by Japanese and ATP III criteria in this population, we determined the incidence of metabolic syndrome using these criteria in each generation from age 20s to 70s in men and women as shown in **Fig. 2**. The incidence of metabolic syndrome using ATP III criteria was about 3 times higher than that by the Japanese criteria. Using both criteria the incidence of metabolic syndrome started to rise in men in their 30s and reached a plateau after their 40s. Meanwhile, the incidence of metabolic syndrome in women started to rise after their 50s using both criteria, indicating the increased prevalence of metabolic syndrome after menopause.

We next examined whether visceral obesity contributed to metabolic abnormalities in this study population. **Fig. 3** shows the difference of lipid profiles and fasting glucose levels with or without visceral obe-

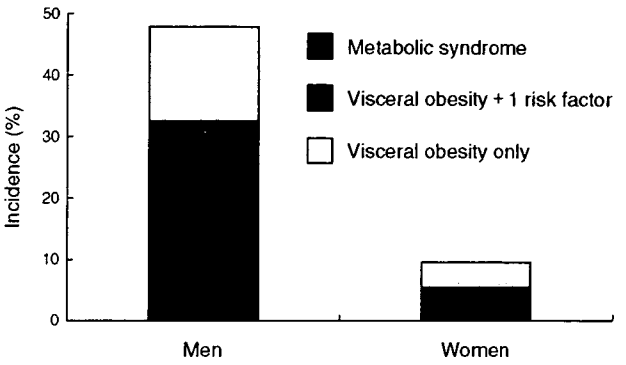

Fig. 1. Incidence of metabolic syndrome and visceral obesity in the lipid survey in 2000.

The percent incidence of metabolic syndrome, visceral obesity plus one risk factor, and visceral obesity in men and women is shown.

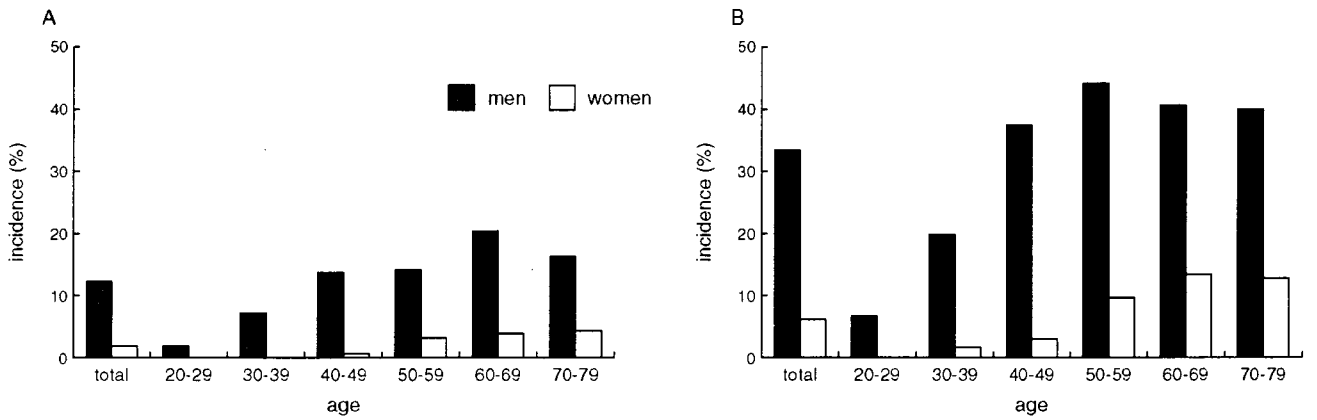

Fig. 2. Incidence of metabolic syndrome in each generation by Japanese and ATP III criteria.

Each column shows the incidence of metabolic syndrome in each generation in men (closed column) and women (open column) by Japanese (A) and ATP III (B) criteria. The incidence in the total population is shown on the left.

Fig. 3. Comparison of metabolic abnormalities with or without visceral obesity.

