Adiponectin and adipocyte fatty acid binding protein in the pathogenesis of cardiovascular disease

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Running head: Adipokines, heart, and blood vessels

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Abstract

The heart and blood vessels are surrounded by epicardial and perivascular adipose tissues respectively, which play important roles in maintaining cardiovascular homeostasis by secreting a number of biologically active molecules, termed “adipokines”. Many of these adipokines function as an important component of the ‘adipo-cardiovascular axis’ mediating the cross-talk between adipose tissues, the heart, and the vasculature. On the one hand, most adipokines [including tumor necrosis factor α, resistin, adipocyte fatty acid binding protein (A-FABP) and lipocalin-2] are pro-inflammatory and causally associated with endothelial and cardiac dysfunction by their endocrine/paracrine actions. On the other hand, adiponectin is one of the few adipokines that possesses multiple salutary effects on the prevention of cardiovascular disease, because of its pleiotropic actions on the heart and the blood vessels. The discordant production of adipokines in dysfunctional adipose tissue is a key contributor to obesity-related cardiovascular disease. This review provides an update in understanding the roles of adipokines in the pathogenesis of cardiovascular disorders associated with obesity and diabetes, and focuses on the two most abundant adipokines, adiponectin and A-FABP. Indeed, data from both animal studies and clinical investigations imply that these two adipokines are prognostic biomarkers for cardiovascular disease and even promising therapeutic targets for its treatment.
As a consequence of over-nutrition and sedentary lifestyle, the prevalence of overweight and obesity has increased dramatically to an epidemic level worldwide. Obesity is closely associated with an increased risk for a cluster of metabolic and cardiovascular disorders, including insulin resistance, type 2 diabetes, hypertension, coronary heart disease and stroke. Thus, an epidemiological survey involving 900,000 participants from several ethnic groups showed that in morbidly obese subjects [with body weight index (BMI) between 4 to 45 kg/m²] compared to those with normal BMI the median survival rate is reduced by eight to ten years, mainly due to increased cardiovascular mortality (111).

The dysfunction of adipose tissue, characterized by infiltration of inflammatory cells and aberrant production of adipokines, is a key link between obesity and cardiovascular disease. Besides its traditional role as storage of excess energy, adipose tissue is a highly dynamic endocrine organ and an important metabolic sensor, participating in the regulation of insulin sensitivity, glucose and lipid metabolism as well as cardiovascular homeostasis (100). Since the discovery of leptin, several adipokines released from adipose tissues have been identified and characterized. They are key components of the “adipo-cardiovascular axis” that mediates the crosstalk between adipose tissue and the cardiovascular system. In obese subjects, enlarged adipose tissue is infiltrated with activated macrophages and several other types of inflammatory cells, leading to augmented production of pro-inflammatory adipokines, including tumor necrosis factor (TNF)α, interleukin (IL)6, monocyte chemoattractant protein (MCP)-1, resistin, leptin, lipocalin-2, adipocyte fatty acid binding protein (A-FABP) and plasminogen activator inhibitor (PAI)-1 (94). By contrast, the production of adiponectin, which exerts beneficial effects on insulin sensitivity and cardiovascular function, is reduced markedly (123). Thus, the aberrant production of adipokines has been recognized as an important contributor to the obesity-related cardio-
metabolic syndrome. The present review aims to discuss how dysfunctional adipose tissue may contribute to the pathogenesis of obesity-related cardiovascular disease and focuses mainly on the role of the two most abundant adipokines, adiponectin and A-FABP.

Adipose tissue, heart and blood vessels

Most blood vessels and the heart are surrounded by adipose tissue. In particular, the heart is covered by two anatomically distinct fat depots, including epicardial adipose tissue located between the myocardium and the visceral pericardium, and pericardial adipose tissue situated outside the inner layer of the pericardium (76). In the human heart, epicardial adipose tissue is found in both ventricles in the atrioventricular and interventricular grooves extending to the apex and along the coronary arteries, and constitutes approximately 20% of the total ventricular weight (40). Pericardial adipose tissue covers 80% of the heart and accounts for 20 to 50% of the weight of the human heart (76). Under normal conditions, epicardial and pericardial adipose fat function as an important local energy source for the heart, in order to maintain its contractile activity, by releasing fatty acids through lipolysis. However, expanded epicardial and pericardial fat are important risk factors for obesity-related cardiac dysfunction.

