastasi, L. Coli, M. Wratten, S. Stefoni, Coronary calcifications in end-stage renal disease patients: a new link between osteoprotegerin, diabetes and body mass index? Blood Purif. **29**(1), 13–22 (2010). doi:10.1159/000245042
123. D.P. Lorant, M. Grujicic, C. Hoebaus, J.M. Brix, F. Hoellerl, G. Schernthaner, R. Koppensteiner, G.H. Schernthaner, Fetuin-A levels are increased in patients with type 2 diabetes and peripheral arterial disease. Diabetes Care **34**(1), 156–161 (2011). doi:10.2337/dc10-0788