Each column shows the mean ± SD of total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, and fasting glucose with or without visceral obesity in men (A) and women (B). $*p < 0.001$

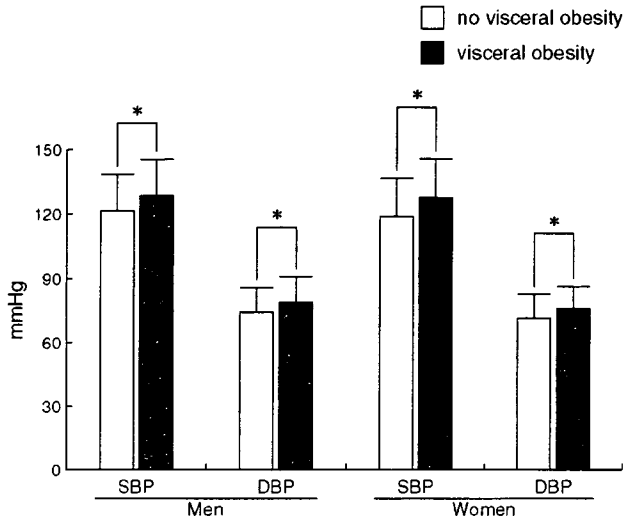

Fig. 4. Comparison of systolic and diastolic pressure with or without visceral obesity.

Each column shows the mean ± SD of systolic and diastolic blood pressure with or without visceral obesity in men and women. $^{*}p < 0.001$

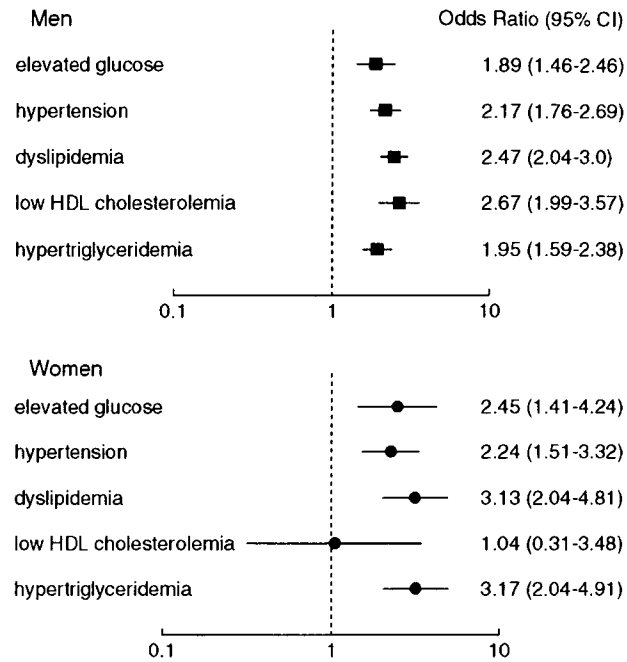

Fig. 5. Effect of visceral obesity on hypertriglyceridemia, low HDL cholesterolemia, dyslipidemia, hypertension, and glucose intolerance in men and women.

Odds ratios and 95% confidence interval are shown for each abnormality in the presence or absence of visceral obesity.

sity in this study population. The levels of total cholesterol, triglyceride, LDL-cholesterol, and fasting glucose were significantly higher, while the level of HDL-cholesterol was significantly lower in the group with visceral obesity than in the group without, indicating the contribution of visceral obesity to these metabolic abnormalities in both men and women. Systolic and diastolic blood pressure was also higher in the visceral obesity group in both genders (**Fig. 4**). We also determined the effect of visceral obesity on the development of each abnormality by calculating the odds ratios and 95% confidence interval (**Fig. 5**). Visceral obesity was significantly associated with the development of each metabolic abnormality in men and women except for low HDL-cholesterolemia in women. When we changed the cutoff of HDL-cholesterol to 50 mg/dL, visceral obesity was significantly associated with low HDL-cholesterolemia in women. The odds ratio was 2.10 and the 95% confidence interval was 1.35-3.27. Among dyslipidemia, hypertension, and glucose intolerance, visceral obesity was most associated with the development of dyslipidemia.

We also determined the age-adjusted difference of lipid profile in the presence or absence of visceral obesity in this population. Even after age adjustment we found a significant difference in total cholesterol, triglyceride, HDL-cholesterol, and LDL-cholesterol in men and in women, except for a difference in LDL-cholesterol in women (**Table 4**).