Indeed, several population-based epidemiological studies, including the Multi-Ethnic Study of Atherosclerosis and the Framingham Heart Study, identified the amount of adipose tissue around the heart as an independent predictor for cardiovascular disease (56, 83). Both the thickness and volume of epicardial fat were increased in patients with coronary artery disease and unstable angina compared to healthy individuals. In patients with coronary artery disease, thickness of the epicardial adipose tissue was related to the severity of the disease, as measured by the Gensini
score (21). In addition, this thickness was associated with subclinical markers of atherosclerosis, including stiffness and intimal media thickness of the carotid artery (67). In morbidly obese individuals, a close association between epicardial fat thickness and diastolic dysfunction has also been observed (41). However, whether or not epicardial adipose tissue is a major source of paracrine factors that modulate myocardial function remains unclear at this stage.

Perivascular adipose tissue surrounds virtually all blood vessels directly in that no fascia separates this fat depot from their adventitia (52). The amount of perivascular fat can be quantified by computed tomography with reasonable reproducibility. In the Framingham heart study, measurement of adipose tissue surrounding the thoracic aorta demonstrated a close correlation of this depot with visceral obesity, diabetes and hypertension (50). In addition, the presence of perivascular adipose tissue is associated with calcification of coronary and abdominal aorta (50).

Both animal studies and clinical investigations demonstrate the profound effects of perivascular adipose tissue on vascular responsiveness and remodelling (52). Indeed, this fat depot secretes a number of bioactive substances that directly regulate vascular function through their paracrine and endocrine actions. In particular, perivascular fat can induce both vasodilatation and vasoconstriction by releasing adipocyte-derived relaxing factor (ADRF) and adipocyte-derived constricting factor (ADCF), respectively (76). ADRF exerts its anti-contractile activity by opening potassium channels of the vascular smooth muscle cells, thus inhibiting responses of various vasoconstrictor agonists [including phenylephrine, serotonin, angiotensin II and U46619 (a thromboxane A2 mimic)] (30). On the other hand, ADCF-dependent vasoconstriction is mediated by reactive oxygen species (ROS) and is blocked by inhibitors of NADPH oxidase (81). In obesity, dysfunction and inflammation of adipose tissue result in impaired production of
ADRF and elevated release of ADCF, thereby leading to vascular dysfunction (30). A number of adipokines with anti-contractile properties, including leptin, adiponectin (see below) and angiotensin (Ang) 1-7 (76), have been proposed as potential candidates for ADRF. However, none of these adipokines fully mimics the vascular effects of ADRF (23) and the precise identity of ADRF (as well as that of ADCF) remains to be elucidated.

Adipose tissue inflammation and cardiovascular disease

Adipose tissue inflammation, characterized by infiltration of macrophages and other types of inflammatory cells, plays a central role in mediating obesity-related cardiovascular disorders. In rodents, transplantation of visceral adipose tissue into apolipoprotein (apo) E−/− mice caused a marked acceleration of atherosclerosis by inducing the production of pro-inflammatory factors (70). Among different types of adipose tissues, perivascular adipose tissue is an important contributor to vascular inflammation because of both its proximity to the blood vessel wall and its pronounced pro-inflammatory properties (11). The secretion of MCP-1, a key pathological cytokine in vascular inflammation and atherosclerosis, was increased by more than 40-fold in perivascular as compared to subcutaneous adipocytes (8). In addition, pro-inflammatory cytokines/adipokines released from other major adipose tissue depots, such as subcutaneous and abdominal fat, may also contribute to vascular inflammation in virtue of their endocrine actions.

The pro-inflammatory factors released from adipose tissue exert adverse effects on the vasculature by both direct and indirect mechanisms. First, a number of pro-inflammatory adipokines and cytokines act in an endocrine manner to induce insulin resistance and metabolic derangement, which are classical risk factors for cardiovascular disease (37). Second, several
chemokines and adipokines, in particular MCP-1 and IL8, induce the recruitment and infiltration of monocytes, lymphocytes and neutrophils into the blood vessel wall to instigate local inflammation (53). Third, many adipokines and cytokines act on endothelial cells to impair NO production and thus endothelium-dependent vasodilatation (31).

