

# Fatty Acid-Binding Protein 4 in Cardiovascular and Metabolic Diseases

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Fatty acid-binding proteins (FABPs), a family of lipid chaperones, contribute to systemic metabolic regulation via several lipid signaling pathways. Fatty acid-binding protein 4 (FABP4), known as adipocyte FABP (A-FABP) or aP2, is mainly expressed in adipocytes and macrophages and plays important roles in the development of insulin resistance and atherosclerosis in relation to metabolically driven low-grade and chronic inflammation, referred to as 'metaflammation'. FABP4 is secreted from adipocytes in a non-classical pathway associated with lipolysis and acts as an adipokine for the development of insulin resistance and atherosclerosis. Circulating FABP4 levels are associated with several aspects of metabolic syndrome and cardiovascular disease. Ectopic expression and function of FABP4 in cells and tissues are also related to the pathogenesis of several diseases. Pharmacological modification of FABP4 function by specific inhibitors, neutralizing antibodies or antagonists of unidentified receptors would be novel therapeutic strategies for several diseases, including obesity, diabetes mellitus, atherosclerosis and cardiovascular disease. Significant roles of FABP4 as a lipid chaperone in physiological and pathophysiological conditions and the possibility of FABP4 being a therapeutic target for metabolic and cardiovascular diseases are discussed in this review.

**Key words:** Adipocyte, Macrophage, Endothelium, Atherosclerosis, Insulin resistance, Adipokine, aP2, A-FABP, Mal1, E-FABP

## Introduction

Several factors, including genetics, behavior and environment, cause visceral fat accumulation, which is associated with obesity-related metabolic disorders<sup>1, 2)</sup>. In the adipose tissue of obesity, adipocytes and several immune cells, especially macrophages, interact with each other and induce insulin resistance, diabetes mellitus, dyslipidemia and hypertension, leading to the development of atherosclerosis<sup>2)</sup> (Fig. 1). Adipose tissue can secrete several hormonal bioactive molecules called adipokines, including adiponectin, leptin, resistin and fatty acid-binding protein 4 (FABP4)<sup>2)</sup>. It has recently been reported that FABP4, mainly expressed in adipocytes and macrophages, plays significant roles in the development of insulin resistance and atherosclerosis via both intracellular and extracellular effects<sup>3-6)</sup> (Fig. 1).

## Fatty Acid-Binding Proteins (FABPs)

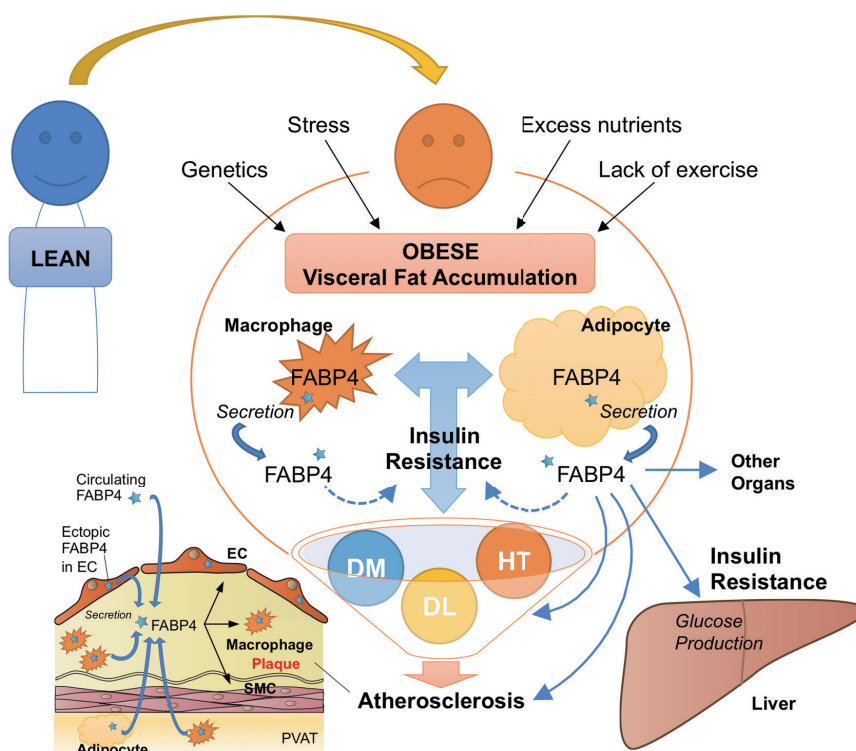
Fatty acid trafficking in cells affects many aspects of cellular function<sup>3)</sup>. Fatty acids act both as an energy source and as signals for metabolic regulations including gene expression, inflammatory and metabolic responses, and growth and survival pathways. Fatty acid-binding proteins (FABPs), a family of intracellular lipid chaperones, regulate lipid trafficking and responses in cells and are linked to metabolic and inflammatory pathways<sup>3-6)</sup>. FABPs are abundantly expressed 14-15-kDa proteins that reversibly bind hydrophobic ligands, such as long-chain fatty acids and other lipids. It has been proposed that FABPs actively facilitate the transport of fatty acids to specific organelles in the cell for lipid oxidation in the mitochondrion or peroxisome, transcriptional regulation in the nucleus, signaling, trafficking and membrane synthesis in the endoplasmic reticulum (ER), and regula-

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**Fig. 1.** FABP4-mediated insulin resistance and atherosclerosis in obesity

Several factors, including genetics, stress, excess nutrients and lack of exercise, induce obesity characterized by visceral fat accumulation. In adipose tissue, adipocytes and several immune cells, especially macrophages, interact with each other and induce insulin resistance, diabetes mellitus (DM), dyslipidemia (DL) and hypertension (HT), leading to the development of atherosclerosis. Fatty acid-binding protein 4 (FABP4), which is mainly expressed in adipocytes and macrophages, plays significant roles in the development of insulin resistance and atherosclerosis via both intracellular and extracellular effects.

