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REVIEW

Small lipid-binding proteins in regulating endothelial and vascular functions: focusing on adipocyte fatty acid binding protein and lipocalin-2

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Dysregulated production of adipokines from adipose tissue plays a critical role in the development of obesity-associated cardiovascular abnormalities. A group of adipokines, including adipocyte fatty acid binding protein (A-FABP) and lipocalin-2, possess specific lipid-binding activity and are up-regulated in obese human subjects and animal models. They act as lipid chaperones to promote lipotoxicity in endothelial cells and cause endothelial dysfunction under obese conditions. However, different small lipid-binding proteins modulate the development of vascular complications in distinctive manners, which are partly attributed to their specialized structural features and functionalities. By focusing on A-FABP and lipocalin-2, this review summarizes recent advances demonstrating the causative roles of these newly identified adipose tissue-derived lipid chaperones in obesity-related endothelial dysfunction and cardiovascular complications. The specific lipid-signalling mechanisms mediated by these two proteins are highlighted to support their specialized functions. In summary, A-FABP and lipocalin-2 represent potential therapeutic targets to design drugs for preventing vascular diseases associated with obesity.

LINKED ARTICLES

This article is part of a themed section on Fat and Vascular Responsiveness. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2012.165.issue-3

Abbreviations

AA, arachidonic acid; A-FABP, adipocyte fatty acid binding protein; AP-1, activated protein-1; apoE$^{-/-}$, apolipoprotein E-deficient; BMI, body mass index; CAD, coronary artery disease; CHD, coronary heart disease; CVD, cardiovascular diseases; E-FABP, epidermal fatty acid binding protein; ER, endoplasmic reticulum; FFA, free fatty acid; hsCRP, high-sensitivity C-reactive protein; HSL, hormone-sensitive lipase; LCFA, long-chain fatty acids; Lcn2-KO, Lipocalin-2 knockout; LDLR$^{-/-}$, low-density lipoprotein receptor-deficient; MCFA, medium-chain fatty acids; NGAL, neutrophil gelatinase–associated lipocalin; NEFA, non-esterified fatty acids; PAI-1, plasminogen activator inhibitor type 1; PKC, protein kinase C; RBP4, retinol binding protein 4; ROS, reactive oxygen species; SCD, stearoyl-CoA desaturase; SLBPs, small lipid-binding proteins; UCP, uncoupling protein

Introduction

Obesity, particularly abdominal obesity, is a major risk factor for cardiovascular and peripheral vascular disease, the number one cause of death worldwide (Roger *et al.*, 2011). Indeed, obesity and the related metabolic syndrome (a cluster of chronic symptoms including insulin resistance, hyperglycaemia, dyslipidaemia, hypertension and systemic low-grade inflammation) predispose individuals to developing cardiovascular dysfunctions (Poirier *et al.*, 2006). The Gothenburg study showed that the waist–hip ratio is an independent predictor of myocardial infarction (Lapidus *et al.*, 1994). The interaction between obesity and coronary heart disease (CHD) has been confirmed by the PROCAM study and the Nurses' Health Study (Manson *et al.*, 1990; Schulte *et al.*, 1999). Abdominal body fat distribution represents a stronger risk factor for CHD than overall obesity (Canoy, 2010). The abdominal diameter index is the superior anthropometric measure with predictive value for the ten-year Framingham CHD risk score (Smith *et al.*, 2005). Obesity also worsens the prognosis of patients with known cardiovascular diseases (CVD) (Dagenais *et al.*, 2005). A meta-analysis of prospective studies with two million participants demonstrated a graded positive relationship of overweight and obesity with the incidence of ischemic stroke, independently from age, lifestyle and other cardiovascular risk factors (Strazzullo *et al.*, 2010).

The causal relationships between abdominal obesity and elevated cardiometabolic risk are complex and not fully understood. A number of mechanisms, including the promotion of insulin resistance and the development of an inflammatory milieu in adipose tissue, play reciprocal roles in the development of cardiovascular dysfunctions (Calabro *et al.*, 2009). The expanded 'inflamed' visceral adipose tissue in obese subjects releases excess free fatty acids (FFAs) and adipokines that act directly on blood vessels and contribute to vascular damage. For example, the elevated circulating levels of pro-inflammatory adipokines, such as TNFα, IL-6, resistin and plasminogen activator inhibitor type 1 (PAI-1), are not only biomarkers associated with but also active participants in the pathogenesis of atherosclerosis and CVD (Inadera, 2008; Gustafson, 2010). Adiponectin, another important adipokine, possesses anti-inflammatory and protective cardiometabolic properties (Li *et al.*, 2010a). However, the expression level of this adipokine declines with increasing obesity. Thus, the local production of adipokines by perivascular adipose depots represents an important mechanistic link between obesity and associated vascular complications (Yudkin *et al.*, 2005; Xu *et al.*, 2010).

In addition to the above mentioned adipokines with hormone-like actions, adipose tissue produces a group of small molecular chaperones, including adipocyte fatty acid binding protein (A-FABP), lipocalin-2 and retinol binding protein 4 (RBP4), which bind and transport various lipophilic substances (Tso *et al.*, 2008). These molecules coordinate lipid responses in cells and are also strongly linked to cardiovascular and metabolic abnormalities (Furuhashi *et al.*, 2007; Wang *et al.*, 2007; Ingelsson and Lind, 2009). This brief review will explore how these lipid binding proteins relate to the pathogenesis of CVD and mediate the biochemical mechanisms underlying lipid-induced vascular abnormalities, especially in the context of endothelial dysfunction.

Small lipid-binding proteins (SLBPs) as key mediators in obesity and CVD

SLBPs are abundantly distributed small polypeptides that can bind hydrophobic ligands (Clarke and Armstrong, 1989; Bass, 1993; Bernlohr *et al.*, 1997; Chmurzynska, 2006; Storch and Thumser, 2010). Most of SLBPs are expressed in multiple cell or tissue types. However, they have divergent tissue-specific distributions, suggesting a functional specificity for different family members. The generic functions of these proteins are to promote cellular flux of poorly water-soluble ligands and facilitate their subsequent metabolic utilization or transformation, or to sequestrate ligands in a manner that limits their association with alternative binding sites within the cell. Although the presence of SLBPs in lipid-metabolizing organs, such as adipose tissue, is likely to be necessary, their physiological functions are largely uncharacterized. Nevertheless, numerous clinical evidence support a role for adipose-derived SLBPs, such as A-FABP and lipocalin-2, in obesity-related metabolic and cardiovascular complications (Table 1 and Table 2).

