Pathological Implication of Adipocytes in AAA Development and the Rupture

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Abdominal aortic aneurysm (AAA) is a vascular disease that involves the gradual dilation of the abdominal aorta followed by its rupture. AAA is closely associated with weakening of the vascular wall due to oxidative stress, chronic inflammation, and degradation of the extracellular matrix. No effective drug therapy is currently available for preventing aneurysm progression or rupture. Adipocytes in the vascular wall are reportedly closely associated with AAA development and rupture. Fiber degradation in the aneurysm wall is enhanced by increased numbers of adipocytes, and rupture risk may increase as well. Recent studies suggested that appropriate control of adipocytes in the vascular wall may be an important strategy to prevent AAA rupture, and further studies may aid in the establishment of a method for preventing AAA rupture by therapeutic drugs or functional foods. In this review, we summarize adipocyte function and the correlation between AAA and adipocytes.

Keywords: abdominal aortic aneurysm, adipocyte, triglyceride, diet, fish oil

Introduction

Abdominal aortic aneurysm (AAA) is a lethal disease that involves the gradual dilation of the abdominal aorta, followed by rupture of the formed aneurysm. No current drug therapy can prevent aneurysm progression or rupture. The detailed mechanisms of AAA development and rupture are not fully understood. We recently demonstrated that the abnormal appearance of adipocytes in the vascular wall is strongly associated with AAA progression and rupture.1,2

The primary roles of adipocytes are storing excess energy as triglycerides (TGs) and supplying fatty acid and glycerol for other organs when needed. Adipocytes secrete biologically active substances called adipocytokines that are vital in glucose and lipid homeostasis. Excess fat accumulation in the adipose tissue causes the aberrant production of adipocytokines and causes risk factors for metabolic diseases and cardiovascular disease in the systemic tissues.3 However, the correlation between adipocytes and AAA is poorly understood. In this review, we describe the basic function and differentiation of adipocytes, their relevance to AAA, and the correlation between dietary habits and AAA.

Ectopic Fat and Diseases

Ectopic fat and diseases

TG is mainly stored in the adipose tissue, and it has been established that ectopic fat deposition is associated with various diseases. Ectopic fat is defined as excess TG accumulation in non-adipose tissue that normally contains little TG, e.g., liver, skeletal muscle, pancreas, pericardial tissue, and perivascular tissue.4 Ectopic TG accumulation in the liver is associated with non-alcoholic fatty liver disease.5 Intracellular TG accumulation in the muscle contributes to diabetes due to a decreased uptake of glucose through insulin secretion impairment.6 It has also been reported that perivascular ectopic fat accumulation causes atherosclerosis by directly affecting secreted substances from the perivascular adipose tissue into the vascular lumen.7 The perivascular adipose tissue produces cytokines, such as monocyte chemotactic protein-1 (MCP-1), that directly promote atherosclerotic plaque formation in the arterial wall.8 In particular, pericardial adipose tissue causes atherosclerosis in the coronary arteries, thereby causing risk factors for angina pectoris and myocardial
The perivascular adipose tissue is reportedly associated with AAA as described in Perivascular adipose tissue and AAA.

Method for detecting TG in the vascular wall
Principle of matrix-assisted laser desorption ionization mass spectrometry imaging
In the evaluation of the biological implications of ectopic fat in various tissues, it is important to identify the distribution of ectopic TG accumulation. However, it is difficult to visualize the TG distribution in the tissues using clinical examination or histological staining because these conventional methods do not distinguish among various types of lipids, such as TGs, cholesterol esters, and phospholipids. At this time, mass spectrometry imaging (MSI) is the most suitable method for the simultaneous visualization of several lipid species in tissue sections. Among the several ionization methods, matrix-assisted laser desorption ionization (MALDI) is the most widely used method to visualize the distribution of lipid species in tissue sections. In this method, the matrix molecules absorb the laser energy and facilitate desorption and ionization of analyte molecules in the tissue. MALDI-MSI is a two-dimensional MALDI-MS technique used to visualize the spatial distribution of molecules in tissue sections. The image of the distribution of molecules of interest is described based on the ion intensity of m/z at each measurement point.