EC, endothelial cell; PVAT, perivascular adipose tissue; SMC, smooth muscle cell.

tion of enzyme activity and storage as lipid droplets in the cytoplasm<sup>3)</sup>.

At least 9 different FABP isoforms have been identified. The FABP family includes liver (L-FABP/FABP1), intestinal (I-FABP/FABP2), heart (H-FABP/FABP3), adipocyte (A-FABP/FABP4/aP2), epidermal (E-FABP/FABP5/mal1), ileal (Il-FABP/FABP6), brain (B-FABP/FABP7), myelin (M-FABP/FABP8), and testis (T-FABP/FABP9) isoforms<sup>3)</sup>. FABPs have about 15% to 70% sequence identity between different isoforms and have almost the same three-dimensional structures showing a cap by the helix-loop-helix region and two orthogonal five-stranded  $\beta$ -sheets by a 10-stranded anti-parallel  $\beta$ -barrel structure<sup>3, 7)</sup>. The fatty acid-binding pocket is located inside the  $\beta$ -barrel. The opening of the binding pocket is framed on one side by the N-terminal helix-loop-helix cap domain, and usually one long-chain fatty acid can be bound to the interior cavity of FABPs except for FABP1, which can bind two fatty acids. Each FABP

has different ligand selectivity and binding affinity for fatty acids because of structural differences<sup>3)</sup>.

### Expression of FABP4

Expression of FABP4 is highly induced during adipocyte differentiation and is transcriptionally controlled by peroxisome proliferator-activated receptor (PPAR)  $\gamma$  agonists, fatty acids, insulin and dexamethasone<sup>8-12)</sup>. Expression of FABP4 is also induced during differentiation from monocytes to macrophages and by treatment with lipopolysaccharide (LPS), phorbol 12-myristate 13-acetate, PPAR $\gamma$  agonists, oxidized low-density lipoprotein and advanced glycation end products<sup>13-17)</sup>. Similar to macrophages, monocyte-derived dendritic cells express FABP4 during differentiation<sup>18)</sup>. Conversely, treatment with omega-3 fatty acids<sup>19)</sup> and sitagliptin<sup>20)</sup> decreases FABP4 expression in 3T3-L1 adipocytes. In macrophages, treatment with atorvastatin<sup>21)</sup> and metformin<sup>22)</sup> reduces FABP4

expression. FABP4 also triggers the ubiquitination and subsequent proteasomal degradation of PPAR $\gamma$  and consequently inhibits PPAR $\gamma$ -related functions, thereby providing a negative feedback loop<sup>23</sup>.

The upstream of the 5' flanking region of the mouse FABP4 gene contains a direct repeat-1 (DR-1)-type PPAR response element (PPRE) at -5.3 kb<sup>24, 25</sup>, a glucocorticoid response element (GRE) at -393 to -385 bp<sup>9</sup>, a CCAAT/enhancer-binding protein (C/EBP)  $\alpha$  binding site at -149 to -130 bp<sup>26</sup>, and an activator protein-1 (AP-1) site at -122 to -116 bp<sup>27</sup>. A functionally significant genetic variation at the FABP4 locus in humans, T-87C polymorphism, has been reported to result in decreased FABP4 expression in adipose tissue due to alteration of the C/EBP and reduced transcriptional activity of the FABP4 promoter<sup>28</sup>.

FABP4 is also expressed in capillary and venous, but not arterial, endothelial cells in a normal condition<sup>29</sup>. Treatment with vascular endothelial growth factor (VEGF)-A via VEGF-receptor-2 or basic fibroblast growth factor (bFGF) induces FABP4 expression in endothelial cells<sup>29</sup>, and FABP4 in endothelial cells promotes angiogenesis<sup>30</sup>. Interestingly, cellular senescence and oxidative stress induce FABP4 expression in microvascular endothelial cells<sup>31, 32</sup>. Furthermore, FABP4 is ectopically induced in injured arterial endothelial cells<sup>33, 34</sup>.

### Fatty Acid Affinity of FABP4

In an assay for fatty acid-binding affinity, FABP4 generally had higher affinity and selectivity for long-chain fatty acids than did albumin<sup>35</sup>. Linoleic acid and  $\alpha$ -linolenic acid, essential polyunsaturated fatty acids, had the highest affinity for FABP4 under a basal condition<sup>35</sup>, suggesting that transport of linoleic acid and  $\alpha$ -linolenic acid is a physiological role of FABP4. Under an oxidative condition, the affinity of FABP4 for most of the fatty acids except for palmitic acid was decreased<sup>35</sup>, indicating that palmitic acid, a saturated fatty acid, has relatively high affinity for FABP4 under a specific condition such as obesity-induced oxidative stress.

### Function of FABP4 in the Cell

Similar to other FABPs, FABP4 is thought to carry fatty acids to several organelles in the cell such as the mitochondrion, peroxisome, ER and nucleus (Fig. 2). The primary sequence of FABP4 does not have a typical nuclear localization signal (NLS) or nuclear export signal (NES) as a potential functional domain<sup>3</sup>. However, the NLS and NES could be