The majority of adipokines, including TNFα, interleukin-1β, PAI-1, resistin, adipocyte fatty acid binding protein (A-FABP) and lipocalin-2, possesses pro-inflammatory properties and exerts detrimental effects on cardiac and vascular functions (53). Only a few adipokines, including adiponectin and adrenomedulin, have anti-inflammatory and cardiovascular protective properties (131).

**Cardiovascular protection by adiponectin**

Adiponectin is one of the most abundant adipokines secreted by adipocytes, accounting for approximately 0.01% of the total plasma protein content in humans (4). It is composed of a NH2-terminal hyper-variable region, followed by a collagenous domain consisting of 22 Gly-X-Y repeats and a COOH-terminal C1q-like globular domain (87). Plasma adiponectin forms three distinct oligomeric complexes, including trimer, hexamer and a high molecular weight (HMW) multimeric isoform that consists of 12-18 protomers (109). Unlike that of most other adipokines, the plasma level of adiponectin is reduced in obesity and related pathologies (123). Epidemiological studies in different ethnic groups have identified low levels of plasma adiponectin, especially its HMW oligomeric complex, as an independent risk factor for type 2 diabetes, hypertension, atherosclerosis and myocardial infarction ((123).

In animals, administration of recombinant adiponectin protects against almost all the major obesity-related disorders, including insulin resistance (5), hypertension (69), dyslipidemia (117),
atherosclerosis (71), non-alcoholic steatohepatitis (115), heart failure (90), airway inflammation (95) and several types of obesity-related cancers (105, 106). Besides its beneficial effects in alleviating insulin resistance and adipose tissue inflammation, adiponectin exerts its cardiovascular protective effects through direct actions on the heart as well as on several types of vascular cells (Figure-1).

**Cardiac actions of adiponectin**

Adiponectin-null mice are more susceptible to pressure overload-induced cardiac hypertrophy and ischemia-reperfusion injury-induced myocardial infarction compared with wild-type mice (89, 90, 99). Furthermore, adiponectin deficiency in mice exacerbates angiotensin II-induced cardiac fibrosis and left ventricular dysfunction as well as doxorubicin-induced cardiomyopathy (26, 45). These pathological changes can be reversed by adenovirus-mediated expression of recombinant adiponectin. The adipokine may also be a key contributor to the beneficial effects of caloric restriction in improving left ventricular function and in reducing infarct size after ischemic injury (93). Protective effects of adiponectin against myocardial ischemia/reperfusion injury have also been observed in pigs (44).

The anti-apoptotic activity of adiponectin represents a key mechanism whereby this adipokine protects against cardiac injury (99) (Figure-2). It is mediated by its ability to activate AMPK (90), as adenovirus-mediated expression of dominant negative AMPK blocks the suppressive effects of adiponectin on ischemia/reperfusion-induced apoptosis in mice and in cultured cardiomyocytes (104). In addition, adiponectin protects against palmitate-induced cardiomyocyte apoptosis by activation of ceramidase, thereby leading to the production of the anti-apoptotic metabolite sphingosine-1-phosphate and the subsequent inhibition of caspase-8 (35). In rat
neonatal left ventricular cardiomyocytes, adiponectin increases cell survival and prevents stress-induced cell apoptosis by activation of the survival kinase Akt (96). Therefore, adiponectin appears to exert its anti-apoptotic effects through activation of distinct signalling pathways under different pathological conditions (Figure-2).

Anti-oxidative/nitrative stress activities represent another important mechanism that accounts for the cardio-protective effects of adiponectin (32, 99, 104) (Figure-2). Adiponectin-null mice exhibit increased formation of nitric oxide, superoxide anions and peroxynitrite in cardiac tissue as compared to wild-type littermates (99). These changes can be reversed by the administration of recombinant adiponectin. In isolated rat hearts, adiponectin improved left ventricular function and increased coronary flow during reperfusion, whereas administration of the nitric oxide synthase inhibitor nitro-l-arginine (L-NNA) abrogated the improvement in myocardial function induced by the adipokine (32). The anti-oxidative/anti-nitrative effects of adiponectin are independent of AMPK, but are perhaps related to its differential regulation of eNOS and iNOS (104). Adiponectin reduces ischemia-/induced protein expression of iNOS and gp91phox, thereby blocking peroxynitrite formation (Figure-2).