Discussion

In this study we determined the incidence of metabolic syndrome in the Japanese general population using a lipid survey performed in 2000 using new Japanese criteria to diagnose metabolic syndrome. We found that 3 times more people were diagnosed with metabolic syndrome using the new ATP Ⅲ criteria than the Japanese criteria and that visceral obesity contributed to metabolic abnormalities, such as dyslipidemia, glucose intolerance, and hypertension.

In our study the incidence of metabolic syndrome in Japanese men and women was 12.1 and 1.7%, respectively. The incidence of metabolic syndrome in our survey is lower than that from the latest National Health and Nutrition survey in 2004. In that survey the incidence of metabolic syndrome in Japanese men and women was 23.0 and 8.9%, respectively. In this national survey they used HbA1c ($\geqq$5.5) instead of FBS to diagnose glucose intolerance. This might explain the difference between the two surveys. This difference also indicates that the cutoff of FBS needs to be changed in the future. Although the mean age and the criteria used were different, Takeuchi *et al.*

Table 3. Incidence of each metabolic abnormality in the presence or absence of visceral obesity

	visceral obesity		no visceral obesity	
	men	women	men	women
hypertriglyceridemia	41.1%	25.4%	22.2%	9.7%
low HDL-cholesterolemia	17.6%	2.3%	7.4%	2.2%
dyslipidemia	45.7%	26.9%	25.4%	10.5%
hypertension	32.8%	33.1%	18.4%	18.1%
elevated fasting glucose	18.4%	14.6%	10.6%	6.2%

Dyslipidemia is defined as hypertriglyceridemia and/or low HDL-cholesterolemia

Table 4. Age-adjusted difference of lipid profile in the presence or absence of visceral obesity

		men		age-adjusted	women		age-adjusted	all		age-adjusted
		no visceral obesity	visceral obesity	*P*	no visceral obesity	visceral obesity	*P*	no visceral obesity	visceral obesity	*P*
T-cho	mean	195.6	205.9		198.8	214.2		197.3	206.9	
	number	994	923	<0.001	1217	130	0.082	2211	1053	<0.001
	SD	33.4	33.4		35.4	33.1		34.6	33.4	
TG	mean	128.7	162.0		88.9	121.7		106.8	157.0	
	number	994	923	<0.001	1217	130	<0.001	2211	1053	<0.001
	SD	119.3	138.8		60.2	51.5		93.7	131.8	
HDLc	mean	57.7	51.7		65.1	59.8		61.8	52.7	
	number	994	923	<0.001	1217	130	0.003	2211	1053	<0.001
	SD	14.2	13.9		14.5	12.5		14.8	14.0	
LDLc	mean	112.1	122.1		111.4	128.0		111.7	122.9	
	number	374	479	0.001	510	71	0.106	884	550	<0.001
	SD	26.0	30.1		29.0	28.8		27.8	30.0	

The mean, the number of samples, and SD are shown. *P* value was obtained by ANCOVA.

reported that the incidence of metabolic syndrome in men in the Tanno and Sobetsu study was 25.3%[11]. The mean age of their study population was 60.3 years, about 15 years older than that in our study population. Other studies reported a similar incidence of metabolic syndrome in Japanese. Considering that the incidence of metabolic syndrome in our population in their 60s was about 20%, the difference of the criteria used contributed to this difference. Similar to our study Urashima *et al.* reported an incidence of metabolic syndrome in Japanese men and women of 14.1% and 1.7%, respectively in central Tokyo[12]. Thus, the current incidence of metabolic syndrome in Japan would be around 15% in men and a few percent in women. In our study we found that about twice as many people with metabolic syndrome had visceral obesity and one risk factor in both men and women, indicating a potential for the incidence of metabolic syndrome to increase in the future. In our previous analysis we showed that the level of triglyceride in men dramatically increased from 1990 to 2000[6]. Therefore, we need to tackle this problem to prevent the increase in metabolic syndrome and cardiovascular disease in Japan.