Structure and function of A-FABP

A-FABP, also termed aP2, ALBP and FABP4, is one of the most abundant intracellular lipid transport proteins in mature adipocytes (Makowski and Hotamisligil, 2004; Xu *et al.*, 2006) and macrophages (Pelton *et al.*, 1999; Fu *et al.*, 2002; Kazemi *et al.*, 2005). A-FABP is also expressed in endothelial cells (Lee *et al.*, 2007; Elmasri *et al.*, 2009). A-FABP belongs to the conserved multi-gene family of the intracellular lipid-binding proteins (Bernlohr *et al.*, 1997). So far, nine tissue-specific cytoplasmic FABPs have been identified. Despite a wide variance in protein sequence, the tertiary structure is common to all members, with each of them forming a characteristic β-barrel that surrounds a hydrophobic core (Bernlohr *et al.*, 1997). The lipid-binding pocket is located inside the barrel, the opening of which is framed on one side by the NH_2-terminal helix-turn-helix domain. All members of the FABP family can reversibly bind hydrophobic ligands known to influence energy metabolism and inflammation, in particular saturated and unsaturated long-chain fatty acids (LCFA), and eicosanoids (Frayn *et al.*, 2005). FABPs regulate lipid metabolism by promoting diffusion, sequestration and transport of LCFA (Flower, 1996). In addition, some of the FABPs are involved in the cellular uptake of LCFA (Bonen *et al.*, 1998). A-FABP has been shown to bind oleic acid and retinoic acid (Matarese and Bernlohr, 1988), arachidonic acid (Veerkamp *et al.*, 1999), as well as 15-deoxy-$\Delta^{12,\ 14}$-prostaglandin J_2 (Simpson *et al.*, 1999). The expression of A-FABP can be induced by both saturated and unsaturated LCFAs (Amri *et al.*, 1991; Distel *et al.*, 1992).

A-FABP may function as a positive factor in fatty acid signalling by directly targeting and delivering fatty acid metabolites to the lipid signal transduction pathway (Tan *et al.*, 2002). Upon association with particular ligands, A-FABP can translocate from the cytosol to the nucleus, where it delivers the ligands to the nuclear receptor PPARγ, thereby

Table 1

A summary of the clinical and genetic studies on the associations of A-FABP levels with obesity, metabolic and cardiovascular diseases

Clinical evidence – medical complications with increased serum A-FABP levels	References
Obesity and metabolic syndrome	Stejskal and Karpisek (2006), Xu *et al.* (2006), Cabre *et al.* (2007), Haider *et al.* (2007), Xu *et al.* (2007), Engl *et al.* (2008), Haluzik *et al.* (2009), Karakas *et al.* (2009), Hong *et al.* (2011), Cabre *et al.* (2008b), Hsu *et al.* (2010), Choi *et al.* (2009), Choi *et al.* (2011), Corripio *et al.* (2010)
CAD and CHD	Bao *et al.* (2011), Rhee *et al.* (2009)
Atherosclerosis	Krusinova and Pelikanova (2008), Hong *et al.* (2011), Miyoshi *et al.* (2010)
Carotid intima media thickness	Yeung *et al.* (2007)
Plaque instability	Agardh *et al.* (2011)
Endothelial dysfunction	Aragones *et al.* (2010)
HIV patient with metabolic syndrome	Coll *et al.* (2008), Escote *et al.* (2011)
Non-alcoholic fatty liver disease	Milner *et al.* (2009), Koh *et al.* (2009), Kim *et al.* (2011)
Renal dysfunction	Cabre *et al.* (2008a), Yeung *et al.* (2009)
Gestational diabetes mellitus	Kralisch *et al.* (2009)
Clinical evidence – increased local tissue expression	**References**
Macrophage/foam cells of human atherosclerotic plaques	Fu *et al.* (2002)
Epicardial adipose tissue in metabolic syndrome patients.	Vural *et al.* (2008)
Genetic studies	**References**
A population genetic study: individuals that carry T-87C polymorphism had reduced risk for CHD and type 2 diabetes	Tuncman *et al.* (2006)
A prospective study: common genetic variants in the FABP4 gene were not associated with increased risk of type 2 diabetes in a multiethnic cohort of postmenopausal women.	Chan *et al.* (2011)

facilitating the ligation with and enhancing the transcriptional activity of the receptor (Tan *et al.*, 2002; Furuhashi and Hotamisligil, 2008). In addition, A-FABP appears to play an important role in lipolysis (Coe *et al.*, 1999; Scheja *et al.*, 1999). Targeted disruption of A-FABP in mice led to a reduction in both basal and hormone-stimulated lipolysis in response to β-adrenergic stimulation (Scheja *et al.*, 1999). The stimulatory effect of A-FABP on lipolysis is possibly mediated by the physical interaction with hormone-sensitive lipase (HSL) (Jenkins-Kruchten *et al.*, 2003; Smith *et al.*, 2004). Notably, only the ligand bound form of A-FABP interacts with the activated, phosphorylated HSL, suggesting that the interaction facilitates the delivery of fatty acids for feedback inhibition of this lipase (Smith *et al.*, 2007). A-FABP knockout mice are partially protected from insulin resistance induced by dietary and genetic obesity, suggesting that this lipid chaperone is also involved in regulating insulin sensitivity (Furuhashi *et al.*, 2008a).

A-FABP in obesity and related medical complications

A-FABP is a secretory protein and its circulating levels are elevated in patients with obesity and several key features of the metabolic syndrome, including adverse lipid profiles [increased serum triglyceride and low-density lipoprotein (LDL)-cholesterol, and decreased high-density lipoprotein (HDL)-cholesterol], hyperglycaemia and hypertension, independently of sex, age and adiposity (Stejskal and Karpisek, 2006; Engl *et al.*, 2008; Hsu *et al.*, 2010). A five-year prospective study including 495 non-diabetic adults demonstrates that individuals with higher A-FABP levels at baseline have a progressively worse cardiometabolic risk profile (Xu *et al.*, 2007). The baseline A-FABP levels predict the development of the metabolic syndrome, independently of adiposity, insulin resistance and other classical risk factors. On the other hand, weight loss by gastric banding reduces circulating levels of

Table 2

A summary of the clinical and genetic studies on the associations of lipocalin-2 levels with obesity, metabolic disease and CVDs

Clinical evidence – medical complications with increased serum lipocalin-2 levels	References
Obesity, inflammation and metabolic syndrome	Wang *et al.*, (2007), Tso *et al.*, (2008), Auguet *et al.*, (2011), Corripio *et al.*, (2010), Cakal *et al.*, (2011), Panidis *et al.*, (2010), Wallenius *et al.*, (2011), Stepan *et al.*, (2010), Moreno-Navarrete *et al.*, (2010)
Atherosclerosis and hypertension	Forsblad *et al.*, (2002), Hemdahl *et al.*, (2006), Anwaar *et al.*, (1998a), Elneihoum *et al.*, (1997), Giaginis *et al.*, (2010), Kasahara *et al.*, (2009)
CAD and CHD	Bolignano *et al.*, (2009a), Tuladhar *et al.*, (2009), Yndestad *et al.*, (2009), Zografos *et al.*, (2009), Sahinarslan *et al.*, (2011), Choi *et al.*, (2008), Lee *et al.*, (2010)
Cardio-renal syndrome	Bachorzewska-Gajewska *et al.*, (2006), Poniatowski *et al.*, (2009), Ling *et al.*, (2008), Bennett *et al.*, (2008), Bolignano *et al.*, (2010); Capuano *et al.*, (2009), McIlroy *et al.*, (2011), Aghel *et al.*, (2010), Comnick and Ishani (2011), Mishra *et al.*, (2005)
Acute kidney injury and chronic kidney disease	Nguyen and Devarajan (2008), Zappitelli *et al.*, (2007), Mitsnefes *et al.*, (2007), Nickolas *et al.*, (2008), Yang *et al.*, (2009), Bolignano *et al.*, (2009b), Di Grande *et al.*, (2009), Haase *et al.*, (2009), Kronenberg, (2009), Rauen *et al.*, (2011), Haase *et al.*, (2011)
Acute cerebral ischaemia	Anwaar *et al.*, (1998b), Elneihoum *et al.*, (1996), Falke *et al.*, (2000)
Clinical evidence – increased local tissue expression	**References**
Adipose tissue of obese subjects	Auguet *et al.*, (2011), Catalán *et al.*, (2009), Fain *et al.*, (2010)
Atherosclerotic plaque, myocardial infarction, abdominal aortic aneurysm	Paulsson *et al.*, (2007), Leclercq *et al.*, (2007), Folkesson *et al.*, (2007), Hemdahl *et al.*, (2006), Te Boekhorst *et al.*, (2011), Yndestad *et al.*, (2009)
Renal epithelial injury	Schmidt-Ott *et al.*, (2006)