In the MALDI-MSI procedure, matrix is firstly sprayed onto a tissue section to form the uniform refined matrix crystals on the tissue section. Next, the laser is radiated to create the ion of biomolecules in the section. After laser irradiation is delivered to the region of interest, the distribution of interest m/z in tissue section can be visualized based on the signal intensity at each measuring point using imaging software.

Detection of ectopic TG by MALDI-MSI
Ectopic TG is characteristically distributed in the vascular walls of the coronary arteries, abdominal aorta, and peripheral arteries. It is well known that the abnormal metabolism of cholesterol is associated with the development of atherosclerosis; on the contrary, the correlation between TG in the vascular wall and vascular diseases is not fully understood. Hirano and our group reported a novel clinical entity named triglyceride deposit cardiomyovasculopathy (TGCV), wherein the accumulated lipid is TG rather than cholesterol ester in atherosclerotic lesions. Primary TGCV is a lethal disease that develops due to a congenital mutation of adipose triglyceride lipase (ATGL). In a subsequent study, we revealed the occurrence of a TGCV-like phenotype in patients without ATGL mutation and named it idiopathic TGCV. MALDI-MSI revealed that abnormal TG accumulation is mainly observed in vascular smooth muscle cells (SMCs) in both primary and idiopathic TGCV. These studies suggest that abnormal TG metabolism in SMCs of the coronary artery could be involved in cardiac failure.

The characteristic distribution of TG in the vascular wall was also observed in the abdominal aorta. In cases of human AAA, MALDI-MSI revealed that TG is characteristically distributed in the adventitial wall, not in the perivascular adipose tissue. A subsequent study revealed that TG in the adventitial wall is derived from adipocytes and suggested that the abnormal appearance of adipocytes in the vascular wall is associated with the development of AAA. The pathological implication of adipocytes in the adventitia is discussed below.

Adipocytes and AAA
Function and differentiation
There are two main kinds of adipose tissue: white adipose tissue (WAT) and brown adipose tissue (BAT). BAT, as a thermogenic tissue, contributes to energy expenditure. Brown fat cells have a unique protein called uncoupling protein 1 (UCP1) that dissipates the proton-motive force that is normally used to produce cellular adenosine triphosphate. Consequently, the energy in the mitochondrial electrochemical gradient is converted into heat. WAT plays a role in the storage of excess energy as TG. In addition, WAT plays a role in regulation of the systemic immune system and energy metabolism through the secretion of various biologically active substances called adipocytokines, such as adiponectin, MCP-1, tumor necrosis factor-α (TNF-α), and resistin. Lipid droplet-accumulated hypertrophic adipocytes due to obesity increase the synthesis and secretion of MCP-1, TNF-α, and resistin and oppositely decrease adiponectin secretion. Excess energy intake and lack of exercise cause obesity and metabolic diseases, which are related to the progression of atherosclerosis.

Adipocytes are derived from mesenchymal stem cells (MSCs), multipotent stromal cells that can differentiate into a variety of cell types, including adipocytes, chondrocytes, and osteoblasts, and have self-renewing abilities. Various factors are reportedly associated with the differentiation process from MSCs to adipocytes. CCAAT/enhancer-binding protein (C/EBP) β and C/EBPδ are highly expressed in preadipocytes in the early stage of induction during their differentiation into adipocytes; subsequently, peroxisome proliferator-activated receptor (PPAR)γ expression is induced. During the later stage of differentiation, C/EBPα expression is induced and C/EBPα interacts with PPARγ. C/EBPs and PPARγ are master regulators of adipocyte differentiation. C/EBPα is an essential tran-
Adipocyte and AAA transcriptional factor that increases insulin sensitivity in adipocytes. C/EBPα and PPARγ induce fatty acid synthase and sterol regulatory element-binding protein-1, which are involved in adipocyte growth.