In this population the incidence of metabolic syndrome in women was one seventh that in men. The incidence of visceral obesity, dyslipidemia, and glucose intolerance in women was one fifth, one third, and one half that in men, respectively. Furthermore, most of the women who satisfied this criteria were more than 50 years old, which means that few women are diagnosed with metabolic syndrome before the menopause. In Japan we adopted a cutoff of waist circumference of 90 cm for women, which is 5 cm more than that for men. This might explain why the incidence of metabolic syndrome in women was much less than in men. In contrast to the cutoff waist circumference in Japan, other criteria, such as in ATP Ⅲ,

generally have a larger cutoff in men than in women; however, our cutoff in women is based on the extensive study by Matsuzawa and his group using CT scan[13-15]. Therefore, in terms of detecting visceral obesity, 90 cm would be appropriate for Japanese women. However, we need to establish another method to select high-risk patients without visceral obesity. Our data also strongly indicate that visceral obesity using our cutoff is associated with metabolic abnormalities even after age adjustment, as shown in **Fig. 5** and **Table 4**. Therefore, we believe that visceral obesity is a useful surrogate marker for metabolic abnormalities and intervention to reduce abdominal circumference would lead to the prevention of cardiovascular disease. However, in terms of the cutoff of HDL-cholesterol, 50 mg/dL might be better than 40 mg/dL from the odds ratio in women (**Fig. 5** and Results) as in the cutoff of the ATP III criteria.

In summary we have shown that the incidence of metabolic syndrome in the Japanese general population is 7.8%, 12.1% in men and 1.7% in women. Intervention is required to prevent metabolic syndrome as well as metabolic abnormalities, such as dyslipidemia, hypertension, and glucose intolerance. The current criteria for metabolic syndrome should be assessed for the better diagnosis of women and elderly people.

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Relationship Between Metabolic Risk Factor Clustering and Cardiovascular Mortality Stratified by High Blood Glucose and Obesity

NIPPON DATA90, 1990–2000

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OBJECTIVE — Metabolic syndrome is diagnosed according to several criteria. Of these, some require glucose intolerance and others require obesity for the diagnosis. We investigated the relationship between metabolic risk factor clustering and cardiovascular disease (CVD) mortality stratified by high blood glucose or obesity.

RESEARCH DESIGN AND METHODS — We followed 7,219 Japanese men and women without a history of CVD for 9.6 years. We defined high blood pressure, high blood glucose, high triglycerides, low HDL cholesterol, and obesity as metabolic factors. The multivariate adjusted hazard ratio (HR) for CVD mortality according to the number of clustering metabolic factors was calculated using the Cox proportional hazards model.

RESULTS — During follow-up, 173 participants died of CVD. The numbers of metabolic risk factors and CVD mortality were positively correlated (P_{trend} = 0.07). The HR was obviously higher among participants with than among those without high blood glucose and clustering of ≥2 other metabolic risk factors (HR 3.67 [95% CI 1.49–9.03]). However, the risk increase was only modest in participants without high blood glucose even if they had ≥2 other metabolic risk factors (1.99 [0.93–4.28]). Conversely, metabolic risk factor clustering was related to CVD mortality irrespective of obesity.

CONCLUSIONS — Our findings suggest that glucose tolerance plays an important role in CVD mortality. Because the prevalence of nonobese participants with several metabolic risk factors was quite high and their CVD risk was high, excluding them from the diagnosis of metabolic syndrome because of the absence of obesity might overlook their risk.

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Abbreviations: CVD, cardiovascular disease; IDF, International Diabetes Federation; NCEP, National Cholesterol Education Program; WHO, World Health Organization.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

The World Health Organization (WHO) states that individual risk factors for cardiovascular disease (CVD) convey greater CVD risk. Furthermore, even though each one of these risk factors alone is not serious, the risk becomes more "powerful" when they are combined (1). Metabolic syndrome is the concept of clustering risk factors comprising insulin resistance, abdominal fat distribution, dyslipidemia, and hypertension (2–5).