A-FABP in obese subjects (Haider *et al.*, 2007). In addition to its role in lipid metabolism and insulin sensitivity, both clinical investigations and animal studies suggest that A-FABP is a central player in mediating obesity-related vascular disease, primarily by inducing insulin resistance and potentiating lipid-induced inflammation (Hoo *et al.*, 2008). Circulating levels of A-FABP are independently associated with measures of endothelial dysfunction (Aragones *et al.*, 2010), coronary atherosclerotic burden (Miyoshi *et al.*, 2010), intima media thickness (Yeung *et al.*, 2007) and CHD (Rhee *et al.*, 2009). A cross-sectional study including 237 diabetic patients demonstrates that serum A-FABP is independently associated with diabetic nephropathy staging and is markedly elevated in patients with macrovascular complications (Yeung *et al.*, 2009). In addition to the aforementioned epidemiological data, genetic studies also support the role of A-FABP as a causative factor of obesity-related medical complications. An early report showed that the interaction between the A-376C polymorphism of A-FABP and the Pro12Ala substitution of PPARγ is a significant predictor of obesity and insulin resistance (Damcott *et al.*, 2004). A functionally significant genetic variation (T-87C) at the A-FABP promoter region in humans has been identified in a population-based genetic study involving 7899 participants (Tuncman *et al.*, 2006). This genetic variation causes reduced transcriptional activity of the A-FABP promoter, resulting in decreased adipose tissue A-FABP mRNA expression. Subjects who are carriers of this polymorphism have much lower serum triglyceride levels and significantly reduced risk for CHD and type 2 diabetes compared with subjects homozygous for the wild-type allele (Tuncman *et al.*, 2006).

Structure and function of lipocalin-2

Lipocalin-2 [also known as 24p3, neutrophil gelatinase-associated lipocalin (NGAL) and siderocalin] is a 25 kDa secretory glycoprotein that belongs to the lipocalin family (Kjeldsen *et al.*, 1993; Goetz *et al.*, 2000). Lipocalins are a diverse family that generally bind small, hydrophobic ligands, but can also bind soluble, extracellular macromolecules and specific cell surface receptors (Flower, 1996). The crystal structures of lipocalin-2 display a typical lipocalin fold, albeit with an unusually large cavity lined with more polar and positively charged amino acid residues (Goetz *et al.*, 2000). Chemotactic formyl-peptides from bacteria have been proposed as ligands of lipocalin-2, but binding experiments and the structure of this protein do not support this hypothesis (Kjeldsen *et al.*, 2000). Although lipocalin-2 can bind weakly to some common ligands of lipocalins, including leu-

kotriene B_4 and platelet activating factor (Bratt *et al.*, 1999; Goetz *et al.*, 2000), its high-affinity endogenous ligand(s) remains to be identified. *In vivo,* lipocalin-2 exists as monomers, homodimers and heterodimers with gelatinase (Kjeldsen *et al.*, 1993; 2000; Flower *et al.*, 2000). A cysteine residue (Cys^{87}) of mouse lipocalin-2 is responsible for its homodimerization and heterodimerization. In addition, one *N*-linked glycosylation o occurs on residue Asn^{65} in both rodent and human lipocalin-2 (Chu *et al.*, 1996; Rudd *et al.*, 1999).

The expression of lipocalin-2 in adipocytes was first described by Lin *et al.* (2001) and subsequently confirmed by both microarray- and proteomics-based studies (Kratchmarova *et al.*, 2002; Wang *et al.*, 2007). Expression of lipocalin-2 was markedly induced following differentiation of pre-adipocytes to adipocytes (Baudry *et al.*, 2006). Although lipocalin-2 was originally identified in mouse kidney cells (Hraba-Renevey *et al.*, 1989) and human neutrophil granules (Kjeldsen *et al.*, 1993), the protein is expressed in many tissues, including liver, lung, thymus, kidney, small intestine and mammary gland. Its expression in epithelial cells can be induced during inflammation and after cell injury. The wide tissue distribution pattern of lipocalin-2 implies diverse functions. Lipocalin-2 plays a key role in the innate immune response to bacterial infection by binding to iron-laden bacterial siderophores and thereby limiting bacterial growth (Flo *et al.*, 2004; Fischbach *et al.*, 2006). Lipocalin-2-deficient mice display an increased susceptibility to bacterial infections due to the failure of iron sequestration. Recombinant lipocalin-2 induces cellular differentiation in the kidney during embryogenesis and protects it from ischaemic injury (Mori *et al.*, 2005). However, a study in lipocalin-2-deficient mice argued against the renal protective role of lipocalin-2 (Berger *et al.*, 2006). In fact, Viau *et al.* (2010) provided evidence suggesting that lipocalin-2 is essential for progression of chronic kidney disease in both animals and humans. Lipocalin-2 is an excellent predictor of acute kidney injury and a biomarker for chronic kidney diseases (Bolignano *et al.*, 2010). In various forms of gastrointestinal injury, lipocalin-2 facilitates mucosal regeneration by promoting cell migration (Playford *et al.*, 2006). *In vitro* studies suggest that lipocalin-2 is important for both cellular apoptosis and survival in various cell types (Tong *et al.*, 2003; 2005). Two putative cellular receptors for lipocalin-2 have been identified (Devireddy *et al.*, 2005; Hvidberg *et al.*, 2005). Megalin, a member of the LDL receptor family, has been shown to bind human lipocalin-2 and to mediate its cellular uptake (Hvidberg *et al.*, 2005). In addition, the brain-type organic cation transporter, a protein with 12 transmembrane helices, has been shown to be the cell surface receptor for mouse lipocalin-2, which selectively mediates apoptosis through modulation of cellular iron homeostasis (Devireddy *et al.*, 2005). Despite the identification of these potential receptors, the precise role of lipocalin-2 in cell survival and death has yet to be determined.