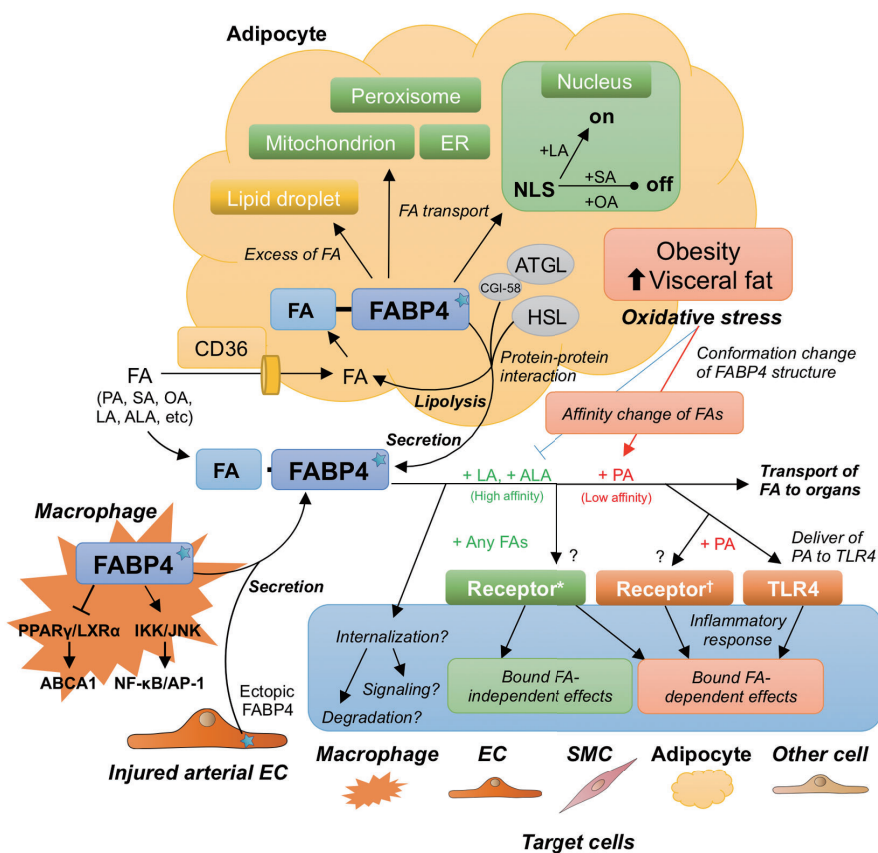
found in the three-dimensional structure of FABP4<sup>36</sup>. The NLS in FABP4 is activated by closure of the portal loop and perturbation of a swinging doorway region<sup>37</sup>. Non-activating ligands, such as oleic acid and stearic acid, protrude from the portal and prevent its closure, leading to masking of the NLS, while activating ligands, such as linoleic acid, troglitazone and anilidonaphthalene sulphonate, expose the NLS<sup>37</sup> (Fig. 2).

The sequence of FABP4 includes a hormone-sensitive lipase (HSL) binding site<sup>38</sup>. A direct protein-protein interaction between FABP4 and HSL in adipocytes regulates lipolysis, and adipocytes in FABP4-deficient mice have decreased lipolysis *in vitro* and *in vivo*<sup>39, 40</sup> (Fig. 2). FABP4 also interacts with comparative gene identification-58 (CGI-58), a potent co-activator of adipose triglyceride lipase (ATGL) that catalyzes the initiating step of intracellular triglyceride hydrolysis<sup>41</sup>. Interestingly, during experimentally induced lipolysis, FABP4-deficient mice have reduced insulin secretion<sup>39</sup>. As metabolic crosstalk between host and pathogen, *Chlamydia pneumoniae*, which needs to obtain nutrients such as ATP and lipids from host cells, infects and proliferates in adipocytes by inducing HSL-mediated lipolysis<sup>42</sup>. Interestingly, *Chlamydia pneumoniae* exploits host FABP4 to facilitate fat mobilization and intracellular replication in adipocytes, suggesting that intracellular pathogens acquire energy via hijacking of the host lipid metabolism pathway<sup>42</sup>.

As another protein-protein interaction, ligand-bound FABP4 binds to Janus kinase 2 (JAK2) and attenuates its signaling<sup>43</sup>. Phosphatase and tensin homolog on chromosome 10 (PTEN), which negatively regulates the phosphoinositide 3-kinase pathway, interacts with FABP4, possibly for regulation of lipid metabolism and adipocyte differentiation<sup>44</sup>. Notably, PTEN-null keratinocytes showed an elevated expression of FABP4, suggesting that PTEN plays a role in the transcriptional regulation of FABP4 expression<sup>45</sup>.

### Phenotype of FABP4 Deficiency

FABP4-deficient mice with high-fat diet-induced and genetic obesity show reduced insulin resistance, but there is no effect of FABP4 on insulin sensitivity in lean mice<sup>46, 47</sup>. Knockdown of the *Fabp4* gene by RNA interference in dietary obese mice increases body weight and fat mass without significant changes in glucose and lipid homeostasis<sup>48</sup>, being similar to the phenotype of FABP4 heterozygous knockout mice on a high-fat diet<sup>46</sup>. The remaining expression of FABP4 might maintain some parts of FABP4 function.



**Fig. 2.** Possible intracellular and extracellular functions of FABP4

FABP4 is abundant in the cytosolic fraction of adipocytes and can bind one long-chain fatty acid (FA), including palmitic acid (PA), stearic acid (SA), oleic acid (OA), linoleic acid (LA) or  $\alpha$ -linolenic acid (ALA). FABP4 facilitates the transport of FAs to specific organelles in the cell such as the mitochondrion, peroxisome, endoplasmic reticulum (ER) and nucleus, regulates enzyme activity, and stores excess FA as lipid droplets in adipocytes. LA-bound, but not SA- or OA-bound, FABP4 can be moved into the nucleus by unmasking of the nuclear localization signal (NLS). The protein-protein interactions of FABP4 with hormone-sensitive lipase (HSL) and comparative gene identification-58 (CGI-58), a potent co-activator of adipose triglyceride lipase (ATGL), regulate intracellular triglyceride hydrolysis, and FABP4 is secreted from adipocytes in a non-classical pathway associated with lipolysis. FABP4 in macrophages inhibits the peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ )-liver X receptor  $\alpha$  (LXR $\alpha$ )-ATP-binding cassette A1 (ABCA1) pathway and induces inflammatory responses through activation of the inhibitor of nuclear kappa B kinase (IKK)-nuclear factor-kappa B (NF- $\kappa$ B) and c-Jun N-terminal kinase (JNK)-activator protein-1 (AP-1) pathways. FABP4 is ectopically induced in injured arterial endothelial cells (ECs). Macrophages and ECs can also secrete FABP4. Secreted FABP4 may act as a carrier of LA and ALA, essential polyunsaturated FAs, to organs because of high affinities for FABP4 under normal conditions. Circulating FABP4 may affect several responses in target cells, including macrophages, ECs, smooth muscle cells (SMCs), adipocytes and other cells, through unidentified receptor-mediated effects in bound FA-dependent and -independent manners and/or possible internalization of FABP4 into the cell. Obesity and increased visceral fat promote oxidative stress. An oxidative stress condition can induce conformation change of the FABP4 structure and decrease affinity of most of the FAs, except for PA, for FABP4. Under a specific condition such as obesity-induced oxidative stress, PA would have relatively high affinity for FABP4, leading to PA-dependent inflammatory responses through unidentified receptors of PA-bound FABP4 and/or delivery of PA to toll-like receptor 4 (TLR4). Receptor\*, an unidentified receptor of non-specific FA-bound FABP4. Receptor†, an unidentified receptor of specific PA-bound FABP4.