Several institutions have established their own diagnostic criteria for metabolic syndrome. The National Cholesterol Education Program (NCEP) considers that each metabolic factor has the same importance (6), whereas the WHO requires impaired glucose tolerance among its criteria to diagnose metabolic syndrome (7). Finally, the International Diabetes Federation (IDF) and the Japanese guidelines require central obesity defined by waist circumference to diagnose metabolic syndrome (8,9). Thus, whether a relationship between metabolic risk factor clustering and CVD mortality differs according to obesity or impaired glucose tolerance, which are both required for a diagnosis of metabolic syndrome, should be determined. Thus, in the present study, we investigated the association between metabolic factor clustering and CVD mortality stratified according to obesity or impaired glucose tolerance in a population-based cohort study in the Japanese general population.

RESEARCH DESIGN AND METHODS

— Cohort studies of the National Survey on Circulatory Disorders, Japan, are referred to as NIPPON DATA (National Integrated Project for Prospective Observation of Noncommunicable Disease and Its Trends in the Aged). NIPPON DATA includes two cohort studies. Baseline data were surveyed in 1980 and in 1990 (NIPPON DATA80 and NIPPON DATA90), and

Table 1—*Means and prevalence of baseline characteristics of 2,999 men and 4,220 women aged ≥30 years (NIPPON DATA90, 1990)*

	Number of metabolic factors					
Baseline risk characteristics	0	1	2	3	4	5
n	1,604	2,657	1,643	942	336	37
Women (%)	67.3	54.3	39.4	35.4	36.9	36.0
Age (years)	44.1 ± 11.0	52.7 ± 13.6	56.0 ± 13.4	56.1 ± 12.5	58.0 ± 13.2	58.6 ± 11.2
BMI (kg/m^2)	20.9 ± 2.0	21.9 ± 2.4	24.1 ± 3.2	25.5 ± 3.1	26.7 ± 2.4	27.8 ± 2.0
Systolic blood pressure (mmHg)	114.9 ± 8.8	137.2 ± 19.7	141.8 ± 19.0	145.8 ± 17.4	149.2 ± 16.4	154.3 ± 18.4
Diastolic blood pressure (mmHg)	71.7 ± 7.5	82.1 ± 11.4	84.3 ± 11.4	86.7 ± 10.8	88.1 ± 11.5	89.7 ± 12.0
Total cholesterol (mg/dl)	194.2 ± 32.0	198.6 ± 36.2	206.0 ± 37.9	217.3 ± 40.8	224.6 ± 42.7	237.8 ± 43.7
Triglycerides (mg/dl)	78 (57–106)	93 (70–127)	127 (91–176)	192 (131–252)	255 (205–346)	269 (214–363)
HDL cholesterol (mg/dl)	63.5 ± 12.8	58.2 ± 14.6	49.5 ± 13.2	42.4 ± 10.9	37.5 ± 7.8	36.2 ± 6.8
Blood glucose (mg/dl)	92.6 ± 13.5	98.4 ± 22.5	105.5 ± 33.0	114.4 ± 43.9	126.5 ± 51.3	196.7 ± 69.7
High blood pressure (%)	0.0	72.1	82.8	93.7	99.4	100
High triglycerides (%)	0.0	2.8	20.0	55.1	89.3	100
Low HDL cholesterol (%)	0.0	16.1	46.0	73.5	93.2	100
High blood glucose (%)	0.0	2.1	11.7	19.2	33.3	100
Drinking						
Never drinker (%)	73.8	64.0	70.9	66.8	73.8	73.0
Ex-drinker (%)	2.3	2.7	3.4	3.8	3.3	10.8
Current drinker (%)	23.9	33.3	25.7	29.4	22.9	16.2
Smoking						
Never smoker (%)	65.8	58.2	61.6	58.1	54.2	56.8
Ex-smoker (%)	8.6	11.2	11.8	12.3	13.7	10.8
Current smoker (%)	25.6	30.6	26.6	29.6	32.1	32.4
Physical activity						
Yes (%)	18.9	20.3	20.6	21.2	19.3	24.3
No for physical problems (%)	3.4	5.3	6.8	7.1	9.0	10.8
No for other reasons (%)	77.7	74.4	72.6	71.8	71.7	64.9

Data are %, mean ± SD, or median (interquartile range). Metabolic factors were defined as follows: obesity as BMI ≥25 kg/m^2, high blood pressure as systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg and/or medication, high blood glucose as nonfasting blood glucose ≥140 mg/dl and/or medication, high triglycerides as nonfasting triglycerides ≥200 mg/dl and/or medication, low HDL cholesterol as HDL cholesterol ≤40 md/dl for men or ≤50 mg/dl for women.

the details of these cohorts have been reported (10–15). Here, we analyzed data from NIPPON DATA90 because the baseline survey of NIPPON DATA80 does not include some important metabolic factors such as HDL cholesterol.

