Table 1 | Clinical gene therapy trials for metabolic diseases

Diseases and transgene	Gene delivery	Vector	Administration route	Trial country	Phase	Number	References
Alpha-1 antitrypsin deficiency							
Alpha-1 antitrypsin	In vivo	Adeno-associated virus	Intramuscular	USA	Phase I	2	60–62
					Phase II	1	
		Naked DNA	Intranasal	USA	Phase I	1	
Cystic fibrosis							
Alpha-1 antitrypsin	In vivo	Naked DNA	Intranasal	USA	Phase I	1	63–66
Cystic fibrosis transmembrane conductance regulator	In vivo	Adeno-associated virus	Intrabronchial	USA	Phase I	2	
					Phase II	2	
			Intranasal	USA	Phase I/II	3	
		Adenovirus	Intrabronchial	France	Phase I/II	1	
				USA	Phase I	3	
			Intradermal	USA	Phase I	1	
			Intranasal	Switzerland	Phase I	1	
				USA	Phase I	4	
					Phase I/II	1	
			Intranasal + intrabronchial	USA	Phase I	1	
		Naked DNA	Intrabronchial	UK	Phase I	1	
			Intranasal	UK	Phase I/II	4	
				USA	Phase I	5	
			Intranasal + intrabronchial	UK	Phase I	1	
Familial hypercholesterolemia							
Low-density lipoprotein receptor	Ex vivo (Hepatocytes)	Retrovirus	Intrahepatic	USA	Phase I	1	16–18
Gaucher's disease							
Glucocerebrosidase	Ex vivo (CD34 + PBC)	Retrovirus	Bone marrow transplantation	USA	Phase I	1	67,68
				USA	Phase I/II	1	
			Intravenous	USA	Phase I	1	
Huntington's disease							
Ciliary neurotrophic factor (CNTF)	Ex vivo (BHK)	Naked DNA	Intracerebral	Switzerland	Phase I	1	69,70
				France	Phase I	1	
Lipoprotein lipase deficiency							
Lipoprotein lipase (LPL)	In vivo	Adeno-associated virus	Intramuscular	Netherlands	Phase I/II	1	19,71,72
				Canada	Phase I	1	
Mucopolysaccharidosis type I (Hurlers syndrome)							
Alpha-L-iduronidase	Ex vivo (BMC)	Retrovirus	Bone marrow transplantation	UK	Phase I/II	1	73,74
	Ex vivo (Fibroblasts)		Intraperitoneal	France	Phase I	1	
Mucopolysaccharidosis type II (Hunter disease)							
Iduronate-2-sulfatase	Ex vivo (PBC)	Retrovirus	Intravenous	USA	Phase I	1	75
Mucopolysaccharidosis type VII							
Beta-glucuronidase	Ex vivo (CD34+PBC)	Lentivirus	Intravenous	USA	Phase I	1	76–78
Ornithine transcarbamylase deficiency							
Ornithine transcarbamylase	In vivo	Adenovirus	Intrahepatic	USA	Phase I	1	20,79
Pompe disease							
Acid-alpha glycosidase	In vivo	Adeno-associated virus	Intramuscular	USA	Phase I/II	1	80–82
Familial lecithin-cholesterol acyltransferase deficiency							
Lecithin-cholesterol acyltransferase	Ex vivo (Adipocytes)	Retrovirus	Subcutaneous	Japan	Phase I	1	42,55,59

Summarized according to the Clinical Trials Database provided by the Journal of Gene Medicine (http://www.wiley.com/legacy/wileychi/genmed/clinical/). Protocol of clinical trial for lecithin-cholesterol acyltransferase deficiency by our group is now under review by Ministry of Health, Labour and Welfare. BHK, baby hamster kidney cells; BMC, bone marrow cells; PBC, peripheral blood cells.

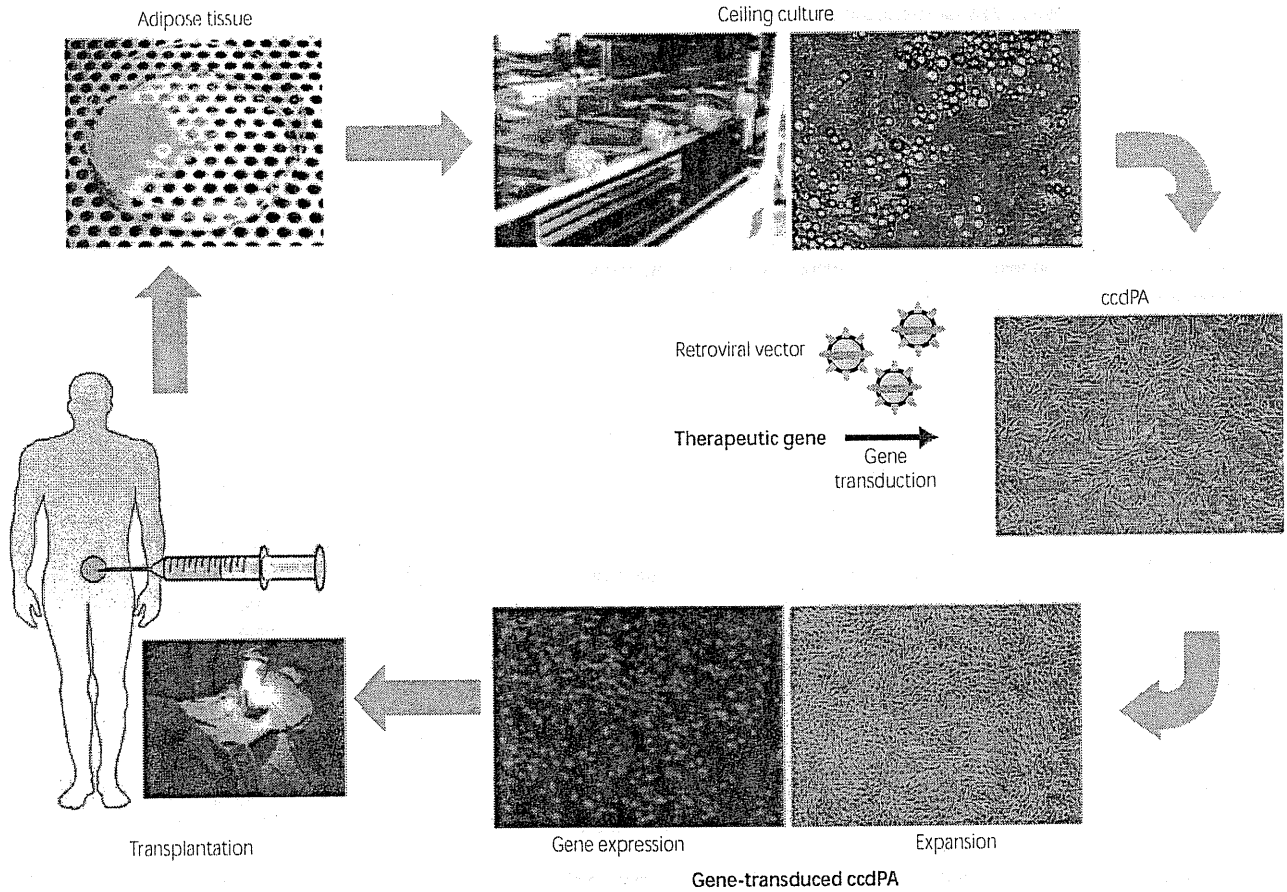

Figure 1 | Therapeutic strategy for adipocyte-based enzyme replacement therapy by ex vivo gene transfer. Adipose tissue is obtained by lipoaspiration from the patient. ceiling culture-derived proliferative adipocytes (ccdPA) are propagated by ceiling culture. The therapeutic gene is transduced by the retroviral vector. ccdPA stably secreting the therapeutic protein are expanded and harvested. Harvested cells are subcutaneously transplanted with the appropriate scaffold.