FABP4 deficiency protects against atherosclerosis in apolipoprotein E (ApoE)-deficient mice<sup>13, 49</sup>. FABP4 in macrophages increases accumulation of cholesterol ester and foam cell formation via inhibition of the PPAR $\gamma$ -liver X receptor  $\alpha$  (LXR $\alpha$ )-ATP-binding cassette A1 (ABCA1) pathway and induces inflammatory responses through activation of the inhibitor of nuclear kappa B kinase (IKK)-nuclear fac-

tor-kappa B (NF- $\kappa$ B) and c-Jun N-terminal kinase (JNK)-AP-1 pathways<sup>50, 51</sup> (Fig. 2). Furthermore, the lack of FABP4 in macrophages decreases redox signaling and inflammasome activation via upregulation of uncoupling protein 2 (UCP2)<sup>52</sup> and sirtuin 3 (SIRT3)<sup>53</sup>. FABP4 in dendritic cells also regulates the IKK-NF- $\kappa$ B pathway and T cell priming<sup>18</sup>.

## Secretion of FABP4

FABP4 lacks a signal peptide in the N-terminal sequence<sup>3)</sup>, which is necessary for the classical secretory pathway, i.e., ER-Golgi-dependent secretion. However, FABP4 is secreted from adipocytes in a non-classical secretion pathway associated with lipolysis, a series of intracellular triglyceride hydrolysis mediated by ATGL, HSL and monoacylglycerol lipase (MGL)<sup>54-56</sup> (Fig. 2). Secretion of FABP4 is also regulated by an intracellular calcium-dependent pathway<sup>57</sup>. Furthermore, FABP4 is secreted partially by microvesicles derived from adipocytes<sup>55, 58, 59</sup>), an established mechanism for unconventional secretion from adipocytes<sup>60</sup>. However, the release of FABP4 via adipocyte-derived microvesicles is a small fraction and conveys a minor activity<sup>55, 58</sup>). In addition, unconventional secretion of FABP4 by endosomes and secretory lysosomes has recently been reported<sup>61</sup>. It has also been confirmed that FABP4 is secreted from macrophages<sup>35</sup>) and vascular endothelial cells<sup>34</sup>) (Fig. 2), though the predominant contributors of circulating FABP4 are adipocytes rather than macrophages and endothelial cells<sup>54, 62</sup>).

## FABP4 as a Bioactive Molecule

FABP4 secreted from adipocytes, macrophages and endothelial cells may have bioactive effects since direct effects of exogenous FABP4 have been demonstrated in various types of cells. Exogenous FABP4 enhances hepatic glucose production *in vivo* and *in vitro*<sup>54</sup>), induces endoplasmic reticulum stress in HepG2 liver cells<sup>63</sup>), inhibits activation of endothelial nitric oxide synthase (eNOS) in vascular endothelial cells<sup>35</sup>), increases proliferation/migration of vascular smooth muscle cells<sup>35</sup>), decreases cardiomyocyte contraction *in vitro*<sup>58</sup>), potentiated glucose-stimulated insulin secretion in pancreatic  $\beta$  cells<sup>64</sup>), and increases breast cancer cell proliferation<sup>65</sup>).

Obesity and increased visceral fat have been reported to promote oxidative stress<sup>66</sup>). FABP4 prefers to bind linoleic acid and  $\alpha$ -linolenic acid for transport of essential polyunsaturated fatty acids in a normal condition, but the affinity of FABP4 would be changed to prefer binding palmitic acid, a saturated fatty acid, probably via conformation change of FABP4 structure, in a condition of obesity-induced oxidative stress<sup>35</sup>) (Fig. 2). Microarray analysis using macrophages treated with recombinant FABP4 in the presence and absence of palmitic acid demonstrated fatty acid-dependent and -independent effects of exogenous FABP4<sup>35</sup>). Notably, treatment of macrophages with recombinant FABP4 in the presence, but not the absence, of palmitic acid significantly increased

inflammatory responses, including chemokine signaling and TNF $\alpha$ -NF- $\kappa$ B signaling pathways<sup>35</sup>). Furthermore, activation of palmitic acid-dependent inflammatory responses by FABP4 was observed in not only in macrophages but also endothelial cells and vascular smooth muscle cells<sup>35</sup>).

Taken together, circulating FABP4 secreted from adipocytes, macrophages and vascular endothelial cells seems to not only carry fatty acids to organs but also act as a bioactive molecule in several target cells, including macrophages, endothelial cells, smooth muscle cells, adipocytes and other cells (Fig. 2). Because of the presence of fatty acid-dependent and -independent effects in cells, there would be both receptors for non-specific fatty acid-bound FABP4 and receptors for specific fatty acid, especially palmitic acid, -bound FABP4. However, possible receptors of FABP4 have not been identified yet, though there was a report that cytokeratin 1 interacts with FABP4 on the endothelial cell membrane<sup>67</sup>). Palmitic acid-bound FABP4 may just deliver palmitic acid to its receptor, toll-like receptor 4 (TLR4). Furthermore, it has been reported that a part of exogenous FABP4 internalizes into the cell<sup>34</sup>), though whether internalized FABP4 has some functions for signaling or whether internalization is a process of degradation remains unclear (Fig. 2). It has recently been reported that cytokeratin 1 facilitates cellular uptake of exogenous FABP4 in endothelial cells, leading to regulation of oxidative and pro-inflammatory effects<sup>68</sup>). It has also been shown that FABP4 binds to megalin, an endocytic receptor expressed in proximal tubule epithelial cells of the kidney, which plays a major role in reabsorption of proteins filtered through glomeruli<sup>69</sup>).