The suppressive effects of adiponectin against ischemia/reperfusion -induced myocardial inflammation are mediated by the induction of cyclooxygenase-2 (COX-2), a rate-limiting enzyme for prostanoid synthesis (84) (Figure-2). Indeed, the pharmacological inhibition of COX-2 in mice reversed the inhibitory effects of adiponectin on myocardial ischemia/reperfusion -induced TNFα production and infarct size (90).

Finally, the beneficial effects of adiponectin on glucose and lipid metabolism may also contribute to the cardio-protection exerted by adiponectin. Indeed, in adult cardiomyocytes,
adiponectin increased CD36-mediated fatty acid uptake and enhanced insulin-stimulated glucose transport by promoting Akt-dependent plasma membrane translocation of GLUT4 (22). The adipokine also stimulated lipoprotein lipase activity via RhoA/Rho-associated protein kinase (ROCK)-mediated actin remodelling (29). Furthermore, adiponectin induced VEGF production, which may also contribute to its cardio-protective effects by promoting angiogenesis (92).

T-cadherin, an adiponectin-interacting partner anchored at the cell surface by glycosyl phosphatidylinositol, plays an indispensable role in adiponectin-induced cardio-protection in mice (17). Indeed, the protective effects of adiponectin against pressure overload-induced cardiac hypertrophy and ischemia/reperfusion-induced myocardial infarction were abrogated in mice lacking T-cadherin (17), suggesting that the latter is a physiological adiponectin-binding receptor that enables the association of the adipokine with cardiac tissue. Both AdipoR1 and AdipoR2 are expressed in cardiac cells (18, 25). In vitro studies demonstrated that the effects of adiponectin on activation of AMPK, Akt and ceramidase are mediated by AdipoR1 and AdipoR2 (77). However, the exact role of these two receptors in the anti-oxidative/nitrative stress and anti-inflammatory actions of adiponectin in cardiomyocytes remain unclear.

Although adipocytes are the major contributor to plasma adiponectin, the adipokine is also expressed in cardiomyocytes (1, 2, 18) and cardiomyocyte-derived adiponectin is biologically active in protecting cells against ischemic injury by paracrine/autocrine activation of adiponectin receptors (110). Adiponectin is expressed in human cardiac cells (96). In patients with dilated cardiomyopathy, the cardiac adiponectin protein expression is down-regulated (96). Taken in conjunction, these findings support an autocrine role for adiponectin in conferring the cardio-protective activities of this adipokine.
Vascular actions of adiponectin

Both loss-of-function and gain-of-function studies in various animal models have demonstrated the protective effects of adiponectin against endothelial dysfunction (91), atherosclerosis (46) and hypertension (69). Adiponectin knockout mice displayed a significantly increased neointimal hyperplasia after carotid injury (60), impaired endothelium-dependent vasodilatation (73), elevated systemic blood pressure (91) and pulmonary hypertension (98). By contrast, elevation of plasma adiponectin by either genetic or pharmacological interventions caused a marked alleviation of atherosclerotic lesions in apoE<sup>−/−</sup> mice (71) and in rabbits with spontaneous atherosclerosis (51), and also caused a significant improvement in endothelial dysfunction and hypertension (34, 123). Adiponectin exerts its vascular protective effects through its direct actions on almost all the major types of cells present in blood vessels, including endothelial cells, endothelial progenitor cells (EPC), macrophages, smooth muscle cells, platelet and leukocytes (Figure-1).

In endothelial cells, adiponectin stimulates NO production by activation of eNOS (12, 13, 33). It induces eNOS phosphorylation at Ser<sup>1177</sup> and also promotes the association between eNOS and heat shock protein 90, a complex required for the maximal activation of the enzyme. APPL1, a multiple domain adaptor protein that transmits adiponectin signalling from its receptor to AMPK, plays an indispensable role in mediating the endothelial actions of adiponectin (14). APPL1 may promote the translocation of LKB1 from the nucleus to the cytosol, thereby increasing the accessibility of LKB1 to its downstream kinase AMPK for further activation (122). Consistent with these in vitro findings, systemic administration of recombinant adiponectin in Sprague-Dawley rats with dietary obesity increased eNOS activity, NO production and relaxation of aortic rings to endothelium-dependent vasodilators (16). Furthermore, adiponectin induced NO-
dependent vasodilatation in resistance arteries of Zucker rats (88). In both coronary arterioles and aortae of db/db diabetic mice, systemic infusion of adiponectin reversed the diabetes-induced impairment in endothelium-dependent relaxations to acetylcholine (121). In addition to eNOS activation, adiponectin suppresses the production of reactive oxygen species induced by high glucose (75), oxidized LDL (65, 79) and palmitate (43) in endothelial cells. The anti-oxidant activity of adiponectin is mediated by cAMP-dependent protein kinase A (PKA) (75) as well as AMPK (43). Furthermore, adiponectin inhibits endothelial cell activation and monocyte attachment, and blocks the interaction between leukocytes and endothelial cells (74).