A total of 8,384 residents (3,504 men and 4,880 women, aged ≥30 years) from 300 randomly selected districts participated in the survey and were followed until 15 November 2000. The participation rate in this survey was 76.5%. Of the 8,384 participants, 1,165 were excluded because of a history of coronary heart disease or stroke (n = 371), information missing at the baseline survey (n = 636), and failure to access because of incomplete residential access information at the first survey (n = 158). The remaining 7,219 participants (2,999 men and 4,220 women) were included in the analysis.

Table 2—*Multiple adjusted HRs and 95% CIs according to the individual components of metabolic risk factor in 2,999 men and 4,220 women aged ≥30 years (NIPPON DATA90, 1990–2000)*

Component of metabolic factor	*n*	HR (95% CI)
Obesity	1,706	0.87 (0.60–1.27)
High blood glucose	579	1.45 (0.99–2.14)
High blood pressure	4,530	2.07 (1.21–3.52)
High triglycerides	1,259	1.42 (0.95–2.11)
Low HDL cholesterol	2,224	0.79 (0.56–1.12)

HRs were estimated by a Cox proportional hazards model adjusted for sex, age, total cholesterol, smoking habits, drinking habits, physical activity, and other components of metabolic factors. Metabolic factors were defined as in the footnote to Table 1. *n* is the number of participants who had the conditions.

Follow-up survey

The underlying causes of death in the National Vital Statistics were coded according to the ICD-9 until the end of 1994 and according to the ICD-10 from the start of 1995 until the end of 2000. Details of these classifications are described elsewhere (10–15). The Institutional Review Board of Shiga University of Medical Science (No. 12–18, 2000) approved this study.

Baseline examination

Nonfasting blood samples were obtained at the baseline survey. The serum was separated and centrifuged soon after blood coagulation. Plasma samples were collected in siliconized tubes containing sodium fluoride and shipped to one laboratory (SRL, Tokyo, Japan) for blood measurements. Plasma glucose was measured enzymatically. Serum triglycerides

Table 3—*Multiple adjusted HRs and 95% CIs according to number of metabolic factors in 2,999 men and 4,220 women ≥30 years (NIPPON DATA90, 1990–2000)*

Number of metabolic factors	*n*	Person-years	Cardiovascular deaths	HR (95% CI)
0	1,604	15,740	8	1.00 (—)
1	2,657	25,398	67	1.93 (0.92–4.03)
2	1,643	15,526	52	1.94 (0.91–4.13)
3	942	8,999	29	2.12 (0.96–4.70)
4	336	3,167	15	2.44 (1.02–5.84)
5	37	361	2	3.27 (0.69–15.50)
				P_{trend} 0.074

HRs were estimated by a Cox proportional hazards model adjusted for sex, age, total cholesterol, smoking habits, drinking habits, and physical activity. Metabolic factors were defined as in the footnote to Table 1.

and total cholesterol were also measured enzymatically, and HDL cholesterol was measured after heparin-calcium precipitation (16).

BMI was calculated as weight in kilograms divided by the square of height in meters. Baseline blood pressure was measured by trained observers using a standard mercury sphygmomanometer on the right arm of seated participants. Public health nurses obtained information on smoking, alcohol consumption, physical activity, and medical history. We divided participants into four categories of smokers (never-smoked, ex-smoker, and current smoker <20 or ≥20 cigarettes/day) and six categories of drinking (never-drinker; ex-drinker; and current drinker of 1, 2, 3, and 4 gou of sake/day; 1 gou [180 ml] is equivalent to 23 g of alcohol) (11). We divided participants into three categories of physical activity (yes or no for physical problems, and no for any other reason).