PREADIPOCYTES WITH HIGH ADIPOGENIC POTENTIAL

Recently, adipose tissue has been demonstrated to be a source of proliferative cells for cell-based therapies, such as regenerative medicine and gene transfer applications. Two types of preparation have been reported to be sources of adipose tissue-derived proliferative cells. One is stromal vascular fractions (SVF), which can be obtained as a sediment by the centrifugation of collagenase-digested fat tissue[9] and is the most commonly used technique. The adherent cells obtained from SVF are now recognized as adipose tissue-derived stem cells (ASC), which are pluripotent and can differentiate to yield various cell types, including cardiomyocytes, chondrocytes and osteoblasts, in addition to adipocytes, thus providing a relatively heterogeneous cell population appropriate for regenerative therapy[51–53]. However, these data show that SVF are heterogeneous, and therefore imply that SVF might not result in a stable therapeutic gene vehicle for gene therapy purposes.

The other cell preparation is obtained from the floating mature fat cell fraction obtained after the centrifugation, followed by a ceiling culture[54]. Because the cells are propagated using the buoyant properties of mature adipocytes in this preparation, the progeny cells are more homogeneous than ASC. Proliferative adipocytes were propagated by the ceiling culture technique from the mature adipocyte fraction, and the cells were designated as ceiling culture-derived proliferative adipocytes (ccdPA)[55]. The ccdPA are nearly homogeneous and show only a trace of mature adipocytes by analysis of surface antigen profiles. On stimulation to induce differentiation, the ccdPA showed increased lipid droplet accumulation accompanied with higher adipogenic marker gene expression compared with the ASC, even after in vitro passaging, suggesting the commitment of ccdPA to the mature adipocyte lineage[56].

GENE-TRANSDUCED ADIPOCYTES AS VEHICLE CELLS

MoMLV-mediated gene transduction in human ccdPA resulted in a high gene transduction efficiency[55]. In search of optimal transplantation conditions, the 3-D long-term culture system using fibrin gel, a tissue sealant utilized in the clinic, was established. The gene-transduced ccdPA spontaneously accumulate lipid droplets without any artificial stimulation in 3-D culture using the fibrin glue (Aoyagi Y, Kuroda M, Asada S, Tanaka S, Konno S, Tanio M, Aso M, Okamoto Y, Nakayama T, Saito Y, Bujo H, unpublished observations, 2010). Interestingly, the fibrinogen concentration was shown to affect the lipid accumulation in the cells. The expression of the transduced gene was correlated with cell differentiation (Aoyagi Y, Kuroda M, Asada S, Tanaka S, Konno S, Tanio M, Aso M, Okamoto Y, Nakayama T, Saito Y, Bujo H, unpublished observations, 2011).

In one study, the insulin gene-transduced cells were propagated, and the efficacy of these cells was evaluated in a diabetic mouse model[35]. The transplantation of the cells improved hyperglycemia and blood HbA_{1c} concentrations in a manner that was dependent on the cell number, without causing hypoglycemia. The plasma insulin concentration was dependent on the implanted cell number, and the systemic effect of the circulating insulin was confirmed by a marked improvement in bodyweight reduction and liver glycogen content. Thus, the autotransplantation of gene-transduced ccdPA could serve as a novel clinical application for a variety of systemic metabolic disorders.

AN EX VIVO GENE THERAPY TRIAL USING EXOGENOUS GENE-TRANSDUCED ADIPOCYTES

Lecithin-cholesterol acyltransferase (LCAT) deficiency has been identified as a genetic metabolic disorder. Cholesteryl ester levels are markedly reduced in lipoproteins, and abnormal cholesterol deposition is observed in the tissues of these patients, who often develop severe complications including corneal opacity, anemia, proteinuria and renal failure[57]. LCAT deficiency is caused by mutations in the lcat gene, and more than 40 different mutations have been identified to date[58]. Protein replacement treatment was suggested to be effective; however, no approach for the permanent correction of the symptoms has been reported.

However, in a previous study, the human lcat gene was transduced into human ccdPA by a retroviral vector. The transduced cells secreted functional LCAT protein in vitro, correlating with the integrated copy number of vector genomes[55]. The secreted LCAT protein clearly ameliorated the disturbed high-density lipoprotein subpopulation profile caused by impaired LCAT function in patients' serum by the in vitro incubation assay, strongly suggesting the feasibility of our strategy[59]. An application of this in vitro assay system to evaluate the responsiveness of patients is now under investigation. The LCAT delivery achieved in the mouse model with the clinically available fibrin scaffold was enough to suggest the efficacy of the ex vivo gene therapy strategy to prevent a poor prognosis in those patients[41].

The potential safety issues related to the ccdPA have been carefully addressed[55]. Gene transduction did not affect the cell growth, adipogenic differentiation or surface antigen profiles of the cells. The averaged integrated copy number was stable during the in vitro expansion process, and clonal expansion was not observed, indicating no predominant growth of gene-transduced cells. The transplantation experiments showed no signs for side-effects.

CONCLUSION

There are high hopes that a successful gene therapy approach can be developed in the future to treat rare genetic defects. Numerous studies have been carried out to develop such treatment strategies, both on the basic level and in the clinic. Although hematopoietic cells are proven target cells for ex vivo gene therapies, especially for immune-related diseases in which those cell functions are primarily affected by the gene defects, they might not be suitable targets for the many metabolic diseases that result in impairment of multiple organs. The physiological functions and applicability of adipose tissue would enable researchers to develop a novel therapeutic strategy to deliver therapeutic proteins systemically.

Mature adipocytes have been explored as a source of target cells for ex vivo gene therapy. Propagated ccdPA would provide an excellent platform for a novel adipocyte-based protein replacement therapy for patients with serum protein deficiencies who require long-term therapeutic protein supplements. A good manufacturing practice production procedure has been established, and the gene-transduced cells can be expanded up to nearly 10^{12} cells from 1 g of fat tissue within 1 month after fat tissue preparation[55]. To further expand the adipocyte-based therapeutic strategy for the supplementation of other proteins, it will be necessary to evaluate the characteristics of ccdPA from various kinds of fat diseases, such as those from subjects with metabolic syndrome, which might affect the secretion function of adipose tissues, and to develop an allogeneic transplantation method for patients with lethal conditions in childhood, as well as to establish the necessary transplantation procedure. After the careful consideration of the safety in combination of efficacy, the novel transplantation therapy developed using adipocytes might be applicable not only for genetic deficiencies, but also for lifestyle-related diseases, including diabetes mellitus.

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Health Education "Hokenshido" Program Reduced Metabolic Syndrome in the Amagasaki Visceral Fat Study. Three-Year Follow-up Study of 3,174 Japanese Employees

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Abstract

Objective The aim of this study was to evaluate the effects of health checkup and the health education "Hokenshido" program based on the concept that visceral fat accumulation causes metabolic syndrome (MetS), leading to cardiovascular disease (CVD).

Methods and Subjects Based on the Japanese definition of metabolic syndrome, in the annual health checkup for general subjects, the measurement of waist circumference and use of "Where am I?" chart on the way to develop atherosclerosis were introduced. The study group comprised 3,174 Japanese employees [2,440 males (46±11 years, mean ± SD), 734 females (43±10 years)], who underwent annual health checkup in 2003, 2004, and 2005. The medical staff provided "Hokenshido" for subjects assessed as having MetS and/or at high risk for CVD.

Results The prevalence of the MetS in 2003, 2004 and 2005 decreased in males (20.8%, 17.2%, 14.4%, $p< 0.001$) and females (3.0%, 2.2%, 1.9%, $p=0.359$), respectively. Among subjects with MetS at baseline, the number of subjects with MetS significantly decreased in males (508, 287, 247, $p<0.0001$) and females (22, 8, 6, $p<0.0001$), respectively. Mean waist loss was 1.6 cm in males (<0.0001) and 1.5 cm in females (<0.001). Among subjects with metabolic syndrome at baseline, the mean waist loss was 2.5 cm in males (<0.0001) and 3.9 cm in females (<0.05). Fatal atherosclerotic vascular events were not recorded in this study period.

Conclusion Health check-up and the "Hokenshido" program reduced the prevalence of the MetS, which might lead to prevention of CVD.

Key words: metabolic syndrome, health checkup, health guidance, Hokenshido, visceral fat accumulation, cardiovascular disease

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Introduction

The metabolic syndrome is a risk factor for atherosclerotic cardiovascular diseases (CVD) (1). Visceral fat accumulation caused by overnutrition and physical inactivity is closely related to glucose intolerance, dyslipidemia, hypertension, and CVD (2-4). Once a diagnosis of the metabolic syndrome is established, management of the condition, lifestyle change as the primary intervention and assessment of cardiovascular risk factors should be adequately conducted to reduce the risk of CVD (5-7).