Lipocalin-2 in obesity and related medical complications

Both clinical and experimental evidence support the causative roles of lipocalin-2 as an inflammatory adipokine in obesity and related medical complications (Wang *et al.*, 2007; Catalán *et al.*, 2009; Esteve *et al.*, 2009; Cakal *et al.*, 2011; Panidis *et al.*, 2010). In human obese subjects, like other insulin resistance-inducing adipokines and cytokines, circulating lipocalin-2 levels are elevated (Wang *et al.*, 2007; Yan *et al.*, 2007; Zhang *et al.*, 2008). High plasma lipocalin-2 levels are also found in pre-pubertal obese children (Corripio *et al.*, 2010). Serum lipocalin-2 levels correlated positively with parameters of adiposity [body mass index (BMI), waist–hip ratio, waist circumference, fat percentage], systolic blood pressure, fasting glucose, insulin, triglycerides, as well as the insulin resistance index as measured by homeostasis model assessment (HOMA-IR) but correlated negatively with HDL-cholesterol (Wang *et al.*, 2007). The positive associations of circulating lipocalin-2 levels with fasting glucose and HOMA-IR remained significant even after adjustment for age, sex and BMI, suggesting that lipocalin-2 represents an independent risk factor for insulin resistance and hyperglycaemia in obese individuals. Furthermore, there is an independent, positive association of lipocalin-2 with serum levels of high-sensitivity C-reactive protein (hsCRP), a well-established marker of chronic inflammation. In obese rodents, increased serum levels of lipocalin-2 are mainly due to the selective augmentation of its expression in adipose tissue and liver (Wang *et al.*, 2007; Yan *et al.*, 2007). Lipocalin-2 expression in adipocytes can be induced by agents that promote insulin resistance. In both obese animal models and humans, treatment with the PPARγ agonist rosiglitazone results in a significant reduction in lipocalin-2 mRNA expression and its circulating protein concentrations. Notably, rosiglitazone-mediated decreases in lipocalin-2 concentrations correlate significantly with increases in insulin sensitivity and decreases in hsCRP. Taken in conjunction, these results suggest that lipocalin-2 can be used as a biomarker for risk stratification of obesity-related metabolic diseases.

In 3T3-L1 adipocytes, forced reduction of lipocalin-2 expression by siRNA enhances insulin-stimulated glucose uptake, whereas addition of exogenous recombinant lipocalin-2 increases glucose production in hepatocytes, suggesting that lipocalin-2 might have a causal role in insulin resistance and hyperglycaemia (Yan *et al.*, 2007). In addition, such causative role of lipocalin-2 in obesity-related metabolic disorders has been demonstrated by animal studies. With aging or during dietary-/genetic-induced obesity, lipocalin-2 knockout (Lcn2-KO) mice show significantly decreased fasting glucose and insulin levels and improved insulin sensitivity compared with their wild-type littermates (Law *et al.*, 2010). Inflammation and the accumulation of lipid peroxidation products are significantly attenuated in the white adipose tissues of Lcn2-KO mice. The differences of adipose fatty acid composition suggest that lipocalin-2 deficiency attenuates the metabolism of arachidonic acid (AA, C20:4 n6), which by contrast is elevated by aging and obesity in wild-type mice. In particular, the inflammatory lipid species metabolized by arachidonate lipoxygenase are significantly reduced in the white adipose tissues of Lcn2-KO mice. Lipocalin-2 elicits its adverse effects by promoting the production of inflammatory lipid species and adipokines from adipose tissue, which in turn magnify the systemic insulin resistance and impair energy homeostasis (Law *et al.*, 2010). Lcn2-KO mice exhibit

impaired adaptive thermogenesis and cold intolerance. Expression of uncoupling protein (UCP)-1, a hallmark of brown adipocyte that is pivotal for cold- and diet-induced thermogenesis, is decreased in the brown adipose tissues of these mice (Guo *et al.*, 2010). Cyclooxygenase, the key enzyme for AA metabolism, plays an essential role during the recruitment of brown adipocytes (Vegiopoulos *et al.*, 2010) and UCP-1 induction (Madsen *et al.*, 2010). These findings suggest that lipocalin-2 deficiency may selectively affect the metabolic pathway related to the production or breakdown of AA. One of the AA metabolites, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (PGJ_2), is a ligand for PPARγ. The response to rosiglitazone is also different between wild-type and Lcn2-KO mice (Jin *et al.*, 2011). However, the detailed molecular events whereby lipocalin-2 modulates AA metabolism warrants further elucidations.

Lipocalin-2 may be involved in the pathogenesis of CVD (Bolignano *et al.*, 2010). Positive associations are found between lipocalin-2 and the visceral fat areas in patients with CHD. Moreover, the abnormalities in lipocalin-2 levels are correlated with the degree of severity, that is, single-, double- or triple-vessel diseases (Lee *et al.*, 2010). Lipocalin-2 plays a pivotal role in the systemic adaptation to chronic heart failure in elderly patients. Those with baseline lipocalin-2 > 783 ng·L^{-1} have a significantly higher mortality than the other subjects (Bolignano *et al.*, 2009a), although it remains to be determined whether or not the increased levels of the adipokine reflect an expression of renal injury during the course of chronic heart failure. Lipocalin-2 has been proposed as a biomarker for the early detection of cardio-renal syndrome in patients with acute heart failure (Alvelos *et al.*, 2010). Above the cut-off value of 170 ng·L^{-1}, the prediction sensitivity is 100% and the specificity is 86.7%. Marked hyper-expression of lipocalin-2 is found in the necrotic areas and the surrounding tissue of the infracted heart. The adipokine may mediate the post-ischaemic inflammation and remodelling responses (Yndestad *et al.*, 2009; Ding *et al.*, 2010). In patients with CHD, the circulating levels of lipocalin-2 increase and are independently associated with systolic arterial blood pressure, insulin resistance and decreased HDL-cholesterol levels (Choi *et al.*, 2008). In response to various types of damage, the vascular wall is over-expressing this protein. In a rat carotid artery injury model, lipocalin-2 is highly up-regulated in the intima after angioplasty (Bu *et al.*, 2006). In both rat and human vascular smooth muscle cells, the mRNA and protein expression of lipocalin-2 can be elevated in an NF-κB-dependent manner. Smooth muscle cell-produced lipocalin-2 is present as mono- and homomeric forms in the cytosol and in a complex containing MMP-9 after secretion (Bu *et al.*, 2006). High lipocalin-2 expression is found in aortic aneurysmal tissue with associated thrombotic lesions and may contribute to an enhanced proteolytic activity (Folkesson *et al.*, 2007). Following acute cerebrovascular events (ischemic stroke), serum lipocalin-2 levels progressively increase and remain high for up to one year (Elneihoum *et al.*, 1996; Anwaar *et al.*, 1998b). If measured one to three days after the event, lipocalin-2 can be used for stratifying the patients according to their mortality risk during the following 4-year period (Falke *et al.*, 2000). Thus, the available evidence suggests that lipocalin-2 can be used as a diagnostic and prognostic marker for patients with overt heart disease.

SLBPs in obesity-induced endothelial dysfunction

Endothelium, the permeable barrier covering the lumen of all vessels, functions to regulate vascular tone and maintain a non-thrombogenic surface (Vanhoutte, 2009). Under healthy conditions, the endothelium promotes vascular homeostasis by producing a balanced array of substances with potent vasodilator, anti-aggregatory, anti-atherosclerotic and anti-inflammatory properties. NO is the main endothelium-derived relaxing (EDRF) and protective factor (Palmer *et al.*, 1987; Furchgott and Vanhoutte, 2007). The loss of normal endothelial function is referred to as 'endothelial dysfunction' and is characterized by impaired endothelium-dependent vasodilatation, imbalanced production of EDRFs and endothelium-derived constricting factors (EDCFs), and reduced regenerative/proliferative capacities (Feletou *et al.*, 2008). Endothelial dysfunction is a well-established major risk factor for CVD (Vanhoutte, 2009). Such dysfunction precedes and contributes to the development of cardiometabolic abnormalities and predicts the occurrence of cardiovascular events. Under pathological conditions such as hypercholesterolemia, hypertension or diabetes, endothelial cells become activated to produce pro-inflammatory and pro-atherosclerotic substances and mediators, which are detrimental to the vascular tone and structure. The activated endothelium promotes the recruitment of circulating leukocytes and facilitates the development of vascular inflammation and atherosclerosis (Rao *et al.*, 2007).