Endothelial progenitor cells are active players in endothelial repair after vascular injury (54). Adiponectin stimulates survival, proliferation and differentiation of bone marrow-derived EPCs (20), and also promotes the migration activities of EPCs through activation of the PI3-kinase/Cdc42/Rac1 signalling pathway (66). On the other hand, AMPK activation is required for the vascular recruitment of EPCs by adiponectin (85), suggesting that the PI3-kinase and AMPK pathways work synergistically to confer this favourable effect of the adipokine. In addition, adiponectin prevents diabetes-induced impairment of EPC function by decreasing high glucose-induced intracellular ROS accumulation (10). In db/db diabetic mice, the lack of adiponectin exacerbates hyperglycemia-induced decreases in the number of circulating EPCs whereas this change is reversed by chronic treatment with recombinant adiponectin. Furthermore, adiponectin blocks high glucose–induced premature senescence of EPCs derived from both human peripheral blood and mouse bone marrow, by reducing activation of p38 MAP kinase and expression of the senescence marker p16INK4A (10).

Adiponectin decreases the proliferation and migration of vascular smooth muscle cells induced by atherogenic growth factors by two distinct mechanisms (3) (107). First, adiponectin interacts
in an oligomerization-dependent manner with different atherogenic growth factors, including heparin-binding epidermal growth factor-like growth factor, platelet-derived growth factor and basic fibroblast growth factor (108), thereby blocking their binding to their respective cell membrane receptors. Second, adiponectin inhibits insulin growth factor-1-induced ERK1/2 activation and proliferation of vascular smooth muscle cells by activation of AMPK (64).

In macrophages, chronic treatment with adiponectin suppresses the production of pro-inflammatory cytokines induced by several stimuli, including lipopolysaccharide (LPS), leptin and resistin (80, 118). The anti-inflammatory effect of adiponectin can be attributed to its ability to suppress NF-κB and ERK1/2 in macrophages (112, 118). Acute treatment with adiponectin triggers the release of TNFα and IL-6 and induces ERK1/2 activation, which subsequently causes an induction of IL-10, an anti-inflammatory cytokine that renders macrophages tolerant to further stimulation by endotoxin or other pro-inflammatory cytokines (78). In addition, adiponectin may exert its anti-inflammatory activity by regulating macrophage polarization (55). Upon stimulation with adiponectin, human monocytes are primed into anti-inflammatory M2 macrophages as opposed to the classically activated M1 phenotype. Incubation of M1 macrophages with adiponectin-treated M2-derived culture supernatant results in a pronounced inhibition in secretion of pro-inflammatory factors such as TNFα and MCP-1. Furthermore, adiponectin inhibits the conversion of macrophages to lipid-laden foam cells by reducing the uptake of acetylated LDL particles (72) and by enhancing the ATP-binding cassette transporter ABCA1-mediated cholesterol efflux (103).

*A-FABP: a key mediator of obesity-related cardio-metabolic syndrome*
A-FABP, also known as P2 and FABP4, is one of the most abundant proteins in mature adipocytes, accounting for approximately 6% of their total cellular protein content (59). In addition, A-FABP is expressed in macrophages (42) and lymphocytes (95). Although A-FABP was originally identified as a cytoplasmic protein, data from both rodents and humans suggests that it is also secreted by adipose tissue into the bloodstream (116). Plasma A-FABP concentrations in humans range from 10 to 100 ng/mL. These levels are much higher than those of several other major adipokines secreted by adipose tissue, including leptin, resistin and TNFα. The putative function of A-FABP is to serve as a lipid-binding chaperone for fatty acids (36). A-FABP is an important player in lipolysis, as both basal (15) and hormone-stimulated lipolysis in response to β-adrenergic activation (86) is impaired in A-FABP-null mice. The latter are partially protected from insulin resistance induced by dietary and genetic obesity, suggesting that the lipid chaperone is also involved in regulating insulin sensitivity (27). Furthermore, both clinical investigations and animal studies identify A-FABP as a central mediator of obesity-related cardiovascular disease, possibly potentiating lipids-induced inflammation (36).