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Table 1. Clinical Characteristics of the Subjects at Baseline

Variables	Male (n=2440)	Female (n=734)
Age, years	45.5±10.6	43.0±9.7
Body weight, kg	69.4±9.8	54.6±8.5
Body mass index, kg/m^2	24.3±3.1	22.3±3.5
Waist circumference, cm	84.9±8.3	78.2±10.0
Triglyceride, mg/dL	174.1±123.1	105.3±75.1
HDL cholesterol, mg/dL	53.7±14.7	65.4±15.4
LDL cholesterol, mg/dL	115.4±29.7	111.7±28.7
Systolic blood pressure, mm Hg	130.3±15.7	119.2±15.6
Diastolic blood pressure, mm Hg	80.1±11.8	71.9±11.9
Plasma glucose, mg/dL	105.3±33.4	98.3±24.7

Data are means±SD. HDL, high-density lipoprotein; LDL, low-density lipoprotein.

In Amagasaki City Office, 7 fatal atherosclerotic vascular events were recorded from year 1995 to 2002 in all -4,000 employees. In addition to the health costs, the insurance costs had also been increasing. Therefore, prevention of CVD was an important and urgent task for the city and employer. To this end, measurement of waist circumference in annual health checkup commenced in year 2003 in these employees, based on the concept that visceral fat accumulation causes the metabolic syndrome. According to the results of the health checkup, a health education "Hokenshido" program, using "Where am I?" chart on the way to develop atherosclerosis, was applied by the medical staff to prevent further development of lifestyle-related diseases and CVD for each subject. We reported previously that the decrease in visceral fat within one year correlated with the decrease in the number of metabolic risk factors (raised blood pressure, dyslipidemia and glucose intolerance) and increase in serum levels of adiponectin (8-17).

The aim of this study was to evaluate the effect of this whole program on the incidence of metabolic syndrome for each year and for each generation of males and females.

Materials and Methods

Participants

This urban area study group comprised 3,174 Japanese [2,440 males (45.9±10.6 years, mean ± SD), 734 females (43.0±9.7 years)] who were employees of the Amagasaki City Office, Hyogo, Japan and had completed the Government-funded annual health checkup every year from 2003 to 2005. The clinical characteristics of the study participants at baseline in year 2003 are shown in Table 1. Of the entire group, 118 (3.7%), 337 (10.6%), and 115 (3.6%) individuals were under treatment for dyslipidemia, hypertension, or diabetes, respectively, at baseline.

All participants gave full informed consent to participate in the study and ethical approval was obtained from committee on the Ethics of Human Research of Osaka University. This trial is registered with number UMIN 000002391 (the Amagasaki Visceral Fat Study).

Anthropometry and laboratory measurements

Height and weight were measured in the standing position. Body mass index was calculated as weight (kg) divided by the square of height in meters (m^2). Waist circumference at the umbilical level was measured in cm with a non-stretchable tape in the late exhalation phase at standing position (18). Systolic and diastolic blood pressure values were measured in the sitting position. Blood was withdrawn fasting or postprandial condition. Biochemical variables were measured with a conventional automated analyzer.

Assessment of risk factors

We defined the metabolic syndrome according to the guidelines for the diagnosis in Japan (19). Abdominal obesity, waist circumference equal to or greater than 85 cm in men or greater than 90 cm in women plus the presence of at least two of the following abnormalities: 1) dyslipidemia; a serum fast triglyceride level over 150 mg/dL and/or a serum high-density lipoprotein (HDL) cholesterol level less than 40 mg/dL, 2) hypertension; systolic blood pressure over 130 mmHg and/or diastolic blood pressure over 85 mmHg and 3) high glucose; serum fast glucose level over 110 mg/dL. Subjects who received specific treatment(s) for each of the above metabolic risk factors were considered positive for that factor. It means that, those who had a risk factor without treatment and those who were on treatment were also included as study subjects. In the case that blood samples were not obtained after >8-hour fasting, we modified 1) to 1)' dyslipidemia; postprandial triglyceride level over 200 mg/dL (20, 21) and/or a serum high-density lipoprotein (HDL) cholesterol level less than 40 mg/dL, 3) to 3)' as high glucose; postprandial serum glucose level over 140 mg/dL (22).

Detailed examination

Oral glucose tolerance test, bicycle ergometer stress test, and carotid artery echography were performed in those subjects with risk factor(s) based on the recommendation of the team physician.

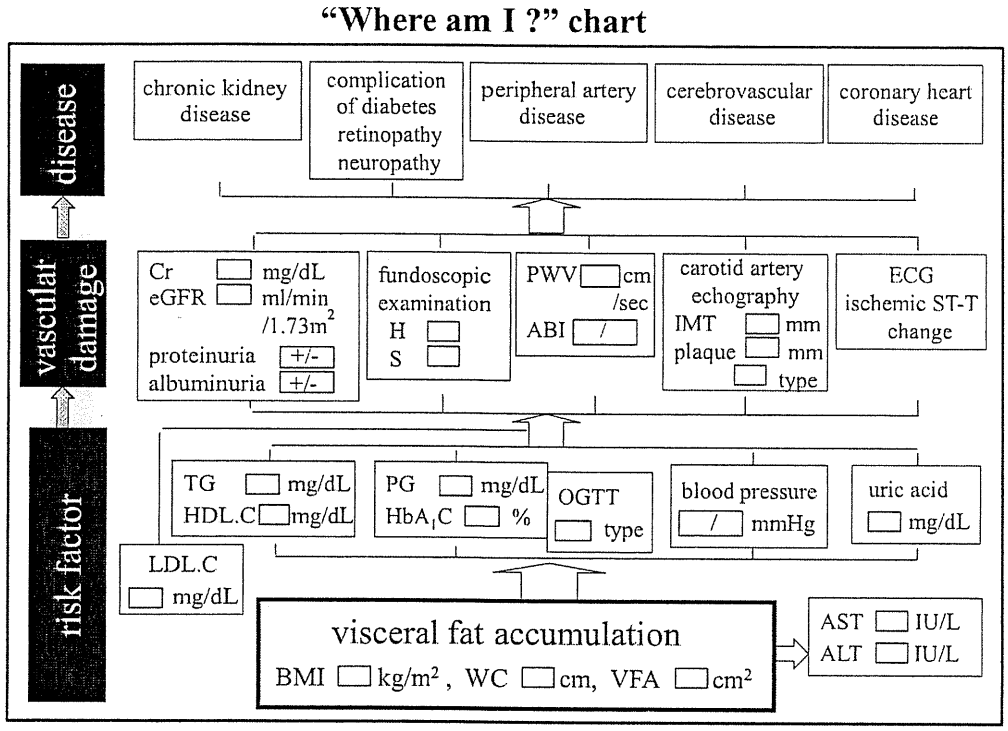

Figure 1. The "Where am I?" chart. All results were transferred into this chart for the individual subject. The metabolic risk factors of vascular damage were displayed at the bottom part of the chart. The results of detailed examination to estimate the current condition of vascular damage were set at the middle of the chart. The status of presence or absence of diseases such as cardiovascular diseases (CVD) was displayed at the top of the chart. BMI: body mass index, WC: waist circumference, VFA: visceral fat area, LDL.C: low-density lipoprotein cholesterol, TG: triglyceride, HDL.C: high-density lipoprotein cholesterol, PG: plasma glucose, OGTT: oral glucose tolerance test, Cr: serum creatinine, eGFR: estimated glomerular filtration rate, PWV: pulse wave velocity, ABI: ankle-brachial index, IMT: intima media thickness

Health guidance ("Hokenshido")

After the health checkup, all of the participants receive the results of the health checkup and "Where am I?" chart (Fig. 1). To enhance understanding, all of the subjects were informed and given the opportunity to attend lectures by public health nurses and medical doctors.