Endothelial dysfunction is a common abnormality in obesity and associated diseases. Impairment of endothelial function is in proportion to insulin resistance and indices of adiposity (de Jongh *et al.*, 2004; Williams *et al.*, 2005). Obesity impairs endothelium-mediated vasodilator responses to increased shear stress, insulin and other neurohumoral mediators (Williams *et al.*, 2002). It is associated with greater arterial stiffness (Wildman *et al.*, 2005). In obese subjects, endothelial dysfunction has been demonstrated in both conduit and resistance arteries (Hashimoto *et al.*, 1998; Avogaro and de Kreutzenberg, 2005; Van Guilder *et al.*, 2006). In patients with normal or mildly diseased coronary arteries, obesity is independently associated with coronary endothelial dysfunction (Al Suwaidi *et al.*, 2001). Administration of a specific blocker of endothelin receptors in obese patients reverses the baseline defect in endothelium-dependent vasodilatation (Mather *et al.*, 2004). The vasoconstrictor response to angiotensin II is greater in obese than in lean men (Nielsen *et al.*, 2004). The distribution of fat, rather than obesity *per se*, appears to negatively influence endothelial function. Impaired flow-mediated endothelium-dependent vasodilatation is found in the brachial artery of subjects with visceral obesity (Hashimoto *et al.*, 1998). In obese children, arterial wall stiffness and endothelial dysfunction are positively correlated to an android distribution of body fat (Tounian *et al.*, 2001). Weight loss in morbidly obese human subjects substantially

improves endothelial dysfunction (Ziccardi *et al.*, 2002; Bigornia *et al.*, 2010).

Mechanisms underlying obesity-induced endothelial dysfunction

While the evidence suggesting that obesity impairs vascular function is convincing, the underlying molecular mechanisms remain largely uncharacterized. Obesity is independently involved in abnormal endothelium-dependent vasodilatation by attenuating NO production (Higashi *et al.*, 2001; Williams *et al.*, 2002). Reduced eNOS expression, the tissue levels of L-arginine (the substrate for NO production) and the cofactors of eNOS (such as FAD, FMN, NADPH and BH_4) have been implicated in causing endothelial dysfunction (Huang, 2003). Insulin stimulates the production of NO by enhancing the phosphorylation of eNOS at Ser^{1177} and decreasing the phosphorylation at Thr^{495} (Muniyappa and Quon, 2007). In insulin-resistant states, on the contrary, the elevated circulating insulin levels promote the production of vasoconstrictors and pro-inflammatory molecules, such as endothelin-1 and PAI-1, in turn contributing to endothelial dysfunction (Steinberg *et al.*, 1996; Kim *et al.*, 2006). Diminished eNOS activity and NO bioavailability render the endothelial cells more susceptible to oxidative stress-induced damage (Bashan *et al.*, 2009). Visceral obesity is also characterized by a NO-independent endothelial vasodilator dysfunction (de Kreutzenberg *et al.*, 2003; Vigili de Kreutzenberg *et al.*, 2003). In obese patients, bradykinin-induced increases in forearm blood flow are blunted irrespective of both NOS and COX inhibition, indicating alternative vasodilator mechanism(s). In particular, endothelium-dependent hyperpolarization is favoured to maintain endothelium-dependent vasodilatation in diet-induced obese animals (Vigili de Kreutzenberg *et al.*, 2003; Chadha *et al.*, 2010).

Lipotoxicity is a key pathogenic link between obesity and endothelial dysfunction. Endothelial lipotoxicity is caused by elevated levels of circulating non-esterified FFAs (NEFAs), which are closely associated with central obesity and insulin resistance (Imrie *et al.*, 2010). Exposure to pathophysiological concentrations of FFAs impairs endothelial function as assessed by both agonist-stimulated and flow-mediated vasodilatation (Steinberg *et al.*, 1997; Steer *et al.*, 2003a). *In vivo* data from both animal and human studies support the concept that acute plasma NEFA elevation leads to increased arterial blood pressure, and epidemiological evidence suggests a link between increased NEFA levels and hypertension (Sarafidis and Bakris, 2007; Umpierrez *et al.*, 2009). Endothelial dysfunction after a high-fat meal in healthy subjects is closely correlated with FFA concentrations, which are derived from lipoprotein lipase-mediated hydrolysis of triglyceride-rich lipoproteins (Austin *et al.*, 2000; Shimabukuro *et al.*, 2007). Fatty acids cause endothelial dysfunction by (1) interfering with eNOS activity and NO production (Davda *et al.*, 1995; Esenabhalu *et al.*, 2003; Lynn *et al.*, 2004); (Rask-Madsen and King, 2007).; (2) impairing insulin's action to stimulate endothelium-dependent vasodilatation (Steinberg *et al.*, 2000; Li *et al.*, 2010b); (3) augmenting α-adrenoceptor-mediated constriction through a COX sensitive mechanism (Stepniakowski *et al.*, 1997); (4) inducing oxidant stress (Lu *et al.*, 1998; Lopes *et al.*, 2003; Tripathy *et al.*, 2003); and (5) promoting vascular cell proliferation and inflammation, etc. (Vacaresse *et al.*, 1999; Artwohl *et al.*, 2004; Weigert *et al.*, 2004; Azekoshi *et al.*, 2010). Elevated FFAs not only affect endothelial function, but also interfere with vascular remodelling. In vascular smooth muscle cells, oleic and linoleic acids activate protein kinase C (PKC), which stimulates NADPH oxidase to generate reactive oxygen species, which in turn negatively affect vascular tone and cell growth (Egan *et al.*, 2001). In addition, increased FFA levels can lead to an impairment of NO-independent vasodilatation by inhibiting potassium channel activity (de Kreutzenberg *et al.*, 2003) and preventing the production of prostacyclin (Jeremy *et al.*, 1983).

The FFA composition appears to be more relevant to endothelial and vascular function than the total amount of NEFAs. Indeed, the serum fatty acid composition predicts endothelial vasodilator dysfunction independently of serum NEFA and cholesterol levels in young, healthy men (Steer *et al.*, 2003b). An acute elevation of LCFA, but not medium-chain fatty acids (MCFA), attenuates endothelium-dependent vasodilatation (Steer *et al.*, 2003a). In men, endothelial function is inversely related to the total proportion of saturated fatty acids, in particular lauric and myristic acid, and positively related to the proportion of α-linolenic acid. The most abundant saturated fatty acid in human plasma, palmitate, induces inflammatory cytokines in endothelial cells and its serum level correlates with those of IL-6 (Staiger *et al.*, 2006). The lipotoxic effects of saturated fatty acids on endothelial cells can be rescued by overexpression of stearoyl-CoA desaturase (SCD)-1 to convert the saturated to monounsaturated fatty acids for incorporation into neutral lipids (Peter *et al.*, 2008). The dietary balance of LCFAs influences the development of endothelial dysfunction and CVD (De Caterina *et al.*, 2006). High intake of linoleic acid-rich oils or fats can lead to cellular oxidative stress and elicit inflammatory responses (Grimble, 1998). Omega-6 fatty acids, especially linoleic acid, cause endothelial cell dysfunction and potentiate TNFα–mediated endothelial injury (Wang *et al.*, 2008), in part due to their properties of easy oxidization (Hennig *et al.*, 2001). By contrast, omega-3 fatty acids, such as eicosapentaenoic and docosahexaenoic acids, reduce cardiovascular events by their antioxidant and anti-inflammatory effects on vascular cells (Psota *et al.*, 2006) and improve endothelial function (Omura *et al.*, 2001). These findings collectively suggest that pharmacological prevention of lipotoxicity in endothelial cells represents a valuable approach for protection against endothelial dysfunction and CVD.