**A-FABP as a biomarker for cardiovascular disease**

Epidemiological studies on different ethnic groups have demonstrated a close association between serum levels of A-FABP and a cluster of obesity-related cardio-metabolic risk factors, endothelial dysfunction and macrovascular complications of diabetes. In both cross-sectional and prospective studies, plasma levels of A-FABP are positively correlated with several key components of the metabolic syndrome, including adverse lipid profiles (increased serum triglyceride and LDL-cholesterol, and decreased HDL-cholesterol), hyperglycemia and hypertension, independently of sex, age and adiposity (38, 63, 97, 114). In addition, plasma levels of A-FABP are positively associated with the pathogenesis of non-alcoholic fatty liver
disease, which is recognized as the hepatic manifestation of metabolic syndrome (61). In a ten-year follow-up study on Chinese subjects, the plasma A-FABP level was found to be a strong predictor of type 2 diabetes independently of the traditional risk factors including obesity, insulin resistance or glycemic indices (102).

Besides their association with metabolic risk factors, plasma A-FABP levels are positively correlated with measures of endothelial dysfunction (113), coronary atherosclerosis (62) and various types of cardiovascular disease (82, 119). Furthermore, in coronary artery disorders, the plasma levels of A-FABP augment as the numbers of stenotic vessels increase, suggesting an etiological role for the adipokine (82). In a cross-sectional study including 237 diabetic patients, plasma A-FABP levels were associated independently with diabetic nephropathy staging, and markedly elevated in diabetic patients with macrovascular complications (119). Furthermore, plasma A-FABP levels are much higher in patients with acute ischemic stroke as compared to their age- and sex-matched controls, and high plasma levels of the adipokine are associated with increased three-month mortality in patients with ischemic stroke, suggesting that A-FABP may serve as a potential prognostic indicator for early mortality (101).

The inflammatory status of atherosclerotic lesions is a major factor triggering acute cardiovascular events (55). In a case control study of an Asian population, the plasma A-FABP level was an independent risk factor for vascular inflammation, as measured by [(18)F]-fluorodeoxyglucose positron emission tomography (PET), which allows non-invasive measurement of atherosclerotic inflammation (120). Finally, the plasma A-FABP levels correlate positively with several pro-inflammatory markers (lipocalin-2, hsCRP, TNFα receptors, interleukin-6), but inversely with adiponectin (114). Taken in conjunction, these clinical findings
support the role of A-FABP as a key pro-inflammatory mediator that links obesity with cardiovascular disease in humans.

A-FABP in the pathogenesis of cardiovascular disease in animals

In line with the clinical findings, data from animal studies also support an etiological role of A-FABP in cardiovascular disease (27). Thus, the secreted form of A-FABP has been identified as a major cardio-depressant factor that confers the suppressive effect of adipocytes on cardiac contractile functions (47). In isolated rat cardiomyocytes, addition of recombinant A-FABP to the extracellular medium acutely depresses shortening amplitude as well as intracellular systolic peak Ca^{2+} in a dose-dependent manner. These findings raise the possibility that increased secretion of A-FABP from adipocytes may serve as a key mediator that links obesity with cardiac dysfunction (47).

A-FABP deficiency resulted in a marked reduction of atherosclerotic lesions along the whole aorta in apoE^{-/-} mice (57) (6). When mice were fed a high-fat atherogenic diet for one year, the survival rate of apo E^{-/-} mice null for A-FABP was 67% higher than those of apo E^{-/-} control mice, due to the increased stability of the atherosclerotic plaques (7). Likewise, pharmacological inhibition of A-FABP also rendered a significant protection against atherosclerotic plaque formation in apoE^{-/-} mice (19, 28).

The pro-atherogenic activity of A-FABP is mediated by its direct actions on macrophages, independently of lipid metabolism and insulin sensitivity (36) (57). Adenovirus-mediated over-expression of A-FABP in human macrophages induced foam cell formation by increasing intracellular cholesterol ester accumulation (24). By contrast, depletion of A-FABP expression prevented oxidized LDL-induced foam cell formation by increasing cholesterol efflux, and also
inhibited IκB kinase/NF-κB activity, thereby leading to reduced expression of both COX-2 and iNOS as well as impaired production of pro-inflammatory cytokines, (57, 58).