In "Where am I?" chart, the metabolic risk factors of vascular damage were displayed at the bottom part of the chart. The results of detailed examination to estimate the current condition of vascular damage were put at the middle (of the chart). The status of presence or absence of diseases such as CVD was displayed at the top (of the chart). The placement of visceral fat accumulation at the bottom (part) implicates that metabolic risk factors, such as dyslipidemia, hyperglycemia, hypertension, and hyperuricemia are "the tip of the iceberg", and that visceral fat accumulation (dysregulation of adipocytokines in abdominal and visceral obesity) should induce the development of the risk factors, leading to atherosclerotic CVD and chronic kidney disease. Through this chart stream, the subjects having visceral fat accumulation can imagine their assumable stage for vascular damage, and be encouraged to alter their problematic lifestyle toward reducing visceral fat and cardiovascular risks. Such subjects are spontaneously helped to identify themselves as high risk subjects using this chart, based on the presence of multiple risk factors with visceral fat accumulation. On these conditions, health education "Hokenshido" program was provided by group and/or individual counseling. Public health nurse and dietitian interviewed and counseled the subjects about their pattern of meal, intake of alcohol, and habit of exercise. Through these processes, the guided subjects could determine the problematic habits which should be altered.

The number of subjects who received individual "Hokenshido" were 429 (13.5%) in year 2003, and 123 (3.9%) in year 2004. In particular, the subjects who could not improve their habit in the initial term were encouraged to repeatedly receive the group and individual lecture. The subjects considered already at high risk for CVD and chronic kidney diseases were referred to consult a cardiologist, neurologist, or nephrologist. Such subjects were continuously on the program to enhance the alteration of their problematic habits.

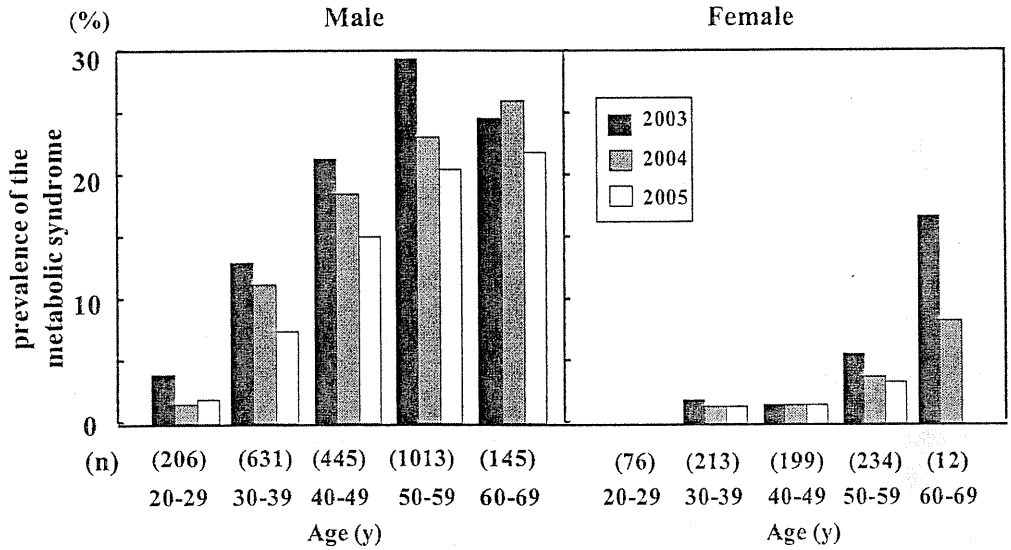

Figure 2. Age-related prevalence of the metabolic syndrome from year 2003 to year 2005. Male (n=2,440), female (n=734). Kruskal Wallis test with a Scheffe's test.

Table 2. Change in Waist Circumferences

		n	Waist circumference, cm			p
			year 2003	year 2004	year 2005	
Male	All	2440	84.9 ± 8.3	84.1 ± 8.4	83.3 ± 8.3	< 0.0001
	'03MS (+)	508	92.8 ± 6.7	91.5 ± 7.6	90.3 ± 7.4	< 0.0001
	'03MS (-)	1932	82.8 ± 7.4	82.1 ± 7.5	81.5 ± 7.5	< 0.0001
Female	All	734	78.2 ± 10.0	76.2 ± 9.4	76.7 ± 9.8	< 0.001
	'03MS (+)	22	98.3 ± 5.5	93.5 ± 6.6	94.4 ± 6.8	< 0.05
	'03MS (-)	712	77.6 ± 9.4	75.7 ± 9.0	76.2 ± 9.3	< 0.001

Data are means±SD.
'03MS (+), with the metabolic syndrome at baseline; '03MS (-), without the metabolic syndrome at baseline.
Kruskal-Wallist test with a Scheffe's test

Stastical analysis

The comparison of prevalence of the metabolic syndrome and risk factors in the 3 year period were analyzed by Kruskal Wallis test with a Scheffe's test. The statistical significance of the differences in the waist circumferences in 3 years were also analyzed by Kruskal Wallis test with a Scheffe's test. All statistical analyses were performed with StatView-J 5.0 (SAS Inc.).

Results

Age-related prevalence of the metabolic syndrome increased from the age of 30 years and was the highest in the 50-59 year age group in males and increased after the age of 50 years in females (Fig. 2). After initiating measurement of waist circumference in annual health checkup, use of "Where am I?" chart, and "Hokenshido", the prevalence decreased among males aged 30-39 years, 40-49 years, and 50-59 years ($p<0.01$), and among females aged 50-59 years, and 60-69 years during the 3-year period of this study.

The prevalence of the metabolic syndrome in 2003, 2004 and 2005 decreased in males (20.8%, 17.2%, 14.4%, $p<0.001$) and females (3.0%, 2.2%, 1.9%, $p=0.359$). Decreased prevalence of the metabolic syndrome in males was associated with significant reductions in the prevalence of abdominal obesity, dyslipidemia, and hypertension ($p<0.0001$). Among subjects with metabolic syndrome at baseline, the number of subjects with metabolic syndrome significantly decreased in males (508, 287, 247, $p<0.0001$) and females (22, 8, 6, $p<0.0001$), respectively.

Significant reductions of waist circumference were seen in males and females (Table 2). Mean waist loss was 1.6 cm in males ($p<0.0001$) and 1.5 cm in females ($p<0.001$). Among subjects with metabolic syndrome at baseline, the mean waist loss was 2.5 cm in males ($p<0.0001$) and 3.9 cm in females ($p<0.05$).

To be noted, during the 3-year period of this study, no fatal atherosclerotic vascular events were recorded.

Discussion

In the present study, we demonstrated that 1) after initiating measurement of waist circumference in annual health

checkup, use of "Where am I?" chart, and "Hokenshido" the prevalence of metabolic syndrome decreased with reductions in risk factors in males and females, 2) significant reductions of waist circumference were seen in males and females, 3) especially among males and females with the metabolic syndrome at baseline, the respective prevalence decreased markedly, and 4) fatal atherosclerotic vascular events were not recorded during the 3-year study period.

Based on the National Nutrition Survey in Japan, the rate of male obesity has been increasing. In this sense, the national campaign to improve the health of all Japanese people, called Kenko (Health) 21st, has not been fully successful. In the current study and program for the city employees, measurement of waist circumference and understanding of "Where am I?" chart seemed to be quite helpful to perceive their health conditions and reconsider the problematic habit. Through "Hokenshido", the guided subjects recognized a problem in their own lifestyle and attempted to reduce visceral fat as a goal to maintain a healthy life. In our Amagasaki Visceral Fat Study (8-17), we reported that the decrease in visceral fat was correlated with the decrease in the number of metabolic risk factors in the general male population (8).

Regarding the lack of fatal CVD events during the three-year study period, improvements of risk factors and possibly also the improvements of adipocytokine dysregulation such as hypoadiponectinemia might stabilize arterial plaque.

A limitation of this study is that majority of blood samples were nonfasting. To enhance annual health checkup for as many employees, such blood sampling policy, either fasting or nonfasting, is allowed by the employer in many work places in Japan. Data were evaluated according to the criteria described in Materials and Methods, dependent on individual fasting or non-fasting conditions. Furthermore subjects with one or two risks without obesity should be also followed-up closely. In this study, 180 (5.7%), 446 (14.1%), and 162 (5.1%) individuals were under treatment for dyslipidemia, hypertension, or diabetes, respectively, in year 2005.