Modulation of endothelial function by SLBPs

Although all SLBPs act as receptors and transporters for hydrophobic ligands, there are differences in their ligand selectivity, binding affinity and mechanisms (Chmurzynska, 2006). Specific SLBPs may possess both unique and overlapping functions depending on cell types, and physiological or pathological conditions (Bass, 1993; Storch and McDermott, 2009). The fatty acid composition may drive the diversified functions of SLBPs in different tissues. For example, the stable tertiary structure of A-FABP complexed with the PPARγ ligand troglitazone and linoleic acid facilitates its nuclear localization (Ayers *et al.*, 2007; Gillilan *et al.*, 2007). By contrast, nuclear translocation does not occur when A-FABP is complexed with oleate or stearate. It is also possible that the needs

of individual cell type determine the selectivity and affinity of SLBPs present at different sites, so that high levels of A-FABP but very low levels of skin-type epidermal FABP (E-FABP) are expressed in adipocytes (Storch and Thumser, 2010). In addition, the functional diversity of SLBPs may be also related to their different protein binding partners: A-FABP interacts with HSL (Smith *et al.*, 2008), whereas lipocalin-2 forms heterodimers with MMP-9 (Rudd *et al.*, 1999).

Based on the information of gene organizations, amino acid sequences as well as the crystal structures, A-FABP and lipocalin-2 are found to be evolutionarily distinct but both can be grouped into a protein family called 'calycins' (Flower *et al.*, 1993), which is characterized by a 'up-and-down β-barrel motif', a key structural feature for hydrophobic ligand binding (LaLonde *et al.*, 1994). However, the ligand binding cavity of A-FABP consists of 10 anti-parallel β-strands, while that of lipocalin-2 contains an eight-stranded motif. The cavity of lipocalin-2 is unusually large and open compared with other SLBPs (Goetz *et al.*, 2000). Unlike the hydrophobic lipid binding pocket of A-FABP (Xu *et al.*, 1992), the binding cavity of lipocalin-2 is lined with polar and positively charged amino acids, suggesting that the lipids with negatively charged functional groups are better ligands of this protein. Polyunsaturated lipids metabolites derived from arachidonic acid show higher binding affinity than highly saturated fatty acids such as arachidic acid and stearic acid (P Fan and Y Wang, unpubl. data). It is possible that lipocalin-2 helps to maintain specific phospholipids pools that may be linked to distinct lipid-mediated signal transductions.

Lipid-derived autacoids play major roles in mediating endothelium-dependent vasoconstriction (Feletou *et al.*, 2010). The EDCFs produced by endothelium include the metabolites of arachidonic acids, the transiently existing endoperoxides, prostacyclin, other prostaglandins and thromboxane A_2. Receptors, such as thromboxane-prostanoid (TP) receptor, mediate EDCF-induced contraction of the vascular smooth muscle cells. EDCF-mediated contraction is exacerbated when NO production is impaired (Vanhoutte *et al.*, 2009). In both cultured human microvascular endothelial cells and regenerated endothelial cells, the induction of A-FABP expression is associated with reduced eNOS phosphorylation and NO bioavailability (Lee *et al.*, 2007; 2011). In apolipoprotein E-deficient ($apoE^{-/-}$) mice, A-FABP expression can be detected in aortic endothelium before the appearance of impaired endothelium-dependent relaxations to acetylcholine, UK14304 (selective α_2-adrenoceptor agonist) and A23187 (calcium ionophore). Treatment with the A-FABP inhibitor, BMS309403, improves endothelial function and eNOS activity, but has no effects on endothelium-independent relaxations (Lee *et al.*, 2011). In type 2 diabetic patients, circulating levels of A-FABP are inversely associated with endothelial dysfunction as measured by peripheral artery tonometry (Aragones *et al.*, 2010), and the ratio of plasma A-FABP/adiponectin is closely correlated with femoral intima media thickness and endothelium-dependent vasodilatation (Xiao *et al.*, 2010). Although A-FABP may act as a lipid sensor to induce cellular stress and endothelial dysfunction, the role of this protein to directly regulate vascular reactivity has not been established. It is not known whether or not elevated A-FABP levels can lead to a dysregulated production of EDCFs. On the other hand, evidence from genetically modified mice suggests that lipocalin-2 deficiency has protective effects against both aging- and dietary obesity-associated endothelial dysfunctions and the imbalanced production of EDRF and EDCFs. Lipocalin-2 acts as a lipid carrier to promote endothelium-dependent contractions and attenuate endothelium-dependent relaxations (Liu *et al.*, 2010). Treatment with this protein induces eNOS uncoupling and elevates COX expression in both intact rat carotid arteries and primary porcine endothelial cell cultures, suggesting that increased expression levels of lipocalin-2 promotes the development of endothelial dysfunction. Considering the differences of their protein structure, ligands and expression profiles, it is highly possible that A-FABP and lipocalin-2 elicit distinctive and specialized functions to promote certain types of endothelial lipotoxicity.

SLBPs in vascular damage and atherosclerosis

Atherosclerosis is considered to be an inflammatory disease, which involves the interplay of prooxidative activities, induction of inflammatory cytokines and adhesion molecules and activation of vascular endothelial cells, all events that promote vascular leukocyte infiltration and plaque development (Ross, 1999). Lipotoxicity is critically involved in the initiation of vascular inflammation, endothelial activation, progression of atherosclerotic lesions and complications such as atherothrombosis, stroke and myocardial infarction (DeFronzo, 2010). Hypertriglyceridaemia and associated high circulating levels of FFAs are important risk factors for atherosclerosis. Indeed, FFAs are independently associated with cardiovascular mortality in patients with clinically overt atherosclerosis (Pilz *et al.*, 2007). NEFAs can induce endothelial cell apoptosis (Dimmeler *et al.*, 2002), which causes the denudation of the endothelium layer and subsequent thrombosis (Durand *et al.*, 2004). This represents a critical mechanism for plaque erosion and a cause of myocardial infarction or stroke. Saturated FFAs are major inducers of endothelial cell apoptosis and inflammatory cytokines. This conclusion is based on the observation that saturated, but not unsaturated, fatty acids induce apoptosis of human coronary artery endothelial cells via NF-κB activation (Staiger *et al.*, 2006). Saturated LCFAs show a higher potency to induce the production of inflammatory cytokines in endothelial cells than MCFAs (Harvey *et al.*, 2010). The mechanisms by which selected fatty acids induce endothelial cell activation and inflammation are not fully understood. The functional membrane lipid rafts microdomains, called caveolae, are required for endothelial cell activation through various oxidative stress and inflammatory pathways, such as NF-κB and MAPK. COX is localized in the lipid raft domain (Wang *et al.*, 2008). Caveolae play a major role in the regulation of endothelial vesicular trafficking as well as the uptake of lipids and related lipophilic compounds. Fatty acids can alter localization and function of caveolae-associated signalling proteins (Frank *et al.*, 2003). In addition, the fatty acid composition in the lipid

rafts may also play a regulatory role in endothelial cell activation and inflammation. Pre-enrichment of caveolae with linoleic acid but not with α-linolenic acid promotes the inflammatory activation process (Chapkin *et al.*, 2008). It is very likely that specific fatty acids either stabilize or perturb caveolar function, thus leading to modifications of caveolae-dependent signalling.