Besides its pro-atherogenic effect in macrophages, A-FABP may contribute to endothelial dysfunction by potentiating lipids-induced impairment in eNOS activation (49). In cultured endothelial cells, insulin-stimulated eNOS phosphorylation and NO production was suppressed by saturated fatty acids, whereas such a suppressive effect was reversed by treatment with the A-FABP inhibitor BMS309403. Furthermore, chronic treatment of apoE<sup>−/−</sup> mice with BMS309403 improved endothelium-dependent relaxations in their aortae studied <i>ex vivo</i>, but did not affect endothelium-independent relaxations (49).

**A-FABP, inflammation and ER stress**

A-FABP may potentiate vascular inflammation by forming a positive feedback loop with C-Jun N-terminal kinases (JNK) and Activator Protein-1 (AP-1) (39). In response to pro-inflammatory stimuli, activated JNK in macrophages increases A-FABP expression by inducing the phosphorylation of c-Jun, which in turn binds to a highly conserved AP-1 <i>cis</i>-element within the A-FABP gene promoter and enhances gene transcription. <i>Vice versa</i>, elevated A-FABP potentiates JNK activation, leading to augmented production of pro-inflammatory cytokines (39). The reciprocal regulation between A-FABP and JNK is also supported by animal studies demonstrating that genetic or pharmacological inhibition of A-FABP suppresses JNK activity in adipose tissue of obese mice and in atherosclerotic lesion areas of apoE<sup>−/−</sup> mice (19, 28). By contrast, genetic inhibition of JNK decreased A-FABP expression in obese adipose tissue.

In macrophages, A-FABP couples toxic lipids to endoplasmic reticulum (ER) stress and inflammation (19) (Figure-3). Indeed, toxic lipids such as palmitate induce A-FABP expression.
as well as ER stress, which in turn leads to JNK activation. Genetic depletion or pharmacological inhibition of A-FABP alleviates ER stress and suppresses JNK activation. Likewise, alleviation of ER stress by the chemical chaperone 4-phenylbutyric acid (PBA) also prevented toxic lipids-induced inflammation in macrophages and reduced atherosclerosis in apoE−/− mice. A lipidomic-based analysis identified steroyl CoA desaturase-1 (SCD1), which detoxifies saturated lipids to monounsaturated lipids, as an intermediate link that couples A-FABP to ER stress (19). A-FABP suppressed SCD1 expression by inhibiting the nuclear receptor LXR-α, thereby inducing accumulation of saturated toxic lipids and ER stress (Figure-3).

Concluding remarks

Adipose tissues modulate cardiovascular homeostasis by secreting a large number of adipokines. Adiponectin and A-FABP are the two most abundant adipokines with opposite effects on insulin sensitivity, cardiac and vascular functions. Adiponectin protects against obesity-related cardiovascular disease through its pleiotropic actions on the heart and vasculature. By contrast, A-FABP mediates obesity-related cardiac and vascular dysfunctions by potentiating lipid-induced inflammation and by acting as a cardiodepressant factor to suppress cardiac contractility. Discordant production of these two adipokines (decreased adiponectin and increased A-FABP) by adipocytes is an important risk factor for the development of cardiovascular disease in both animals and humans. Therefore, these two adipokines represent prognostic biomarkers and promising therapeutic targets. Indeed, pharmacological agents that increase adiponectin production or inhibit A-FABP activity effectively treat obesity-related vascular and cardiac dysfunction in rodents.
Both adiponectin and A-FABP are downstream targets of PPARγ in adipocytes. PPARγ agonists such as thiazolidinediones increase plasma levels of both adiponectin and A-FABP in humans and rodents (9, 123). Data from adiponectin-null mice provide compelling evidence demonstrating that the therapeutic effects of thiazolidinediones on insulin sensitivity and vascular dysfunction are mediated by the induction of adiponectin. However, the clinical use of certain of those drugs has been suspended, because of potential cardiac risk (68). As A-FABP plays a detrimental role in both cardiac and vascular dysfunctions, it is possible that the adverse effects of thiazolidinediones are mediated in part by the induction of A-FABP in adipocytes. Therefore, pharmacological agents that selectively increase adiponectin production or enhance adiponectin signalling may avoid their detrimental cardiac effects.