In addition to C/EBPs and PPARγ, downstream factors such as hormones and cytokines are associated with the regulation of adipocyte differentiation. For example, it has been reported that insulin-like growth factor I, insulin, and Akt protein are promoting factors, whereas interleukin (IL)-1, TNF-α, and p42/p44 MAP kinase are inhibitory factors in adipocyte differentiation. Krüppel-like factors (KLFs), a large family of C2H2 zinc-finger transcription factors that regulate the apoptosis, proliferation, and differentiation of various cells, are reportedly associated with the adipocyte differentiation process. For example, KLF15 promotes adipocyte differentiation and induces expression of the insulin-sensitive GLUT-4. KLF5 is induced in the early stage of adipocyte differentiation by C/EBPβ and C/EBPδ and activates the Pparg2 promoter, functioning in concert with the C/EBPs. In addition, a recent study reported that the actin cytoskeleton dynamics induce adipocyte differentiation. In this report, PPARγ transcription is promoted by the nuclear export but not the nuclear import of megakaryoblastic leukemia 1. However, the cascade of adipocyte differentiation-related transcriptional factors is not fully understood, and unknown factors appear to be involved in the mechanisms underlying adipocyte differentiation.

Development of novel AAA animal model
The histopathology of AAA is characterized by the infiltration of inflammatory cells and degradation of vascular fibers such as elastin and collagen. Inflammatory cells, such as monocytes, macrophages, and neutrophils, release inflammatory cytokines such as MCP-1, TNF-α, and IL-6 that activate matrix metalloproteinases (MMPs) that degrade the extracellular matrix. Next, increased MMP proteins disrupt the elastin and collagen fibers that play an important role in maintaining vascular wall integrity and elasticity. In particular, MMP-2 and MMP-9 activation is especially associated with aneurysm formation via elastin and collagen degradation.

We previously evaluated the content and distribution of heme B, a blood marker, in the vascular wall of human AAA surgical specimens using MALDI-MSI. As a result, a significant reduction in heme B content in lesion sites of the vascular wall was observed compared to normal sites. These results suggested that blood flow in the vascular wall is decreased in the region with aneurysmal formation. Next, a histopathological analysis revealed that the adventitial vasa vasorum (VV) wall of the AAA sac (dilated area) was obstructed (Figs. 1A and 1B). The luminal area of the VV in the sac adventitia was significantly smaller than that in the non-dilated neck adventitia (Fig. 1C). These results suggest that VV obstruction causes hypoperfusion of the AAA wall. Next, by developing the artificial vascular hypoperfusion-induced animal model, we demonstrated that hypoperfusion of the vascular wall causes the development of AAA. The vascular wall of the formed AAA in the model showed inflammatory cell infiltration, accelerated MMP expression, elastin and collagen fiber destruction, and intraluminal thrombus in concordance with the pathological features of human AAA. Because this model has artificially induced hypoperfusion in the vascular wall to form AAA, we named it the hypoperfusion-induced AAA model. Of note, the model shows not only aortic dilation but also spontaneous AAA rupture. Our data suggested that hypoxia and/or under-
nutrition in the tissue due to hypoperfusion by adventitial VV arteriosclerosis can cause AAA formation and rupture.

The induction of artificial hypoperfusion for the abdominal aortic wall in a rat is shown in Fig. 2. First, the infra-renal aorta was exfoliated from the perivascular tissue (Fig. 2A), then vessels branching from the abdominal aorta were ligated with a 5-0 silk string to block the blood supply (Fig. 2B). The abdominal aorta was ligated with a 5-0 silk string just below the renal artery and just above the bifurcation of the aorta to block the aortic blood flow (Fig. 2C). A plastic catheter, shortened to 8–10 mm in length, was inserted via a small incision adjacent to the renal artery branches (Fig. 2D). The incision was then repaired with a 6-0 monofilament string and the abdominal aorta was ligated with a 5-0 silk string together with the plastic catheter (Fig. 2E). Finally, the 5-0 silk string that blocked the blood in the aorta was untied and blood flow was initiated again (Fig. 2F). Using this treatment, hypoperfusion is induced in the vascular wall only while blood flow is maintained in the vascular lumen. About 4 weeks after the abdominal aortic wall ligation, aneurysm formation was observed (Fig. 2G). The AAA rupture rate was about 10% under normal diet condition versus about 40% under a high-fat diet condition (Fig. 2H). In both cases, aneurysm rupture was observed about 2 weeks after the abdominal aortic wall ligation.