Collectively, regular health checkups and "Hokenshido" program, which is based on the concept that visceral fat accumulation causes metabolic syndrome, effectively reduced the prevalence of the metabolic syndrome and various risk factors, which might lead to the prevention of CVD.

The authors state that they have no Conflict of Interest (COI).

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Review

The Concept of Metabolic Syndrome: Contribution of Visceral Fat Accumulation and Its Molecular Mechanism

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Although abdominal obesity or visceral obesity is considered to be one of the components of metabolic syndrome and to have an important role in a cluster of cardiovascular risks, there is no consensus about the definition and diagnostic criteria for this syndrome, probably because there is considerable disagreement about the location and definition of abdominal obesity or visceral obesity.
In this review article, the important role of visceral fat accumulation in the development of a variety of lifestyle-related diseases is shown, including cardiovascular disease based on our clinical studies using CT scans, and the mechanism of these disorders is discussed, focusing on adipocytokines, especially adiponectin.
The importance of diagnosing metabolic syndrome, in which visceral fat accumulation plays an essential role in the development of multiple risk factors, should be emphasized because lifestyle modification for the reduction of visceral fat may be very effective for the reduction of risks of this type, namely metabolic syndrome in the narrow sense.

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Key words; Multiple risk factor clustering syndrome, Visceral fat, Adipocytokines (adipokines), Adiponectin

Introduction

According to the World Health Report 2002 by the World Health Organization, cardiovascular diseases based on over-nutrition and physical inactivity are rapidly increasing in developing countries as well as developed countries, and comprise almost 30% of all causes of death in the world[1]. Atherosclerosis, which is present in the background of cardiovascular diseases, occurs and develops not by a single factor, but by complex of a variety of risk factors. Among them, it is well known that hypercholesterolemia, especially hyper-LDL-cholesterolemia, plays the most important role in the development of atherosclerosis. Hypercholesterolemia has been managed throughout the world since effective cholesterol-lowering drugs such as statins were developed in the past 20 years; however, it is also true that cardiovascular disease occur in subjects without hypercholesterolemia. Although diabetes mellitus, hypertension and lipid disorders, such as hypertriglyceridemia or low HDL-cholesterol, have been recognized as risk factors for atherosclerosis, the contribution of each factor is considered to be weaker than hypercholesterolemia; however, in the past 20 years, clinical and epidemiological studies have demonstrated that the coexistence of these risk factors is a strong risk factor and the multiple risk factor clustering syndrome has become as important as hypercholesterolemia in the background of cardiovascular diseases. Recently, the concept of metabolic syndrome, which almost corresponds to multiple risk factor clustering syndrome, has been noted all over the world. In this review, the mechanism of multiple risk factors cluster in one individual and why this state is so atherogenic are discussed.

In addition, the purpose of diagnosing metabolic

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syndrome is to identify the type in which lifestyle modification to reduce visceral fat takes priority over drug treatment to reduce multiple risks.

Multiple Risk Factors Clustering Syndrome

The Framingham study is a well-known epidemiological study which clearly demonstrated the significance of hypercholesterolemia as a major risk factor for the occurrence of coronary heart disease[2]; however, this study also suggested that the clustering of plural risk factors appeared to raise coronary heart disease risk[3]. Several clinical concepts of multiple risk factor clustering syndrome were proposed around 1990 since Prof. Reaven proposed the concept of Syndrome X, in which insulin resistance is considered to play the leading role in the clustering of cardiovascular risks, such as hyperglycemia, hypertriglyceridemia, low HDL-cholesterol and hypertension, and also in the development of atherosclerosis[4]. Prof. DeFronzo proposed a similar concept, named insulin resistance syndrome[5]. In 1990, Prof. Kaplan proposed a multiple risk factor clustering syndrome, named the deadly quartet, in which upper body obesity was adopted as one of the four components in addition to hypertriglyceridemia, hypertension and hyperglycemia[6]. In Japan, the study group for the Association between Host Origin and Atherosclerotic Diseases supported by the Japanese Labor Ministry investigated the medical records of 10 years of annual health checks in 94 patients with acute myocardial infarction from 1991 to 1992 among 122,051 employees from 31 industries compared with 191 age-matched controls without coronary artery disease in the same industries. It was demonstrated that subjects with a combination of 3 or more risks out of obesity, hyperglycemia, hyperlipidemia and hypertension had an increased relative risk for acute myocardial infarction of 10.56 times[7]. These findings suggest that multiple risk factors clustering may become a strong cardiovascular risk in Asian countries as well as Western countries.

So the question is: do these common disorders gather coincidently in one individual or do some key factors play a role in the development of a variety of disorders?

The disorders which compose multiple risk factor clustering syndrome do not occur from a single cause, but are considered to occur from a complex of genetic and environmental factors. For example, some genetic factors may be involved in the development of type 2 diabetes mellitus, but a clear genetic disorder has been clarified in only a few types; therefore, type 2 diabetes mellitus develops on the basis of wide-ranging factors, including genetic and environmental factors, especially over-nutrition together with physical inactivity and its consequence, obesity. The etiology of hypertension and dyslipidemia is considered to be as complicated as that of diabetes mellitus and obesity is also involved as a common etiology. In some incidences of multiple risk factor clustering syndrome, diabetes mellitus, dyslipidemia and hypertension may gather coincidently in one individual; however, in most cases, obesity might act as a mutual key player for the development of each component. We can easily understand that this syndrome is rapidly increasing with the increase of obesity all over the world, including Asian countries as well as Western countries. As previously mentioned, insulin resistance has been considered to be a key player in multiple risk factor syndrome[4, 5]. There is no doubt that insulin resistance is one of the main causes of type 2 diabetes mellitus in multiple risk factor clustering syndrome. In addition, several epidemiological studies have shown the association of insulin resistance with dyslipidemia or hypertension; therefore, insulin resistance might play an important role in metabolic syndrome. However, the etiology of insulin resistance has not been fully implicated in Syndrome X or insulin resistance syndrome. It may be natural for obesity to be present upstream of insulin resistance as well as hyperglycemia, dyslipidemia and hypertension in multiple risk factor clustering syndrome[8].

Visceral Fat Syndrome

Although common health problems, such as diabetes mellitus, dyslipidemia and hypertension and their clusters, are closely correlated to over-nutrition and its typical consequence, obesity, previous studies on the morbidity of obesity have indicated that the severity of obesity-related diseases does not necessarily correlate to the extent of body fat accumulation, but is closely related to body fat distribution. Several classifications of obesity concerning body fat distribution have been proposed in order to distinguish the possible mechanisms of obesity-related diseases. An ancient Japanese artist showed great insight into the morbidity of obesity 800 years ago when he painted a picture of an obese woman with the title, "A very obese woman who can hardly walk" in the old Japanese picture scroll, "Yamai Zoshi" which means an illustrated scroll for various diseases. Compared with the figure of an obese girl painted by Renoir, she has marked adiposity around her abdomen (**Fig. 1**).

At the end of the 1940s, Prof. Vague noted that, "Fat excess is dangerous because of its metabolic com-

Fig. 1. Classical paintings of obese women.

plications and a woman normally has twice a man's fat mass, i.e. the mass of an obese man. Though she is often as obese as a man or is fatter, she dies later and less often from metabolic complications of obesity." He proposed a classification of obesity into android type and gynoid type in 1947[9]. His classification was based on the brachio-femoral adipomuscular ratio (BFAMR) and subjects with higher BFAM were designated to be android type in whom metabolic complications were more prevalent. Although his classification is not exactly the same as the current classification, he is no doubt a pioneer of recognizing high-risk obesity based on fat distribution.

In the early 1980s, Prof. Bijorntorp proposed a classification between central obesity and peripheral obesity, and Prof. Kissebah proposed a classification between upper body segment obesity and lower body segment obesity based on the waist/hip ratio[10, 11]. Our group developed a method for fat analysis using CT scans which enabled us to analyze adipose tissues in the body cavity in 1983, and we noticed a marked variation in fat distribution between subcutaneous fat and intraabdominal visceral fat[12, 13].