## Circulating FABP4 Level

The concentration of FABP4 is highest among levels of FABP1-5 under a physiological condition in a general population without medication<sup>70</sup>). FABP4 level is significantly higher in females than in males, possibly due to the larger amount of body fat in females than in males since there is an independent and strong correlation between FABP4 level and adiposity<sup>70, 71</sup>). In addition, androgen may partially contribute to the gender difference in serum FABP4 levels<sup>72</sup>). Circulating FABP4 levels are significantly correlated with norepinephrine levels during exercise testing<sup>73</sup>), being consistent with FABP4 secretion from adipocytes via  $\beta$ -adrenergic-mediated lipolytic mechanisms<sup>54-56</sup>). Loss of body weight by exercise training<sup>74</sup>) and bariatric surgery<sup>75, 76</sup>) induces a significant reduction in FABP4 concentrations.

Gene and protein expression levels of FABP4

have been reported to be higher in subcutaneous adipose tissue than in visceral adipose tissue of both lean and obese subjects<sup>77</sup>), but associations of subcutaneous adipose tissue and visceral adipose tissue assessed by multidetector computed tomography with circulating FABP4 levels were reported to be almost the same<sup>78</sup>). One possible reason for the discrepancy is that visceral adipose tissue is more metabolically active and sensitive to lipolysis than is subcutaneous adipose tissue<sup>79</sup>), leading to the predominant contribution of visceral adipose tissue to the lipolysis-mediated secretion and circulating level of FABP4. The main source of circulating FABP4 is adipocytes rather than macrophages and endothelial cells<sup>54, 62</sup>). However, it has been reported that circulating FABP4 was detected in patients with lipodystrophy despite adipose tissue loss in contrast to other adipokines including leptin and adiponectin<sup>80</sup>).

Multiplex proteomics demonstrated that FABP4 level was strongly associated with kidney function decline over a period of 5 years<sup>81</sup>). Serum FABP4 level was shown to be negatively correlated with estimated glomerular filtration rate<sup>70</sup>), suggesting that FABP4 is eliminated from circulation mainly by renal clearance. It has recently been reported that circulating FABP4 is eliminated by the kidney via glomerular filtration followed by megalin-mediated reabsorption<sup>69</sup>). FABP4 level in hemodialysis patients with end-stage kidney disease was about 20-times higher than that in controls with normal renal function and was decreased by 57.2% after hemodialysis<sup>82</sup>).

Increased circulating FABP4 levels have been shown to be associated with obesity<sup>71</sup>), metabolic syndrome<sup>83</sup>), insulin resistance<sup>70, 84</sup>), type 2 diabetes mellitus<sup>85</sup>), hypertension<sup>86</sup>), dyslipidemia<sup>87</sup>), atherosclerosis<sup>88</sup>), left ventricular diastolic dysfunction<sup>89</sup>) and heart failure<sup>90</sup>) (**Fig. 3**). Xanthine oxidoreductase (XOR), a rate-limiting and catalyzing enzyme of uric acid formation in purine metabolism, is involved in an increase in reactive oxygen species<sup>91</sup>), and plasma XOR activity has been shown to be a novel biomarker of metabolic disorders<sup>92, 93</sup>). A cohort study demonstrated that plasma XOR activity is independently associated with levels of adipokines, including FABP4<sup>94</sup>). Circulating FABP4 is independently associated with the level of PCSK9, which binds to and degrades the low-density lipoprotein (LDL) receptor, suggesting associations with hypercholesterolemia and cardiovascular risk<sup>95</sup>). Furthermore, the basal FABP4 level is independently associated with change in carotid intima-media thickness, a marker of atherosclerosis, per year, indicating that FABP4 level predicts progress of atherosclerosis<sup>96</sup>). It has also been reported that serum FABP4 level predicts long-term cardiovascular events

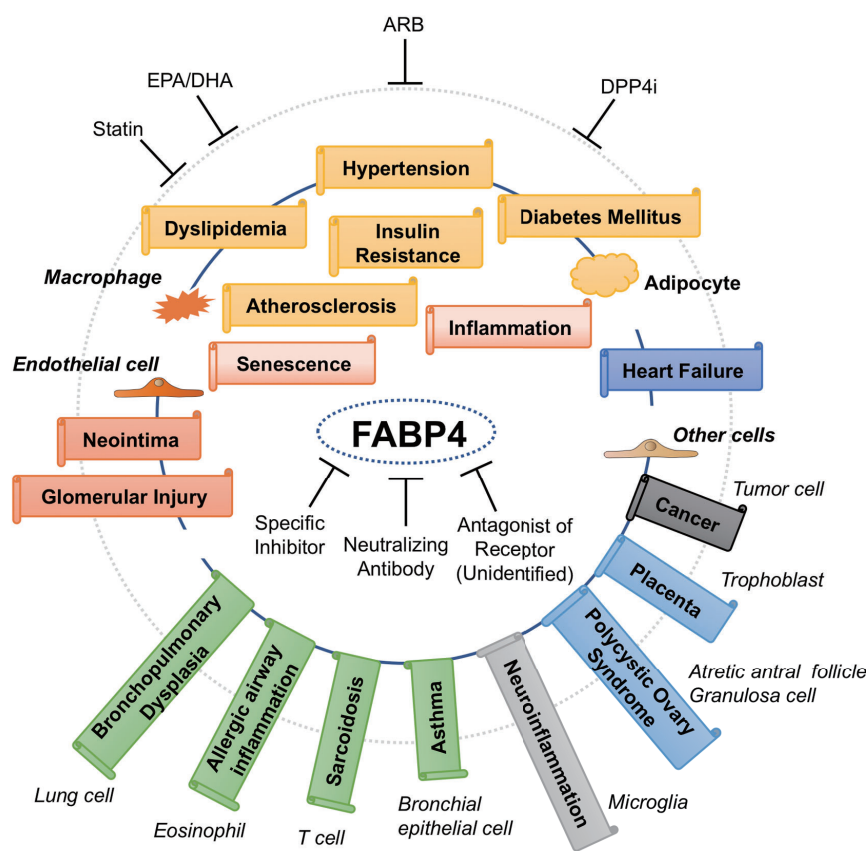
and mortality<sup>82, 97-99</sup>).