The targeted disruption of the A-FABP gene not only provides significant protection against both dietary and genetic obesity-associated insulin resistance and metabolic abnormalities but also leads to marked alleviation of inflammation and atherosclerosis associated with apoE$^{-/-}$ mice (Maeda *et al.*, 2005; Makowski and Hotamisligil, 2005). Ablation of A-FABP in macrophages alone causes a significant reduction of atherosclerotic lesions in the apoE$^{-/-}$ mouse aorta (Makowski *et al.*, 2001). The A-FABP deficiency-mediated protection against atherogenesis persists even when the apoE$^{-/-}$ mice are fed with a hypercholesterolemic Western diet (Boord *et al.*, 2002). The survival rates of apoE$^{-/-}$ mice null for A-FABP in both adipocyte and macrophage are much higher than those of apoE$^{-/-}$ controls, and this can be primarily attributed to the increased stability of the atherosclerotic plaques (Boord *et al.*, 2004). Consistent with the above data, an orally active small-molecule inhibitor of A-FABP show a strong protective effect against severe atherosclerosis and type 2 diabetes in mouse models (Furuhashi *et al.*, 2007), further supporting the causative role of A-FBAP in the development of vascular inflammation and atherosclerotic lesions. Although A-FABP expression is up-regulated in injured endothelial cells, its impact on the progression of atherosclerotic lesions is not known. The available evidence suggests that the effects of A-FABP in promoting atherosclerotic formation are mediated at least in part by its direct actions on macrophages, independently of lipid metabolism and insulin sensitivity (Layne *et al.*, 2001). A-FABP expression in macrophages is induced by several atherogenic and inflammatory factors, such as oxidized LDL (Fu *et al.*, 2002) and Toll-like receptor activators (Kazemi *et al.*, 2005), and is suppressed by the cholesterol-lowering drugs statins (Llaverias *et al.*, 2004). Adenovirus-mediated over-expression of A-FABP in human macrophages induces foam cell formation by increasing intracellular cholesterol ester accumulation (Fu *et al.*, 2002). On the other hand, depletion of A-FABP expression in macrophage prevents oxidized LDL-induced foam cell formation, increases cholesterol efflux and suppresses inflammatory responses and cytokine production (Makowski *et al.*, 2001; Makowski and Hotamisligil, 2005). Treatment of THP-1 macrophages with an A-FABP inhibitor decreases the production of the inflammatory cytokines in a way similar to that observed in A-FABP-deficient macrophage cell lines (Furuhashi *et al.*, 2007). The inflammatory responses of macrophages require A-FABP, which promotes cytokine production via JNK and AP-1 (activator protein-1) (Hui *et al.*, 2010). A-FABP is also necessary for macrophage endoplasmic reticulum (ER) stress response to inflammatory signals (Storch and Thumser, 2010). In both humans and animals, ER stress is present in macrophages of atherosclerotic plaques (Ozcan *et al.*, 2004). A-FABP acts as a lipid sensor to induce cellular stress. It couples toxic lipids to ER stress and induces inflammation in macrophages *in vitro* and *in vivo* (Erbay *et al.*, 2007). Toxic lipids (e.g. palmitate) induce A-FABP expression and concurrently mitigate ER stress, leading to subsequent JNK activation. In apoE$^{-/-}$ mice, both ER stress and A-FABP expression co-exist in macrophages of the atherosclerotic lesion areas (Erbay *et al.*, 2009). Genetic depletion of A-FABP or chemical inhibition of this lipid chaperone leads to alleviation of ER stress and attenuation of JNK activation. Similarly, attenuation of ER stress using the chemical chaperone 4-phenylbutyric acid (PBA) also prevents toxic lipid-induced inflammation in macrophages and reduces atherosclerosis in apoE$^{-/-}$ mice (Erbay *et al.*, 2009). SCD-1 converts toxic saturated lipids to mono-unsaturated lipid moieties and alleviates lipid-induced ER stress (Erbay *et al.*, 2009), whereas A-FABP suppresses SCD-1 expression by inhibiting the nuclear receptor liver X receptor-α. Taken in conjunction, these findings uncover a lipid-signalling network modulated by A-FABP to induce ER stress, inflammation and atherosclerosis (Hoo *et al.*, 2008).

Both A-FABP and lipocalin-2 are pro-inflammatory factors that link obesity with vascular disease and are involved in the pathogenesis of atherosclerotic plaque. The serum levels of A-FABP are positively correlated with those of lipocalin-2 (Xu *et al.*, 2007; Tso *et al.*, 2008; Choi *et al.*, 2009; Milner *et al.*, 2009; Fain *et al.*, 2010). Expression of lipocalin-2 is markedly induced by a variety of pro-inflammatory stimuli, including lipopolysaccharide (LPS), IL-1β, IL-17, TNFα, dexamethasone and hyperglycaemia (Meheus *et al.*, 1993; Cowland *et al.*, 2003; Pawluczyk *et al.*, 2003; Vizzardelli *et al.*, 2006). IL-1β induces mRNA expression of lipocalin-2 through activation of NF-κB (Bu *et al.*, 2006; Cowland *et al.*, 2006). Elevated serum lipocalin-2 concentrations are closely associated with a variety of acute and chronic inflammatory conditions, such as infection (Draper *et al.*, 2006), stroke (Anwaar *et al.*, 1998b; Falke *et al.*, 2000) and acute renal injury (Mishra *et al.*, 2005; Trachtman *et al.*, 2006; Schaub *et al.*, 2007). An augmented expression of lipocalin-2 is also detected in the local inflammatory loci of lung inflammation and rheumatoid arthritis (Shen *et al.*, 2005; Cowland *et al.*, 2006). In a murine heart transplantation model, a marked elevation in both mRNA and protein expression of lipocalin-2 is observed following ischaemia and reperfusion injury (Aigner *et al.*, 2007). In addition to its role in acute inflammatory reactions, lipocalin-2 is an important player in atherosclerosis. A marked elevation in lipocalin-2 expression can be detected in the atherosclerotic plaques of both apoE$^{-/-}$ and LDL receptor-deficient (LDLR$^{-/-}$) mice that spontaneously develop atherosclerosis (Hemdahl *et al.*, 2006). In a rat carotid artery injury model, lipocalin-2 is highly induced in the intima after angioplasty, as a consequence of NF-κB activation (Bu *et al.*, 2006). In both atherosclerotic plaques and the intima of injured vessels, lipocalin-2 is co-localized with MMP-9, a key protease involved in inflammation and atherosclerosis. The interaction between lipocalin-2 and MMP-9 may modulate proteolytic activity during the vascular inflammatory process (Yan *et al.*, 2001). Consistent with these animal-based observations, immunohistochemistry conducted on human carotid endarterectomy specimens and control tissues from the internal mammary artery also show an increased expression of lipocalin-2 and its colocalization with MMP-9 in atherosclerotic plaques (Elneihoum *et al.*, 1996; 1997).