Using the CT scan method for fat analysis, we demonstrated the contribution of visceral fat accumulation to the development of metabolic disorders, including glucose intolerance and hyperlipidemia[14, 15]. Visceral fat accumulation is associated not only with quantitative changes in serum lipids and lipoproteins, but also with qualitative changes in lipoproteins, such as small dense LDL[16]. The steady-state plasma glucose method by our group clearly showed that visceral fat obesity has greater insulin resistance than subcutaneous fat obesity[17, 18].

In addition to these metabolic disorders, we have demonstrated that in premenopausal women, visceral fat accumulation correlates closely with systolic blood pressure[19]. In hypertensive people, we reported a close correlation between the extent of visceral fat reduction, not subcutaneous fat reduction, and a lowering of blood pressure after weight reduction[20].

Visceral fat accumulation relates not only to the development of cardiovascular risks, but also relates directly to the development of cardiovascular disease. Several studies, including ours, have shown that visceral adiposity determined by CT scanning is related to coronary artery disease even in mildly obese individuals[21]. Visceral fat accumulation is also related to the development of cardiac dysfunction and sleep apnea syndrome[22, 23]. From this evidence, we can conclude that visceral fat accumulation is a major risk of cardiovascular disease as well as metabolic diseases.

Metabolic Syndrome

As shown above, visceral fat accumulation might be present upstream of a variety of disorders, including cardiovascular disease; therefore, we have proposed the concept of visceral fat syndrome on the basis of our clinical researches as a similar concept to metabolic syndrome.

The concept of metabolic syndrome has been proposed by several committees, although there has been considerable disagreement over the terminology and diagnostic criteria related to this multiple risk factor clustering syndrome. The first formalized concept of metabolic syndrome was proposed by a consultation group on the definition of diabetes for the World Health Organization (WHO) as a high-risk status with multiple risk factors for cardiovascular disease. This group emphasized insulin resistance as the major underlying factor and required evidence of insulin resistance[24]. The other major criteria came from the National Cholesterol Education Program Adult Treatment Panel Ⅲ (ATP Ⅲ) in 2001. ATP Ⅲ adopted abdominal obesity estimated by waist circumference instead of BMI as one of five factors in addition to elevated triglyceride, reduced HDL-cholesterol, elevated blood pressure and fasting glucose as the basis of establishing the diagnosis, although it did not require abdominal obesity as an essential component[25]. In 2005, the International Diabetes Federation (IDF) attempted to reconcile the different clinical definitions and made abdominal obesity necessary as an essential factor required in the diagnosis with particular emphasis on waist measurement as a single screening tool. The remaining four risk factors were identical to those provided by ATP Ⅲ. Since the definition and diagnostic criteria were proposed by IDF, a general agreement seemed to be reached that metabolic syndrome is des-

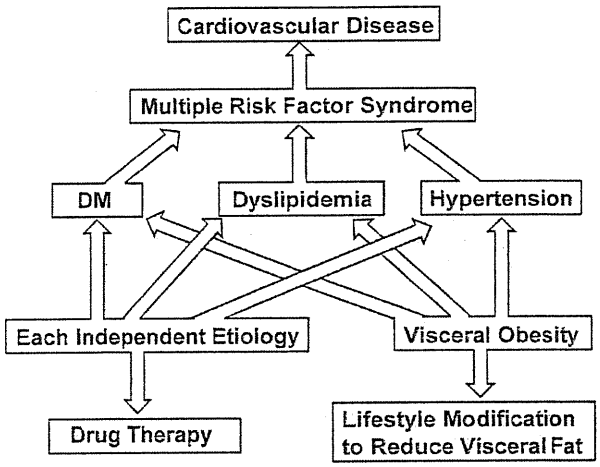

Fig. 2. Background and treatment of multiple risk factor clustering syndrome.

ignated as a multiple risk factor syndrome induced by abdominal or visceral obesity; however, there have been disagreements about the location and cutoff point of the waist circumference in recent years[26]. IDF and AHA/NHLBI representatives held discussions in an attempt to resolve the remaining differences in the definitions of metabolic syndrome and agreed that abdominal obesity should not be a prerequisite for diagnosis but that it is one of five factors, requiring the presence of any three of five risk factors. This recent definition is exactly the same as ATP III criteria for multiple risk factor clustering syndrome[27]. These controversies may have asisen from a misunderstanding of the significance of waist circumference and the location of visceral obesity in the pathophysiology of multiple risk factor clustering syndrome. Waist circumference is not a medical marker like blood pressure or triglyceride, but only a surrogate marker of visceral fat accumulation. Although waist circumference has been shown to correlate to visceral adiposity more strongly than BMI, it estimates total abdominal fat, including subcutaneous fat, as well as visceral fat; therefore waist circumference does not correlate with other risks, such as fasting blood glucose, triglyceride, HDL-cholesterol and blood pressure.

In Japan, metabolic syndrome has been designated to be a multiple risk factor clustering syndrome which is caused by visceral fat accumulation and in which lifestyle intervention to reduce visceral adiposity should take priority over drug treatment (**Fig. 2**). In other words, we diagnose metabolic syndrome in subjects with multiple risk factor syndrome if their visceral fat areas determined by CT scan is over 100 cm^2, and we treat them by lifestyle intervention. The Japanese Committee for the definition of metabolic syndrome adopted a cutoff point of visceral fat area of 100 cm^2 for both men and women because the number of risks increase over this point in men and women equally. Waist circumference corresponding to visceral fat of 100 cm^2 is 85 cm in men and 90 cm in women. Although many different cutoff points have been adopted by different organizations and different countries, the Japanese waist circumference threshold is the only one to be estimated from visceral fat area thresholds for morbidity[28]. Women have physiologically more subcutaneous fat than men on average, which

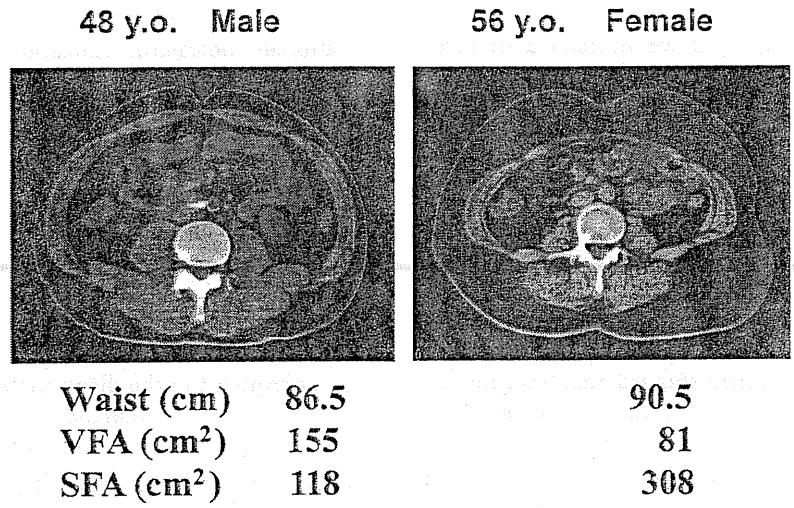

Fig. 3. Comparison of fat distribution between men and women.

makes the waist circumference larger in subjects with equal visceral fat (**Fig. 3**).

A Joint Scientific Statement on metabolic syndrome for "harmonizing metabolic syndrome"[29], published in Circulation in 2009, concluded that, in the interim, national cutoff points can be used. According to ATP III criteria and a recent statement from the joint committee, abdominal obesity is not an obligatory component and three abnormal findings out of five components would qualify a person for metabolic syndrome. This concept of metabolic syndrome may therefore include multiple risk factor clustering syndrome in which visceral fat-independent risks cluster in one individual coincidently. In this case, lifestyle intervention is less effective and drug treatment may be necessary for each risk. Therefore, multiple risk factor clustering syndrome should be divided into two types: in which visceral fat accumulation plays a key role in the development of multiple risks and cardiovascular disease (metabolic syndrome in the narrow sense), and in which multiple risks may gather coincidently. The purpose of diagnosing metabolic syndrome caused by visceral fat accumulation is to select subjects with multiple risk factors in which lifestyle modification to reduce visceral adiposity has priority over drug treatment (**Fig. 2**). The Japanese Committee for the Definition and Diagnosis of Metabolic Syndrome adopted the criteria for metabolic syndrome in the narrow sense, which is caused by visceral fat accumulation (**Fig. 4**).