Pathological implication of the abnormal appearance of adipocytes in the AAA wall

We previously reported that adipocytes abnormally appear in the AAA wall in humans and a hypoperfusion-induced AAA model (Fig. 3). We demonstrated that the number of adipocytes in the adventitial wall of the ruptured wall was significantly increased compared to the non-ruptured AAA wall using the hypoperfusion-induced AAA model (Fig. 4). The collagen fiber around vascular adipocytes was significantly decreased compared with the area without adipocytes, while the inflammatory cell in-
filtration and MMP levels were increased around the adipocytes compared to the area without adipocytes. Many adipocytes were observed along the tear in the adventitial wall of the ruptured wall in a hypoperfusion-induced AAA model. Interestingly, the amount of TG in the adventitia of the human AAA wall was correlated with AAA diameter. These data strongly suggested that the presence of adipocytes in the vascular wall is associated with AAA development and/or rupture.

**Figure 5** shows our hypothesis of the mechanism underlying abnormal-appearing adipocyte-related AAA rupture. Stenosis of the VV occurs in the vascular wall and triggers the degradation of vascular fibers with inflammation and abnormal adipocyte appearance. Because hypertrophic adipocytes cause chronic inflammation in the tissue by inducing the secretion of biologically active substances such as MCP-1, MMP-2, and MMP-9, it is possible that these adipocytokines secreted by adipocytes are associated with vascular wall weakness. Namely, the increased numbers of inflammatory hypertrophic adipocytes and recruited inflammatory cells increase the MMP-2 and MMP-9 levels and the consequent destruction of collagen fibers surrounding the adipocytes. In this hypothesis, vulnerable areas are developed in the aneurysm wall by the increased number of adipocytes. In other words, rupture risk may increase as the number of adipocytes increases in the vascular wall. Recently, Doderer et al. reported that adipocytes were characteristically observed in the AAA wall by the comparative pathological analysis of AAA and popliteal artery aneurysm, the latter of which rarely ruptures. Krueger et al. reported the increased expression of proteolytic and inflammatory factors around the adipocytes in the adventitia in human AAA patients. The findings of their report support our hypothesis that adipocytes in the vascular wall can induce AAA rupture.

There are many unknown points surrounding the mechanism underlying the appearance of adipocytes and its pathological implication in AAA. Preexisting AAA model animals do not demonstrate the abnormal appearance of adipocytes. The hypoperfusion-induced AAA model is the only model that features adipocytes in the vascular wall. This model may play a complementary role in studying the black box in AAA development.

**Perivascular adipose tissue and AAA**

Inflammatory cell infiltration in perivascular adipose tissue was reported in several experimental AAA animal models. Li et al. reported that endothelin-1 plays a role in AAA formation by increasing inflammatory cell infiltration and MMP-2 in the perivascular fat. Blomkalns et al. reported that CD 14 induces adventitial macrophage precursor recruitment and that perivascular adipose tissue is associated with CD 14 expression. Sakaue et al. demonstrated that angiotensin II type 1a receptor in perivas-
Fig. 4 Adipocyte accumulation in the vascular wall of a hypoperfusion-induced model. (A–F) Representative images of hematoxylin–eosin staining (A, C, D, F: scale bar=200 µm; B, E: scale bar=500 µm). (G–L) Representative images of Oil Red O staining (G, J: scale bar=100 µm; H, K: scale bar=500 µm; I, L: scale bar=200 µm). The square area in the middle panels is magnified in the bottom panels. (M) Quantification of adipocyte number in the non-ruptured and ruptured groups. (N) Quantification of adipocyte-containing area in the non-ruptured and ruptured groups. *P<0.01 versus non-ruptured group. N.D.=not detected. Reproduced from a figure previously published in Sci. Rep. Reprinted by permission from Macmillan Publishers Ltd. Copyright 2016.