Perivascular adipose tissue and epicardial fat have recently been proposed to influence vascular function and the pathogenesis of vascular disease<sup>100, 101</sup>). FABP4 mRNA expression in epicardial adipose tissue is profoundly increased compared with its expression in paraaortic adipose tissue in patients with metabolic syndrome<sup>102</sup>). FABP4, mainly derived from epicardial fat, is locally enriched in the pericardial cavity of cardiovascular disease patients<sup>103</sup>). The coronary veno-arterial difference in FABP4 levels in the aortic root and coronary sinus was shown to be an independent predictor of the severity of coronary stenosis after adjustment of conventional risk factors<sup>35</sup>). FABP4 levels are significantly elevated during the early hours after onset of acute myocardial infarction and are robustly increased in out-of-hospital cardiac arrest survivors, probably due to rapid lipolytic release of FABP4 from epicardial fat by adrenergic overdrive that accompanies acute cardiovascular disease<sup>104</sup>).

Several drugs can modify circulating FABP4 levels (**Fig. 3**). Treatment with atorvastatin<sup>105</sup>), a hydroxymethylglutaryl-CoA reductase inhibitor, several angiotensin II receptor blockers<sup>106</sup>), omega-3 fatty acid ethyl esters containing eicosapentaenoic acid and docosahexaenoic acid<sup>19</sup>), and sitagliptin<sup>20</sup>), a dipeptidyl peptidase-4 inhibitor, reduces FABP4 concentrations. On the other hand, treatment with pioglitazone, a PPAR $\gamma$  agonist known as an insulin-sensitizing thiazolidinedione, increases FABP4 levels<sup>107</sup>), presumably due to direct activation of PPAR $\gamma$  since the FABP4 gene promoter includes the PPRE<sup>24, 25</sup>). Treatment with canagliflozin, a sodium-glucose cotransporter 2 (SGLT2) inhibitor, paradoxically increased serum FABP4 level in some diabetic patients despite amelioration of glucose metabolism and adiposity reduction, possibly via induction of catecholamine-induced lipolysis in adipocytes, and patients in whom FABP4 level was increased by canagliflozin had significantly smaller improvements of insulin resistance and hemoglobin A1c than did patients with decreased FABP4 level<sup>108</sup>). The increased FABP4 induced by PPAR $\gamma$  agonists or SGLT2 inhibitors may act as a carrier of linoleic acid and  $\alpha$ -linolenic acid, as a physiological function. Therefore, it is important to enforce diet therapy for reducing accumulation of visceral fat and to prevent binding of FABP4 to palmitic acid, especially in the treatment with a PPAR $\gamma$  agonist and/or an SGLT2 inhibitor.

### Ectopic Expression of FABP4

FABP4 is expressed in endothelial cells of capillaries and small veins but not arteries under a physio-



**Fig. 3.** Chain of FABP4-associated pathological conditions

FABP4 is expressed not only in adipocytes and macrophages but also in several types of tissues and cells under physiological and pathophysiological conditions and may contribute to several aspects of metabolic and cardiovascular diseases as well as renal, respiratory, neurological, gynecological and oncological diseases. Several drugs, including a statin, eicosatetraenoic acid (EPA)/docosahexaenoic acid (DHA) agent, angiotensin II receptor blocker (ARB) and dipeptidyl peptidase 4 inhibitor (DPP4i), can decrease FABP4 levels. Specific inhibitors and neutralizing antibodies of FABP4 and antagonists of unidentified FABP4 receptors may be candidates of therapeutic strategies for several FABP4-associated diseases.

logical condition<sup>29</sup>). FABP4 in capillary endothelial cells is involved in transendothelial fatty acid transport into fatty acid-consuming organs<sup>109</sup>). FABP4 is ectopically induced in regenerated arterial endothelial cells after endothelial balloon denudation<sup>33</sup> and wire-induced vascular injury<sup>34</sup>. Neointima formation after wire-induced vascular injury is significantly decreased in FABP4-deficient mice compared with that in wild-type mice<sup>34</sup>. Intermittent hypoxia also increases the expression of FABP4 in human aortic endothelial cells<sup>110</sup>). FABP4 is expressed in the aortic endothelium of old, but not young, ApoE-deficient atherosclerotic mice, and chronic treatment with BMS309403, a small molecule FABP4 inhibitor, significantly improves endothelial dysfunction in old ApoE-deficient mice<sup>111</sup>). Both FABP4 and FABP5 are also involved in cellular senescence of vascular endothelial

cells<sup>31, 32</sup>) (Fig. 3). FABP4 secreted from vascular endothelial cells increases gene expression of inflammatory cytokines in cells, promotes proliferation and migration of vascular smooth muscle cells, and decreases phosphorylation of eNOS in vascular endothelial cells, which are attenuated in the presence of an anti-FABP4 antibody<sup>34</sup>). Ectopic expression of FABP4 under a pathological condition, but not physiological expression of FABP4, in the endothelium may contribute to the pathogenesis of atherosclerosis and vascular injury.

