Abstract: In atherosclerotic disease, multiple risk factor syndrome characterized by abnormal glucose tolerance, hypercholesterolemia, hypertension, etc., has drawn increasing attention. We show that the accumulation of intraperitoneal visceral fat is the most important factor in the onset of multiple risk factor syndrome and demonstrate the concept of “visceral fat syndrome.” To clarify the molecular mechanism, we studied the biological characteristics of adipocytes and found that it acts to store excess energy as conventionally believed and excretes diverse physiologically active substances such as cytokines, growth factors, compliments etc. PAI-1, related to thrombus formation, was actively synthesized in visceral fat. When visceral fat accumulated, PAI-1 was excreted into the blood and operated to induce factors of atherosclerotic disease. We discovered adiponectin, a collagen-like protein, and showed that it prevents diabetes and atherosclerosis onset. Adiponectin is also shown to markedly decrease in the blood in the obese subjects especially with visceral fat accumulation. We showed that excess and decreased excretion of adipocytokines associated with adipocyte accumulation plays an important role in the onset of multiple risk factor syndrome. We therefore propose an adipocentric hypothesis that places adipocytes at the center of the mechanism underlying common diseases.

Key words: Adipocytokine; Adiponectin; Aquaporin adipose; Adipocentric hypothesis

Introduction

The pathogenesis of atherosclerotic diseases, which are leading causes of death in the Japanese population, involves many factors and is extremely complex. Epidemiological studies have been conducted for many years to identify the risk factors for this group of diseases and to...
clarify the mechanisms involved in the onset and progression of vascular lesions associated with atherosclerotic diseases. To date, elevated serum cholesterol was believed to be the most important risk factor. The significance of increased levels of low-density lipoprotein (LDL), which transports cholesterol, has also been demonstrated. It would not be an exaggeration to state that phenomena that underlie the onset and progression of atherosclerosis, such as expression of adhesion factors on the surface of endothelial cells, migration and proliferation of smooth muscle cells, and cholesterol accumulation in macrophages, have all been clarified primarily through studies focusing on oxidative LDL.

However, studies conducted in Japan to analyze the background of patients with atherosclerotic diseases revealed that there are not many cases in which hypercholesterolemia was found as the only factor related to the disease. More often, a combination of relatively weak risk factors, e.g., hypertriglyceridemia, abnormal glucose tolerance, and hypertension, plays a crucial role in the onset of atherosclerotic diseases, such as myocardial infarction, cerebral infarction, etc. For example, in the study conducted by the Ministry of Labor study group (composed of the author and other investigators), which addressed the host factors involved in the pathogenesis of work-related atherosclerosis, it was found that the risk of development of ischemic heart disease was 30- or more times higher in individuals having three or four of the risk factors, namely, obesity, abnormal glucose tolerance, hypertriglyceridemia, and hypertension, as compared to individuals without any of the four risk factors.3

Visceral Fat Accumulation Underlies the Development of the Multiple Risk Factors

The significance of the multiple risk factor syndrome in the onset of atherosclerotic diseases has been attracting close attention in Western countries. This syndrome is also called syndrome X, or the death quartet (upper body obesity, diabetes mellitus, hypertriglyceridemia, and hypertension). However, the exact nature of this syndrome, and the mechanism underlying the onset and progression of atherosclerosis associated with this syndrome have not yet been fully clarified.2

Based on the unsupported knowledge that insulin resistance probably underlies its development, syndrome X is sometimes referred to as insulin resistance syndrome. To date, however, it has not yet been established whether insulin resistance, i.e., resistance of cells to the insulin effects, or compensatory hyperinsulinemia in insulin resistant state is responsible for the onset of the multiple risk factors and the development of atherosclerotic vascular lesions. Even assuming that insulin resistance plays an important role in the development of this syndrome, it remains unknown as to how such resistance develops in the first place.

We have been studying the mechanism of development of diseases associated with obesity. Our study was motivated by the finding that while some individuals weighing even up to 200 kg do not show any signs of diabetes mellitus or hyperlipidemia, in others, diabetes mellitus or hyperlipidemia either develop or deteriorate following a body weight increase of only one kg. We considered that it is of crucial importance to explain this difference. In 1983, we reported for the first time, a method of analyzing the volume of adipose tissue in vivo using CT scans. This technique does not only allow accurate measurement of the body fat volume (the initial goal of this study), but also the quantification of fat tissue in body lumens.3 Our study revealed that the body fat volume determined using this technique is not a superior index for morbidity to the conventional BMI (body mass index) based on body weight. On the other hand, analysis of the fat distribution, in particular, of intraabdominal visceral fat accumulation, has demonstrated that visceral fat accumulation is closely related to the patho-
VISCERAL FAT SYNDROME

Table 1 lists the diseases associated with visceral fat accumulation, as reported by the author and co-workers to date. Considering that accumulation of visceral fat forms the basis for the development of various risk factors for atherosclerotic diseases (including insulin resistance), the presence of multiple risk factors in the same individual cannot be deemed to be an accidental occurrence. It can probably be explained if we consider that visceral fat accumulation underlies the development of all these risk factors. It has been shown that visceral fat accumulation may be seen not only in obese individuals, but also in individuals of normal weight. It has also been confirmed that even in normal-weight individuals, the accumulation of visceral fat is associated with abnormal glucose metabolism, hyperlipidemia, etc., and with the development of coronary vascular diseases. We thus conclude that in both obese and non-obese individuals, visceral fat accumulation serves as the basis for the development of the multiple risk factor syndrome (including resistance to insulin), and propose that the condition therefore be called “visceral fat syndrome.”