Fig. 5 Proposed mechanism of abdominal aortic aneurysm (AAA) rupture. Hypoperfusion in the aortic wall due to adventitial vasa vasorum arteriosclerosis may cause the appearance of adipocytes in the vascular wall. The recruitment of macrophages around adipocytes may increase matrix metalloproteinase (MMP)-2 and MMP-9 levels and degrade collagen fibers surrounding them. The weak vascular wall around adipocytes could increase the susceptibility to AAA rupture. Reproduced from a figure previously published in J. Oleo Sci. with slight modification. Permission is obtained from Japan Oil Chemists’ Society. Copyright 2017.
cicular adipose tissue is involved in aneurysm formation.\textsuperscript{43} Inflammatory cells are observed in both human perivascular adipose tissue-adjacent AAA and experimental AAA animal models.\textsuperscript{44} In the Framingham Offspring and Third Generation cohorts, periaortic fat volume was reportedly associated with dimension in the thoracic and abdominal aortae.\textsuperscript{45} These studies strongly suggested that perivascular adipose tissue plays a role in AAA development. Thus, it is of interest to clarify the relationship between perivascular adipose tissue and adipocytes in the AAA wall.

**Serum TG levels and AAA**

TG levels in the adventitia but not the intima and media are reportedly correlated with AAA diameter in humans.\textsuperscript{1} Increases in TG levels in the adventitia strongly suggest an increased number of adipocytes or their hypertrophy in the adventitia. The mortality risk of AAA rupture is reportedly correlated with serum TG levels in clinical research.\textsuperscript{46,47} Increased serum TG levels might induce adipocyte hypertrophy in the vascular wall. However, we reported that aortic diameter was not correlated with serum TG levels in AAA patients and a hypoperfusion-induced AAA model.\textsuperscript{1,2} Further studies are needed to validate the correlation between serum TG levels and AAA.

**Diet and AAA**

Although it is important to evaluate the effects of dietary factors on AAA development for preventing rupture, little is known about it. Some groups recently reported that differences in lipid nutrition could be associated with AAA development and rupture. This review is focused on the correlation between lipid nutrition and AAA development.

**Fatty acid species and their biological functions**

It is well established that an excess fat intake causes cardiovascular and metabolic diseases. However, the effect of fat on diseases differs between fatty acid species that are the major components of fat. Fatty acids are classified into saturated acids, monounsaturated acids, and polyunsaturated acids (PUFAs) based on the number of unsaturated bonds. PUFAs are further classified into n-3 PUFA and n-6 PUFA according to the position of the double bond. Both n-3 PUFA and n-6 PUFA are essential fatty acids that cannot be synthesized by the body. Representative n-3 PUFAs are α-linolenic acid (18:3 n-3), eicosapentaenoic acid (EPA) (20:5 n-3), and docosahexaenoic acid (DHA) (22:6 n-3), while representative n-6 PUFAs include linoleic acid (18:2 n-6), γ-linolenic acid (18:3 n-6), and arachidonic acid (20:4 n-6). An epidemiology study revealed that Greenlanders who take fish oils, which are rich in n-3 PUFAs, had a low incidence of atherosclerosis, cerebral infarction, and myocardial infarction compared with Danish, who take almost the same amount of saturated fatty acid.\textsuperscript{48} The epidemiology study led to a wide range of studies on the bioactivities (e.g., anti-inflammatory and hypolipidemic) of n-3 PUFAs. Eicosanoids derived from n-3 PUFAs reportedly have anti-inflammatory effects,\textsuperscript{49} and n-3 PUFAs suppress de novo TG synthesis as antagonists of liver X receptor α.\textsuperscript{50,51} Due to their preferable bioactivities, n-3 PUFAs are widely used as supplements or drugs.