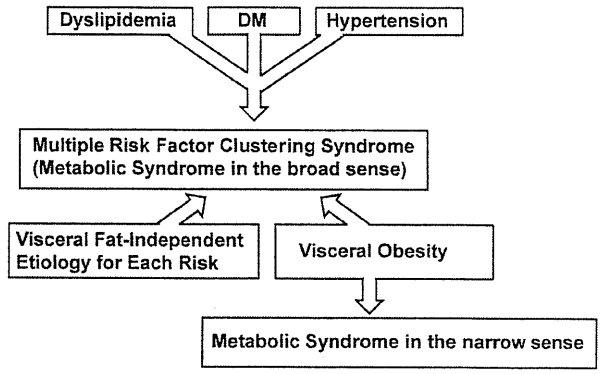

Fig. 4. Concept of metabolic syndrome in the broad and narrow senses.

The Japanese government started a new health policy by providing a specific health checkup followed by specific counseling for subjects diagnosed with metabolic syndrome according to the Japanese criteria from 2008. Health insurers were made responsible for conducting a specific checkup and counseling and approximately 56 million people aged 40-74 years old covered by the public health insurance scheme are the subjects in Japan (**Fig. 5**). We expect the reduction of lifestyle-related diseases, including cardiovascular disease and Government expects to control the increased medical costs of lifestyle-related diseases by this nationwide project. One of the results of a pilot study

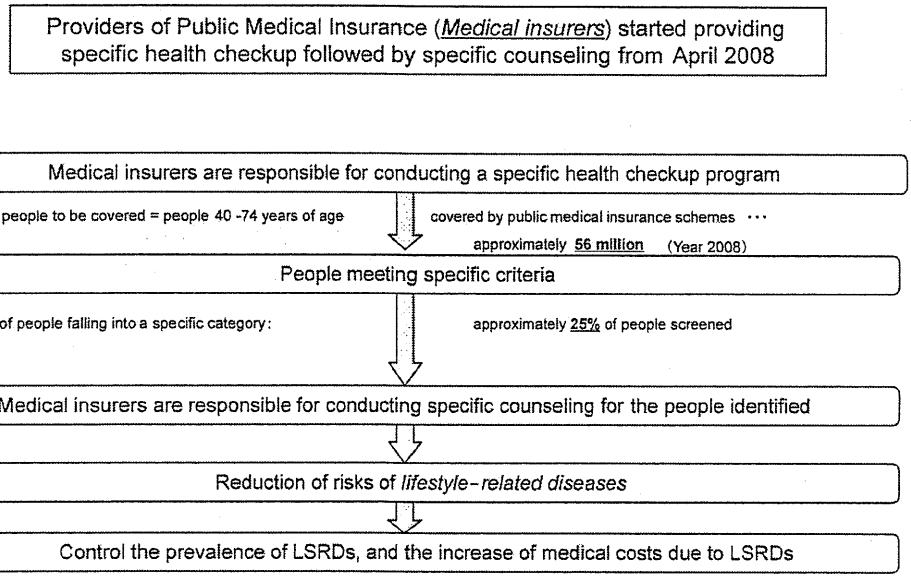

Fig. 5. Specific health checkup system and specific counseling system performed by the Japanese Government.

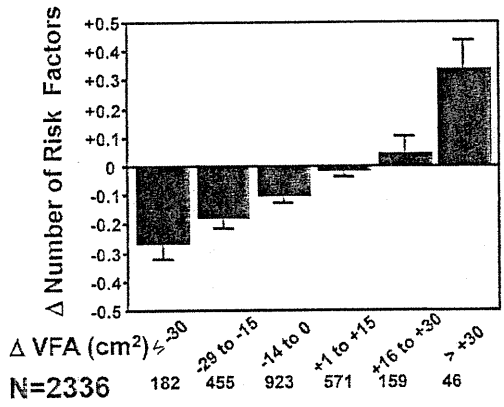

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Fig. 6. Correlation between the reduction of visceral adiposity and the reduction of cardiovascular risks.

performed in one urban city is shown in **Fig. 6**[30]. The extent of reduction of visceral adiposity clearly correlated with the improvement of risk factors.

Metabolic Syndrome and Adipocytokines (Adipokines)

An important question is why visceral fat accumulation causes common disorders; more importantly, why is this syndrome so atherogenic? In order to answer these questions, we have investigated the functions of adiposetissue, which has been traditionally regarded as a tissue passively storing excess energy in the form of triglycerides.

To elucidate the molecular mechanism of visceral fat-related diseases, particularly those in metabolic syndrome, we have investigated the biological characteristics of visceral adipose tissue and subcutaneous adipose tissue by analysis of the gene-expression profile compared with that of other mesenchymal cells. We systematically analyzed active genes by constructing a 3′-directed complementary DNA library in which the messenger RNA population was faithfully reflected. We found an unexpectedly high frequency of the genes encoding secretory proteins in adipose tissue, most of which are important bioactive substances. Of the gene group classified by functions and subcellular localization, approximately 20% of all genes in subcutaneous adipose tissue encode secretory proteins. This frequency rises to about 30% in visceral adipose tissue (**Fig. 7**)[31]. We classified these adiposetissue-derived bioactive substances as adipocytokines.

We found that the genes encoding plasminogen activator inhibitor type 1 (PAI-1) and heparin binding epidermal growth factor-like growth factor are highly expressed in adipose tissue[32, 33]. PAI-1 messenger RNA concentrations increased up to 10-fold in visceral adipose tissue during the development of fat accumulation in ventromedial hypothalamic-lesioned rats, which is an experimental animal model of obesity. In subcutaneous adipose tissue, concentrations remained unchanged. In addition to the animal model, we demonstrated that plasma levels of PAI-1 were significantly correlated with visceral adiposity, assessed by CT scanning, in humans. Circulating PAI-1 is deemed a strong risk factor for thrombotic diseases, including acute myocardial infarction, in metabolic syndrome. Heparin binding epidermal growth factor-like growth factor, a potent factor for smooth-muscle-cell proliferation, secreted from accumulated adipose tissue could also have some significance in vascular remodeling in obesity[33].

When we started the comprehensive genetic analysis of human adipose tissue, 40% of the expressed genes were previously unknown[34]. The gene expressed most abundantly in adipose tissue, which we named adipose most abundant gene transcript-1, apM-1, was a novel gene[35]. The molecule encoded by apM-1 possesses a signal peptide, collagen-like motif and globular domain, and has notable homology with collagen X, VIII and complement factor C1q. We termed the collagen-like protein adiponectin. The mouse homolog of adiponectin has been cloned as ACRP30[36]. We established a method to measure plasma adiponectin levels using an enzyme-linked immunosorbent assay. The average levels of adiponectin in human plasma are extremely high, up to 5-10 μg/mL[37]. Plasma concentrations are negatively correlated with BMI, whereas leptin increases with BMI. The negative correlation of adiponectin levels and visceral adiposity is stronger than between adiponectin levels and subcutaneous adiposity.

The mechanism by which plasma levels are reduced in individuals with visceral fat accumulation is not yet clarified. Co-culture with visceral fat inhibits adiponectin secretion from subcutaneous adipocytes. This finding suggests that some inhibiting factors for adiponectin synthesis or secretion are secreted from visceral adipose tissue[38]. Tumor necrosis factor-α was reported to be a strong inhibitor of adiponectin promoter activity[39]. The negative correlation between visceral adiposity and adiponectin levels might be explained by the increased secretion of this cytokine from accumulated visceral fat as at least one mechanism.