Pathogenesis of the Visceral Fat Syndrome, with Emphasis on the Molecular Mechanisms Underlying the Development of Atherosclerosis

The visceral fat syndrome can be deemed as being essentially identical to syndrome X, or the deadly quartet. Its pathogenesis has been ex-
plained primarily on the basis of insulin resistance and hyperinsulinemia. However, it is difficult to explain the mechanism of onset of all the risk factors on the basis of insulin resistance or hyperinsulinemia. From the two viewpoints mentioned below, the authors have attempted to clarify the mechanisms by which visceral fat accumulation, which probably underlies the development of insulin resistance, may be involved in the onset of diverse diseases.

1. Significance of free fatty acids and glycerol released from adipocytes

It is known that the adipocytes which constitute adipose tissue store excess energy in the form of triglycerides; these triglycerides are hydrolyzed to supply free fatty acids (FFA) and glycerol systemically when energy is needed, or in the case of starvation. This reaction is known to be more active in visceral fat than in subcutaneous fat. As compared to subcutaneous fat, visceral fat allows earlier expression of lipoprotein lipase (LPL) and glucose transporter (Glut4), which are involved in energy uptake, and of acyl coenzyme A (CoA) and acyl synthetase (ACS) which are involved in fat synthesis, in cases of obesity. It is also known that during exercise, the expression of the aforementioned molecules decreases sharply.6,7)

We may say that visceral fat cells possess a high potential for synthesizing and decomposing fat, and that in individuals with excess visceral fat, the release of FFA and glycerol, which are products of (triglyceride) hydrolysis, occurs in amounts corresponding to the volume of visceral fat accumulated. Intrapitoneal visceral fat (mesenteric fat) can be anatomically characterized as being in direct linkage with the liver through the portal vein. Therefore, FFA and glycerol, which are released from the visceral fat, flow directly into the liver. It is known that FFA entering the liver stimulate fat synthesis and suppress insulin catabolism, which results in the onset of peripheral hyperinsulinemia.

We have shown that besides acting as a precursor of fat synthesis, FFA also serve as ligands for the peroxisome proliferator activated receptor (PPAR)-alpha and hepatic nuclear factor (HNF), which are expressed in the liver, and are involved in the gene transcription of several enzymes such as ACS, in a way similar to other physiologically active substances; these result in enhanced transcriptional activity of microsomal triglyceride transfer protein (MTP, a rate-limiting protein involved in the secretion of very low density lipoprotein, VLDL), and the onset of hyperlipoproteinemia.8)

Although glycerol had not attracted attention in this connection before, we discovered an adipocyte-specific glycerol channel (aquaporin adipose, AQPap)9) among the genes encoding the expression of proteins in adipocytes. AQPap has been reported to be abundantly expressed in the presence of visceral fat pools and has been shown to play a principal role in the molecular mechanism involved in glycerol release from adipocytes. Aquaporin-9 (AQP9), which had recently been discovered in the liver, serves as a portal of entry for glycerol into the liver. It seems that glycerol, which is released in large

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Fig. 2 Enhanced glycerol transfer and glucose release from the liver in the presence of visceral fat accumulation

HSL: hormone-sensitive lipase
TG: triglyceride (Source: Reference 10)
amounts via AQPap in the presence of visceral fat pools, enters the liver via AQP9, and is converted into glucose by glycerokinase and other enzymes, which are eventually released from the liver. Thus, a new mechanism for the onset of hyperglycemia in the presence of visceral fat pooling was proposed (Fig. 2).

2. Significance of adipocytokine, a physiologically active substance secreted from adipocytes

To elucidate the molecular characteristics of adipocytes, we conducted analyses of genes encoding proteins expressed in adipocytes by means of large-scale random sequencing, in collaboration of the Osaka University Institute for Molecular and Cellular Biology. Of all the genes encoding protein expression in adipocytes, about 60% remain unknown and novel genes. When the remaining known genes were classified by their function and subcellular localization, it was revealed that an unexpectedly large number of genes encoding secretory proteins are expressed abundantly in adipocytes. Many of these proteins were physiologically active. Genes encoding such proteins accounted for 20% of all genes found in subcutaneous fat tissue and 30% of all the genes found in visceral fat tissue. It was thus shown that adipocytes, which were conventionally thought of as cells that store energy, are actually endocrine cells.