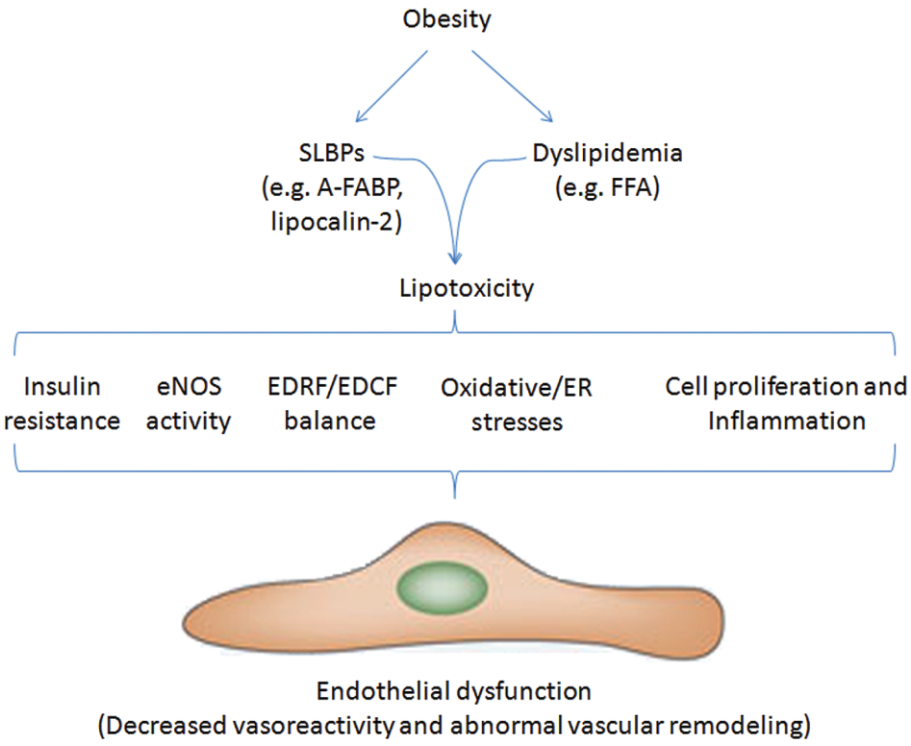

Figure 1

SLBPs, including A-FABP and lipocalin-2, facilitate the development of obesity-induced endothelial dysfunction by promoting lipotoxicity, in turn modulating various signaling pathways that are involved in maintaining the vascular homeostasis.

Increased levels of lipocalin-2 and the lipocalin-2/MMP-9 complex are associated with a high lipid content, high numbers of macrophages, high IL-6 and IL-8 levels, and a low smooth muscle cell content in human atherosclerotic lesions (Te Boekhorst *et al.*, 2011). A similar neutrophil lipocalin-2/MMP9 over-expression can be found in atherosclerotic plaques, particularly those with intramural haemorrhagic debris and central necrosis (Hemdahl *et al.*, 2006; Leclercq *et al.*, 2007). Since augmented lipocalin-2 expression is found in atherosclerotic plaques and myocardial infarction, the adipokine may serve as a novel imaging target for the detection of high-risk plaques using high-resolution MR imaging (Te Boekhorst *et al.*, 2011). Furthermore, serum levels of lipocalin-2 in patients with atherosclerosis predict their mortality in a 4-year follow-up study (Anwaar *et al.*, 1998b). Despite this information, the causal role of lipocalin-2 in vascular remodelling and plaque instability during the development of atherosclerosis remains to be established and clarified. Especially, uncontrolled effects of renal disease in atherosclerotic patients need to be further explored and taken into consideration (Giaginis *et al.*, 2010).

Lipocalin-2 and A-FABP as therapeutic targets for obesity-associated CVD

By binding with specific lipids, SLBPs elicit biological functions to facilitate intra-cellular lipid trafficking and signalling, as well as inter-organ communications. They regulate the composition and partitioning of lipids and have a profound impact on cellular metabolism. Under pathological conditions, these lipid chaperones play a key role in inflammatory responses by coupling lipotoxicity to organelle function. The sub-specialized functionality, the structural diversity and the tissue and temporal specificity make SLBPs druggable targets for numerous chronic diseases including obesity, diabetes and atherosclerosis.

CVD is the major causes of mortality and morbidity in ageing population. Together with the obesity pandemic, it imposes a significant burden on our social and healthcare system. Effective strategies for the prevention and treatment of these costly medical complications are urgently needed. Because A-FABP and lipocalin-2 are causally involved in obesity-related vascular dysfunctions, targeting these two SLBPs represents a promising strategy for the treatment of obesity-associated CVD. Indeed, a number of A-FABP inhibitors have already been identified, including carbazole-based and indole-based inhibitors, benzylamino-6-(trifluoromethyl) pyrimidin-4(1*H*) inhibitors and a biphenyl azole inhibitor (also known as BMS309403) (Furuhashi and Hotamisligil, 2008). Among these A-FABP inhibitors, BMS309403 possesses multiple beneficial therapeutic effects in rodent models (Erbay *et al.*, 2007; Furuhashi *et al.*, 2007). This compound interacts with the fatty acid binding pocket within the interior of A-FABP to inhibit binding of endogenous fatty acids (Sulsky *et al.*, 2007). The compound is orally active, potent and highly selective for A-FABP over other isoforms of FABP. In

macrophages, treatment with BMS309403 prevents toxic lipids-induced ER stress, JNK activation, production of pro-inflammatory cytokines and reduces foam cell formation (Hoo *et al.*, 2008; Hui *et al.*, 2010). In animal models, oral administration of BMS309403 improves insulin sensitivity and glucose tolerance associated with both dietary and genetic obesity (Furuhashi *et al.*, 2007). Furthermore, BMS309403 markedly reduced the extent of atherosclerotic lesion in apoE$^{-/-}$ mice (Furuhashi *et al.*, 2007) and also reversed the impairment in endothelial NO production and vasodilatation (Lee *et al.*, 2011). These beneficial effects of BMS309403 are accompanied by inhibition of JNK activity. However, whether such A-FABP inhibitors are effective in humans remains to be determined.

The crystal structure of lipocalin-2 shows distinctive features from those of A-FABP. The size and shape of the lipocalin-2 calyx, as well as the low relative affinity to most of the known ligands of A-FABP, suggest that a complete different chemical or genetic screening approach needs to be adopted. Chemical screens combined with crystallography and fluorescence detection reveal a complex of lipocalin-2 that binds iron together with a small metabolic product called catechol (Bao *et al.*, 2010). The formation of the complex blocks the reactivity of iron, permits its transport in the circulation and facilitates recycling in endosomes. The lipocalin-2-catechol-Fe(III) complex represents an unforeseen endogenous siderophore for iron traffic in aseptic tissues. These results may provide a ligand-defined approach for designing novel inhibitors of lipocalin-2.

Conclusion

Clinical and experimental studies consistently demonstrate that A-FABP and lipocalin-2 exert detrimental effects on endothelial and vascular function. The elevated production of these two SLBPs in obesity contributes to the pathogenesis of endothelial dysfunction, hypertension and atherosclerosis (Figure 1). Both adipokines have been proposed to be useful therapeutic targets for obesity-related vascular diseases. Indeed, pharmacological agents that inhibit A-FABP are effective in treating vascular disease in animals. Despite the promising advances, whether or not the same effects can be produced in larger animals and humans is unknown. Further studies on these two proteins may not only reveal the missing links between adipose tissue and the vasculature but may also bring novel insights to develop therapeutic agents for treating vascular diseases associated with obesity.

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