Plasma adiponectin concentrations are lower in

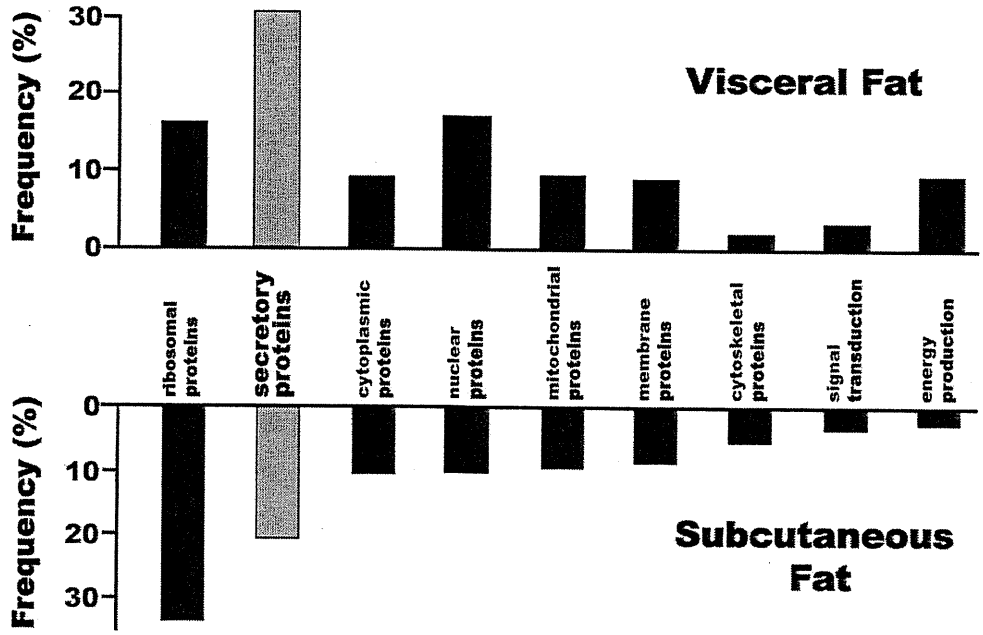

Fig. 7. Profiles of expressed genes in adipose tissue classified by gene function and subcellular localization.

people who have type 2 diabetes mellitus than in BMI-matched controls[40]. The plasma concentrations have been shown to correlate strongly with insulin sensitivity, which suggests that low plasma concentrations are related to insulin resistance[41]. In a study of Pima Indians, individuals with high levels of adiponectin were less likely than those with low concentrations to develop type 2 diabetes. High adiponectin concentration was therefore a notable protective factor against the development of type 2 diabetes[42].

Studies on adiponectin knockout mice support observations in humans. KO mice showed no specific phenotype when they were fed a normal diet but a high-sucrose and high-fat diet induced a marked elevation of plasma glucose and insulin levels. Notable insulin resistance, estimated by an insulin tolerance test during the high-sucrose with high-fat diet, also developed in knockout mice[43]. The supplementation of adiponectin by adenovirus transfection clearly improved this insulin resistance[44]. Plasma levels of adiponectin are also decreased in hypertensive humans, irrespective of the presence of insulin resistance[45]. Endothelium-dependent vasoreactivity is impaired in people with hypoadiponectinemia, which might be at least one mechanism of hypertension in visceral obesity[46].

Most importantly, plasma concentrations of adiponectin are lower in people with coronary heart disease than in controls, even when BMI and age are matched[47]. A case-control study performed in Japan demonstrated that the group with hypoadiponectinemia with plasma levels less than 4 μg/mL had an increased risk of CAD and multiple metabolic risk factors, which indicates that hypoadiponectinemia is a key factor in metabolic syndrome[48]. A prospective study by Pischon *et al.*[49] confirmed that high adiponectin concentrations are associated with a reduced risk of acute myocardial infarction in men. In addition to hypoadiponectinemia accompanied with visceral fat accumulation, genetic hypoadiponectinemia caused by a missense mutation has been reported, which also exhibited the clinical phenotype of metabolic syndrome[50].

This clinical evidence showed that hypoadiponectinemia is a strong risk factor for cardiovascular disease.

Antiatherogenicity of adiponectin is also demonstrated in animal experiments. Adiponectin knockout mice developed more severe intimal thickening by endothelial injury than wild-type mice[51]. In addition, overexpression of human adiponectin by adenovirus transfection attenuated plaque formation in apolipoprotein E-KO mice[52].

A large amount of adiponectin flows with the blood stream and therefore comes into contact with the vascular walls throughout the body. It is important

to know how adiponectin interacts with vascular cells. Immunohistochemical examination with antibodies to adiponectin showed no adiponectin protein in the untreated normal vascular walls in rabbits. Markedly positive immunohistochemical staining was detected, however, in balloon-injured vascular walls. Since adiponectin has the ability to bind subendothelial collagens, such as collagen V, VIII and X, endothelial injury may prevent adiponectin from entering the subendothelial space through binding to these collagens[53].

Cell biological studies have demonstrated that adiponectin has multiple, potent anti-atherogenic functions. When the endothelial barrier is injured by attacking factors such as oxidized LDL, chemical substances and mechanical stress, adiponectin accumulates in the subendothelial space of vascular walls by binding to subendothelial collagen, at which point anti-atherogenic properties of adiponectin become apparent. The protein suppresses monocyte attachment to vascular endothelial cells by inhibiting the expression of adhesion molecules, such as vascular cell adhesion molecule 1, intracellular-adhesion molecule 1 and E-selectin, via the inhibition of NF-κB activation[54]. Adiponectin also attenuates growth factor-induced proliferation of vascular smooth muscle cells by inhibiting mitogen-activated protein kinase[55]. Adiponectin suppresses foam-cell formation by inhibiting the expression of scavenger receptor class A[56].

Acute coronary syndromes are considered to determine the prognosis of cardiovascular disease in which the vulnerability of plaque is an important determinant of plaque rupture. In this process, matrix metalloproteinase secreted from macrophages is thought to play an important part in plaque vulnerability. Tissue inhibitor of metalloproteinase is thought to act as a protector of against plaque rupture by inhibiting matrix metalloproteinase. Adiponectin increases the expression of messenger RNA and protein production of tissue inhibitor of metalloproteinase in macrophages via the induction of interleukin-10 synthesis. This finding suggests that adiponectin protects against plaque rupture by inhibiting matrix metalloproteinase function through the induction of interleukin-10-dependent production of tissue inhibitor of metalloproteinase[57].

The final prognosis of cardiovascular disease depends on cardiac function, and visceral fat accumulation was reported to be related to cardiac dysfunction.

Shibata *et al.* have demonstrated that adiponectin-deficient mice showed enhanced concentric hypertrophy and increased mortality under pressure overload. These phenomena were associated with increased extracellular signal-regulated kinase and diminished AMP-activated protein kinase signaling in the myocardium. Adenovirus-mediated supplementation of adiponectin attenuated cardiac hypertrophy in response to pressure overload[58].

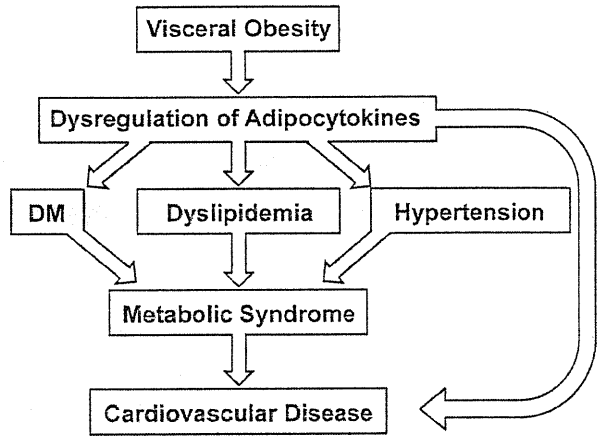

Fig. 8. Concept and pathogenesis of metabolic syndrome.

From these results, the pathophysiology and molecular mechanism of metabolic syndrome is summarized in **Fig. 8.** Visceral fat accumulation caused deregulation of adipocytokine production and secretion, especially the reduction of adiponectin, which is the main mechanism of the development of multiple risks and also direct a mechanism of cardiovascular diseases.

Summary

In this review article, the concept of metabolic syndrome is discussed. If we recognize that this syndrome is a multiple risk factor clustering syndrome caused by visceral fat accumulation, we can easily understand why plural disorders gather in one individual and what kind of molecular mechanism acts in the clustering of multiple risks. In visceral fat syndrome, namely metabolic syndrome in the narrow sense, it is natural that lifestyle modification to reduce visceral fat is the primary measure to prevent the development of cardiovascular diseases as well as its risks, including diabetes mellitus. The Japanese government policy for preventive medicine according to the Japanese concept of metabolic syndrome is also introduced. The reduction of cardiovascular disease and diabetes mellitus is expected by the new government project against metabolic syndrome in the near future.