The physiologically active substances which we named adipocytokines found in visceral fat include: (1) Cytokines such as tumor necrosis factor (TNF)-alpha and interleukin (IL)-6, (2) growth factors such as heparin-binding epithelial growth factor (HB-EGF), (3) various complements, (4) factors acting on the fibrinolytic system such as plasminogen activator inhibitor (PAI)-1, and (5) vasopressors such as angiotensinogen. It is considered that an increase or decrease in the volume of adipose tissue, depending on the nutritional status, may altered the secretion of these bioactive substances and affects the host defense system and metabolic regulation, and may thus be related to pathogenesis of diseases (Fig. 3). It has been shown, for example, that excessive expression of TNF-alpha in adipocytes in obese individuals impairs insulin signal transduction and is thus related to insulin resistance and the pathogenesis of diabetes mellitus.

We then paid attention to the expression of PAI-1 in adipose tissue in view of the report that blood PAI-1 levels are elevated in patients with the multiple risk factor syndrome or syn-

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**Fig. 3 Physiologically active substance derived from adipose tissue (adiopocytokine) and the adipocentric hypothesis (Source: References 11 and 20)**
drome X, and the fact that PAI-1 has been reported to be closely involved in thrombus formation and the development of atherosclerosis. In our study of clinical cases, a significant positive correlation was found between blood PAI-1 levels and the amount of visceral fat as measured in CT scans. When we studied the expression of the PAI-1 gene in adipose tissue during the course of development of obesity in obese rats, we found that PAI-1 mRNA expression in visceral fat increased markedly as fat accumulated. This suggests the possibility that enhanced synthesis and secretion of PAI-1, which occur in association with visceral fat accumulation, are related to the onset of vascular lesions. This provides a direct mechanism for the onset of vascular lesions in association with the visceral fat syndrome.12)

Among the unknown genes detected in adipocytes, a gene specific to adipocytes which was expressed in abundance, was found to encode a secretory protein.13) This protein, which was composed of 244 amino acids, possessed a collagen-like motif (G-X-Y), and was homologous to complement Clq, collagen X and collagen VII. We named this protein “adiponectin” and developed a technique for measuring its levels in the blood. Adiponectin is synthesized and secreted only by adipocytes. Unlike leptin, which is another secretory protein specific to adipocytes, adiponectin is found in lower levels in the blood of obese individuals, and its blood level had a strong negative correlation with the amount of visceral fat accumulation.14) It has also been reported that the expression of adiponectin mRNA was markedly decreased in accumulated visceral fat. In addition, plasma levels of adiponectin were lower in patients with diabetes mellitus coronary artery disease when body mass index was matched.

To examine the relationship between low blood adiponectin levels and the development of disease, we conducted a biological study of vascular cells in relation to the pathogenesis of atherosclerosis. The study showed that adiponectin suppresses the expression of adhesion molecules, such as TNF-alpha-dependent vascular cell adhesion molecule (VCAM-1), intercellular adhesion molecule (ICAM)-1 and E-selectin, on vascular endothelial cells. This, in turn, suppresses the adhesion of monocytes to the endothelial cells.15,16) Regarding the mo-
molecular mechanisms involved in this action of adiponectin, it has been demonstrated that adiponectin suppresses the phosphorylation of inhibitor-κB (I-κB) and thus regulates the actions of nuclear factor-κB (NF-κB), resulting in suppressed transcription of the aforementioned adhesion molecules. Adiponectin has a strong affinity for binding to collagen I, III, and V, which are matrix proteins found in the vascular wall. In fact, it has been confirmed that adiponectin, which is not normally expressed in the subendothelial layer, was accumulated in the subendothelial layer of the carotid artery of rats following balloon-induced injury of the artery. In addition to its effects on endothelial cells, adiponectin also acts on vascular smooth muscle cells to suppress their proliferation, and on macrophages to suppress fat accumulation in them.

As described above, adiponectin is secreted from adipocytes into the blood and seems to play a role resembling that of a fireman, i.e., binding to the injured vascular wall to repair them or suppress the onset of atherosclerosis (Fig. 4). Decreased secretion of adiponectin in the presence of obesity (especially visceral fat accumulation) can facilitate the onset of atherosclerosis.

Conclusion—Proposal of Adipocentric Hypothesis

We have shown that adipocyte accumulation, especially accumulation of visceral fat, promises to be as an important theme of medical research to clarify the pathogenesis of vascular diseases which is as important target as the pathogenesis of cancer in the 21st century, and have established the disease entity “visceral fat syndrome”. As described in this paper, visceral fat accumulation can promote the development of diverse diseases associated with daily habits or lifestyle through inducing excessive or reduced secretion of adipocyte-derived factors, such as FFA, glycerol and adipocytokines. It is possible that the presence of a combination of these diseases can lead to the development of atherosclerosis. Another important finding is that abnormal secretion of adipocytokines such as PAI-1 and adiponectin can directly induce vascular lesions. This probably explains why the visceral fat syndrome may serve as the major basis for the development of atherosclerotic diseases. In conclusion, we emphasize the importance of the adipocentric hypothesis, which places adipocyte accumulation at the center of the mechanisms underlying the onset of various common diseases, including atherosclerosis (Fig. 2).

REFERENCES