In normal kidneys, FABP4 is expressed in endothelial cells of the tubulointerstitial peritubular capillary and vein in both the cortex and medulla, but not in glomerular or arterial endothelial cells, under a normal physiological condition<sup>29</sup>). Ectopic expression of FABP4 in endothelial cells and macrophages of the glomerulus is associated with progression of protein-

uria and renal dysfunction<sup>112)</sup> (**Fig. 3**). It has also been reported that FABP4 is expressed in glomerular mesangial cells of diabetic nephropathy<sup>113)</sup>. Among the FABPs, FABP1 (L-FABP) is expressed in proximal tubular epithelial cells in the kidney, and urinary FABP1 reflects damage of proximal tubular epithelial cells<sup>114)</sup>. Urinary FABP4 has been proposed to be a novel biomarker reflecting glomerular damage<sup>115)</sup>, and determination of both FABP1 and FABP4 in urine may enable better characterization of renal injury.

Evidence for the involvement of FABP4 in several kinds of diseases and conditions has been accumulating<sup>3-6)</sup> (**Fig. 3**). FABP4 has been shown to be expressed in lung and bronchoalveolar cells in bronchopulmonary dysplasia<sup>116)</sup>, lung lavage cells in sarcoidosis<sup>117)</sup>, bronchial epithelial cells<sup>118)</sup> and vascular endothelial cells<sup>119)</sup> in asthma, eosinophils in allergic airway inflammation<sup>120)</sup>, microglia cells in neuroinflammation<sup>121)</sup>, resident macrophages/microglial cells and endothelial cells of the hyaloid vasculature in the immature retina of proliferative retinopathy<sup>122)</sup>, pulmonary artery endothelial cells in pulmonary thromboembolism<sup>123)</sup>, hepatocytes in liver ischemia/reperfusion injury<sup>124)</sup>, human placental trophoblasts during placental development<sup>125)</sup>, and granulosa cells of the ovary in polycystic ovary syndrome<sup>126, 127)</sup>. Furthermore, FABP4 has been detected in human ovarian cancer cells at the adipocyte-tumor cell interface, and FABP4 deficiency in mice substantially impaired metastatic tumor growth<sup>128)</sup>. FABP4 can substantially increase the metastatic potential of ovarian cancer cells<sup>129)</sup>. FABP4 expression has also been detected in lipoblasts of lipoblastoma and liposarcoma<sup>130)</sup>, glioblastoma<sup>131)</sup> and urothelial carcinomas<sup>132)</sup>, breast cancer<sup>133, 134)</sup>, prostate cancer<sup>135)</sup>, hepatocytes<sup>136, 137)</sup> and hepatic stellate cells<sup>138)</sup> in hepatocellular carcinoma, oral and cervical squamous cell carcinoma<sup>139, 140)</sup> and non-small cell lung cancer<sup>141)</sup>.

### FABP4 as a Therapeutic Target

Several series of FABP4 inhibitors have been synthesized<sup>3, 142-144)</sup>. The specific FABP4 inhibitor BMS309403 is an orally active small molecule that interacts with the fatty acid-binding pocket within the interior of FABP4 to inhibit binding of endogenous fatty acids<sup>3, 142, 143)</sup>. Treatment with BMS309403 has been shown to improve insulin resistance, diabetes mellitus, fatty liver disease and atherosclerosis in experimental models<sup>143)</sup>, indicating that chemical inhibition of FABP4 could be a therapeutic strategy against several aspects of metabolic syndrome (**Fig. 3**). Recent studies have also demonstrated that neutralization of secreted FABP4 with an antibody to FABP4

could be a feasible approach for treatment of insulin resistance, type 2 diabetes mellitus and vascular injury<sup>34, 54, 145, 146)</sup> (**Fig. 3**). Furthermore, treatment with antagonists of receptors for FABP4, especially PA-bound FABP4, would be a novel therapeutic strategy, though receptors for FABP4 have not been identified yet.

### FABP5 as an FABP4-Related Lipid Chaperone

FABP5, another FABP known as epidermal FABP (E-FABP), psoriasis-associated FABP (PA-FABP) or mal1, is expressed most abundantly in epidermal cells of the skin but is also present in several tissues and cells including adipocytes<sup>3)</sup>. FABP4 and FABP5 have 52% amino acid similarity and bind to several fatty acids with similar affinity and selectivity. The amount of FABP4 in adipocytes is about 100-fold larger than that of FABP5 in adipocytes<sup>147)</sup>. Both FABP4 and FABP5 are also expressed in macrophages and dendritic cells, though the amount of FABP4 in adipocytes is about 10,000-fold larger than that in macrophages<sup>118)</sup>. The stoichiometry of FABP4 and that of FABP5 are nearly equal in macrophages under physiological conditions<sup>13)</sup>. FABP4 deficiency induces a strong compensatory increase of FABP5 in adipose tissue but not in macrophages or dendritic cells<sup>13, 18, 46)</sup>. Other than adipocytes and macrophages, FABP5 is coexpressed with FABP4 in microvascular endothelial cells in the heart and kidney<sup>29, 109)</sup> and even in endothelial cells of larger blood vessels<sup>148)</sup>. Similar to FABP4, FABP5 facilitates transendothelial transport of fatty acids into fatty acid-consuming organs<sup>109)</sup>.

Expression of FABP5 in macrophages is increased by treatment with toll-like receptor (TLR) agonists: LPS, a TLR4 agonist, and zymosan, a fungal product that activates TLR2<sup>149)</sup>. Expression of FABP5 in endothelial cells is induced by cellular senescence and H<sub>2</sub>O<sub>2</sub>-induced oxidative stress<sup>31, 32)</sup>. In contrast to FABP4, FABP5 is not induced by VEGF-A or bFGF in endothelial cells<sup>148)</sup>. FABP5, similar to FABP4, in endothelial cells promotes angiogenic responses, but FABP5 can also exert opposing effects on endothelial survival, indicating that the balance between FABP4 and FABP5 in endothelial cells may be important for the regulation of angiogenic versus quiescent phenotypes in blood vessels<sup>148)</sup>.