**Effect of fatty acid nutrition on AAA development and rupture**

The administration of triolein, a kind of TG that consists of monounsaturated acid and oleic acid, increased the hypertrophic adipocyte accumulation in the vascular wall and the AAA rupture rate in hypoperfusion-induced AAA model rats.\textsuperscript{1} In addition, the administration of a high fat diet that is rich in saturated acids also increased hypertrophic adipocyte accumulation in the vascular wall and the AAA rupture rate.\textsuperscript{34} These results suggested that an excess fat intake causes AAA development. In contrast, the administration of fish oil, which contains the other kind of TG, significantly decreased adipocyte numbers and sizes in the vascular wall and the rupture risk compared to triolein administration.\textsuperscript{1} A pathological analysis showed that macrophage infiltration, protein expressions of MMP-2 and MMP-9, and collagen degradation were significantly suppressed in the area with adipocytes in the vascular wall in the fish oil-administered group compared with those of the triolein-administered group.\textsuperscript{31} These results can be attributed to the suppressive effect of TG synthesis and the anti-inflammatory effect of n-3 PUFAs in fish oil. In addition, the suppressive effect of n-3 PUFAs on the development of AAA was reported in other AAA animal models.\textsuperscript{52–55} Wang et al. reported that EPA treatment attenuates CaCl\textsubscript{2}-induced AAA formation and aortic calcification.\textsuperscript{52} Yoshihara et al. reported that EPA and DHA suppress angiotensin II infusion-induced AAA development induced in apolipoprotein E-deficient (Apoe\textsuperscript{−/−}) mice.\textsuperscript{53} We reported that EPA-rich fish oil prevents the AAA development induced by hypoperfusion of the vascular wall.\textsuperscript{54} Kamata et al. reported that EPA attenuates CaCl\textsubscript{2}-induced AAA development by activating Gpr-120/Ffar-4 in Osteoprotegerin knockout mice.\textsuperscript{55} Pope et al. reported that resolving DHA metabolites has a suppressive effect on the development of murine AAA by inducing M2 macrophage polarization.\textsuperscript{56,57} N-3 PUFAs prevented AAA development and rupture in experimental animal models by multiple mechanisms.\textsuperscript{53,58,59} Aikawa et al. recently reported a negative correlation between serum EPA level and AAA diameter in AAA patients.\textsuperscript{60} Because it was a cross-sectional survey, the causal relationship among these factors remains unknown. However, that study showed...
rupture. Wang et al. reported that nicotine infusion in smoking increases the incidence of aneurysm growth and matrix. We reported that the oral administration of leads to degradation and weakening of the extracellular is related to MMP-2 expression due to nicotine, which activating AMP-activated protein kinase by acti-

Effect of fish oil on nicotine-induced elastin degradation
Several population-based studies have established that smoking is an independent risk factor for AAA. Most AAA patients have a history of smoking, and continued smoking increases the incidence of aneurysm growth and rupture. Wang et al. reported that nicotine infusion in Apoe−/− mice induced AAA. They also demonstrated that the phosphorylation of activator protein 2α by activating AMP-activated protein kinase α2 in vascular SMCs is related to MMP-2 expression due to nicotine, which leads to degradation and weakening of the extracellular matrix. We reported that the oral administration of nicotine increases MMP activity and elastin and collagen fiber degradation in the rat abdominal aortic wall. We recently reported that dietary EPA-rich fish oil can suppress elastin fiber degradation in nicotine-administered mice by suppressing oxidative stress and MMP-12 expression in the vascular wall.

Conclusion
In this review, we focused on adipocytes and AAA. From the standpoint of AAA development and rupture, adipocytes in the vascular wall and not in the visceral fat seem important. Abnormal adipocyte accumulation in the vascular wall could be a novel target for preventing AAA development and rupture. Further studies may lead to the establishment of a therapeutic drug or functional food method for preventing aneurysmal rupture.

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References
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