Although the pathophysiological roles of A-FABP and adiponectin have been characterized in great details, further studies are needed to investigate the cellular mechanisms underlying their effects on the cardiovascular system. In particular, whether or not the two adiponectin receptors (AdipoR1 and AdipoR2) mediate the cardiac and vascular protective effects of adiponectin in animals has not been determined so far. The pathological pathways that induce adiponectin resistance, which has been observed in cardiac and vascular cells in obesity (48), remain poorly understood. Whether or not the effects of adiponectin and A-FABP on heart and vasculature can be attributed to their paracrine actions from epicardial and perivascular adipose tissue, or endocrine actions from other fat depots, remains unclear. Further investigations of these exciting fields will provide an important knowledge base for the rational design of adiponectin agonists or A-FABP antagonists to combat the escalating epidemic of obesity-related medical complications.
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Figure Legends

Figure 1: Pleiotrophic actions of adiponectin on the cardiovascular system. Adiponectin released from adipocytes can act in either an endocrine or paracrine manner to modulate the functions of cardiomyocytes, endothelial cells (ECs), endothelial progenitor cells (EPCs), macrophages, leukocytes and vascular smooth muscles (VSMCs). eNOS: endothelial nitric oxide synthase; NO: nitric oxide; ROS: reactive oxygen species.

Figure 2: Adiponectin exerts its cardio-protective effects by activation of multiple signalling pathways in cardiomyocytes: (1) the anti-apoptotic effect is mediated by activation of ceramidase, Akt and AMPK; (2) the anti-oxidative/nitrative stress activity is attributed to its ability to decrease the expression of inducible nitric oxide synthase (iNOS) and gp91 (a subunit of NADPH oxidase); (3) the anti-inflammatory action is mediated by activation of Sphingosine kinase-1 (Sphk1) and cyclooxygenase-2 (COX-2). In addition, adiponectin stimulates (4) lipid uptake via AMPK-mediated up-regulation of CD36, and (5) glucose uptake via Akt-induced plasma membrane translocation of glucose transporter 4 (GLUT4). S1P: sphingosine-1-phosphate; VSMCs: vascular smooth muscle cells.

Figure 3: A-FABP potentiates lipid-induced inflammation and ER stress in macrophages. Toxic lipids, in particular saturated fatty acids released from adipocytes, induce A-FABP expression via activation of Toll-like receptor-4 (TLR4). Increased A-FABP potentiates endoplasmic reticulum (ER) stress by decreasing the activity of Liver X receptor α (LXRα) and stearoyl-Coenzyme A desaturase 1 (SCD1), thereby altering lipid composition. Augmented ER stress
causes activation of c-Jun N-terminal kinase (JNK), which in turn instigates inflammation and also enhances A-FABP expression.
Adipose tissue

↓ Hypertrophy and hyperplasia
↓ Oxidative/nitrative stress
↑ Fatty acid/glucose uptake
↓ Apoptosis and fibrosis
↓ Inflammation

Cardiomyocytes

↓ Mobilization
↑ Adhesion
↑ Migration
↓ Senescence
↑ Circulating number

EPCs

Macrophages

↓ Activation
↓ Foam cell formation
↑ M1→M2 polarization

VSMCs

↓ Proliferation
↓ Migration

Leukocytes

↓ Activation

EC activation
↑ eNOS/NO bioavailability
↓ Apoptosis
↓ Oxidative stress

Leukocyte-EC interaction
Fig. 2

Adipose tissue

Adiponectin

Globular
Trimer
Hexamer
HMW

S-S

AdipoR1/R2
Cardiomyocyte

Cardiomyocyte

Adipose tissue

COOH

T-cadherin

COOH

NH2

Sphk-1
COX-2
TNFα

Inflammation

Peroxynitrite

iNOS
gp91

Ceramide
Ceramidase
Caspase-8
Akt

AMPK

Lipid Uptake

Glucose Uptake

Apoptosis

Glut4 Translocation

Glucose

CD36

Oxidative/Nitrative stress

(1)

(2)

(3)

(4)

(5)
Fig. 3

Adipocyte → Toxic lipids → TLR4 → Macrophage

Mono-unsaturated lipids → Inflammation

ER stress

A-FABP → LXR-α → SCD-1

JNK

LXR-α → SCD-1