FABP5 transgenic mice in adipose tissue on a high-fat diet show enhanced basal and hormone-stimulated lipolysis and reduced insulin sensitivity<sup>150, 151)</sup>. On the other hand, FABP5 deficiency mildly increases systemic insulin sensitivity in dietary and genetic obesity mouse models<sup>150)</sup>. Ablation of FABP5 suppresses



atherosclerosis in LDL receptor-deficient mice on a western-style hypercholesterolemic diet, and the anti-atherosclerotic effect of FABP5 deletion is associated with reduction of inflammatory response<sup>152</sup>).

Secretome analyses showed that FABP5 is also secreted from cells<sup>62, 111, 153</sup>), though the mechanism remains unclear. Transcriptome and metabolome analyses showed that exogenous FABP4 and FABP5 differentially affect transcriptional and metabolic regulation in adipose-derived stem cells near adipocytes<sup>153</sup>). Circulating FABP5 has been reported to be detected at levels of about one tenth or less of circulating FABP4 concentrations, and FABP5 levels are associated with components of metabolic syndrome, although the correlation is not as strong as that of FABP4<sup>70, 154</sup>). Interestingly, the concentration of FABP5, but not FABP4, is negatively and independently correlated with cholesterol efflux capacity from macrophages, the first step in the reverse cholesterol transport pathway, suggesting a potential biomarker for residual risk of atherosclerosis<sup>155</sup>).

### Phenotype of Combined Deficiency of FABP4 and FABP5

Mice with combined deficiency of FABP4 and FABP5 (*Fabp4*<sup>-/-</sup>*Fabp5*<sup>-/-</sup>) exhibit protection against type 2 diabetes, fatty liver disease and atherosclerosis more than do FABP4- or FABP5-deficient mice<sup>156-158</sup>). The effects of FABP4 and FABP5 on atherosclerosis are mainly due to their actions in macrophages<sup>13, 152</sup>). On the other hand, actions of FABP4 and FABP5 in adipocytes and those in macrophages have distinct roles in regulation of insulin sensitivity through metabolic and inflammatory responses<sup>62</sup>). Calorie restriction prevents age-related metabolic disease and extends life span<sup>159</sup>), and it shares many molecular features in combined deficiency of FABP4 and FABP5, but *Fabp4*<sup>-/-</sup>*Fabp5*<sup>-/-</sup> mice do not have increased longevity<sup>160</sup>), indicating that extension of a metabolically healthy span in the absence of calorie restriction can be uncoupled from lifespan. It has also been demonstrated that *Fabp4*<sup>-/-</sup>*Fabp5*<sup>-/-</sup> mice have defective uptake of fatty acid via capillary endothelial cells of the heart and skeletal muscle with compensatory up-regulation of glucose consumption in those tissues during fasting<sup>161</sup>). Furthermore, *Fabp4*<sup>-/-</sup>*Fabp5*<sup>-/-</sup> mice show impaired thermogenesis after cold exposure during fasting<sup>162</sup>).

Lipidomic analyses showed increased *de novo* lipogenesis by induction of stearoyl-CoA desaturase-1 (SCD-1) and fatty acid synthase in adipose tissue of *Fabp4*<sup>-/-</sup>*Fabp5*<sup>-/-</sup> mice, leading to identification of increased palmitoleate (C16:1n7), an unsaturated free

fatty acid, as an adipose tissue-derived lipid hormone, referred to as 'lipokine', that can decrease fatty liver and increase glucose uptake in skeletal muscle<sup>163</sup>). Deletion of FABP4 in macrophages also increases *de novo* lipogenesis pathways through LXR $\alpha$ -mediated SCD-1 activation, resulting in production of palmitoleate and resistance to ER stress<sup>164</sup>). Unsaturated fatty acids including palmitoleate modulate histone deacetylation, resulting in decreased basal and LPS-induced expression levels of FABP4 in macrophages<sup>165</sup>). Treatment with palmitoleate prevents atherosclerosis in ApoE-deficient mice in relation to reduced ER stress and inflammasome activation<sup>166</sup>). In a human study, palmitoleate level was positively correlated with insulin sensitivity assessed by euglycemic-hyperinsulinemic clamp studies after adjustment of age, gender and adiposity<sup>167</sup>). The level of the *trans* isomer of palmitoleate, an exogenous source of C16:1n7, was associated with lower insulin resistance and lower incidence of diabetes mellitus<sup>168</sup>).

### Perspectives and Conclusion

FABP4 is mainly expressed in adipocytes and macrophages and plays important roles in the development of insulin resistance and atherosclerosis in relation to metabolically driven low-grade and chronic inflammation, referred to as 'metaflammation'<sup>2</sup>) (Fig. 1). FABP4 is involved in the regulation of inflammatory and metabolic processes in target cells (Fig. 2). The presence of FABP4 in cells may be beneficial for storing energy in adipocytes, for acting on an immune response in macrophages against pathogens, and for trafficking of fatty acids in capillary endothelial cells. Additionally, secreted FABP4 in association with lipolysis during fasting may regulate hepatic glucose production for survival in a famine. In the contemporary life-style with excessive caloric intake and decreased energy expenditure, the presence and induction of FABP4 or enhanced secretion of conformation-changed FABP4, which can bind to palmitic acid with a relatively high affinity<sup>35</sup>), may be rather disadvantageous for regulating inflammatory or metabolic homeostasis. In such conditions, inhibition of FABP4, neutralization/elimination of secreted FABP4 or the use of possible antagonists for unidentified receptors of FABP4 could be an effective therapeutic strategy against metabolic and cardiovascular diseases and possibly other diseases (Fig. 3). Further studies are obviously needed to investigate whether chemical or other types of inhibition/neutralization of FABP4 and blocking receptors of FABP4 can be safely used in humans and to show the efficacy of agents for metabolic and cardiovascular diseases. Furthermore, other

than FABP4, several types of FABP inhibitors have been identified<sup>169, 170</sup>, but there have been few detailed examinations using those inhibitors in *in vivo* and *in vitro* studies. Not only FABP4 but also other FABPs, including FABP5, may offer targeting opportunities as a class for prevention or treatment of other diseases. Much work is still needed to determine the precise applications and indications for other isoforms.

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### Conflict of Interest

None.

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