We defined metabolic factors as follows: obesity, BMI ≥25 kg/m^2; high blood pressure, systolic blood pressure ≥130 mmHg, diastolic blood pressure ≥85 mmHg, administration of antihypertensive agents, or any combination of these; and high blood glucose, serum glucose ≥140 mg/dl, medication for diabetes, or both. Because our samples were nonfasting, the postload blood glucose level for diagnosis of impaired glucose tolerance was ≥140 mg/dl (17). We defined high triglycerides as nonfasting serum triglyceride ≥200 mg/dl and also as taking medication for dyslipidemia. Low HDL cholesterol was defined as serum HDL cholesterol ≤40 mg/dl for men and ≤50 mg/dl for women.

Statistical analysis

Continuous variables were compared using ANOVA, and the χ^2 test was used to compare the dichotomized variables to examine differences in baseline characteristics of participants according to the numbers of clustering metabolic factors.

The multivariate adjusted hazard ratio (HR) of all CVD mortality for each group was calculated using the Cox proportional hazards model adjusted for age, sex, total cholesterol, smoking, drinking, and physical activity category. When we calculated HR for an individual component of a metabolic factor, we further adjusted for other components of the metabolic factor. We used nonobese participants without any metabolic factor or participants with neither a metabolic factor nor high blood glucose as references in analyses stratified by obesity or high blood glucose (required component by the IDF and WHO, respectively). Because leaner participants also have a higher CVD mortality risk in Japan, we further analyzed a data subset excluding leaner participants (BMI <18.5 kg/m^2) (18,19).

All CIs were estimated at the 95% level. $P < 0.05$ was considered significant. The Statistical Package for the Social Sciences (version 11.0J; SPSS Japan, Tokyo, Japan) was used to perform all analyses.

RESULTS — Table 1 shows the baseline characteristics of the study participants according to the numbers of metabolic factors. Total person-years were 69,170, and the mean follow-up period was 9.6 years. During follow-up, 625 participants died of all causes and 173 died of CVD. Table 2 shows the multiple adjusted HRs and 95% CIs according to individual components of metabolic risk factors.

Table 3 shows the number of deaths, multiple adjusted HRs, and 95% CIs according to various numbers of metabolic factors. The HRs for CVD mortality were higher in the group with more metabolic factors, but the trend was not statistically significant ($P_{trend} = 0.074$). The relationship between numbers of risk factors and CVD mortality did not differ according to sex ($P_{interaction} = 0.70$). We therefore combined men and women in the following analyses. The tendency for HR to be higher in those with more metabolic factors was similar for heart disease (three risk factors: HR 2.08 [95%CI 0.67–6.48]; four risk factors: 3.97[1.24–12.72]; five risk factors: not applicable) and stroke (three risk factors: 2.07 [0.67–6.37]; four risk factors: 1.23 [0.30–5.05]; five risk factors: 6.26 [CI, 1.13–34.60]) mortality. The HR tendency for all-cause mortality was similar, but the number of clustering metabolic factors was not significantly related to all-cause mortality (three risk factors: 1.16 [0.81–1.65]; four risk factors: 1.18 [0.77–1.80]; five risk factors: 1.44 [0.57–3.63]).

Table 4 shows multiple adjusted HRs (95% CI) due to the number of metabolic factors except high blood glucose stratified by high blood glucose. The HRs trended to increase in both groups (with and without high blood glucose). The HR for CVD in participants with ≥3 metabolic factors but high blood glucose was modest and not statistically significant. Conversely, HRs were obviously higher for participants with high blood glucose and ≥2 other metabolic factors than those for participants with neither metabolic factors nor high blood glucose. The risk increases were statistically significant.

Table 4 also shows multiple adjusted HRs (95% CI) for CVD mortality according to the number of metabolic factors other than obesity stratified by obesity. The relationship between HRs and the numbers of metabolic factors was positive in both obese and nonobese groups. This relationship was unchanged when participants with lower BMI (≥18.5 kg/m^2) were excluded.

CONCLUSIONS — We found that metabolic factor clustering was positively associated with CVD mortality in the general Japanese population. The risk increase in participants with both high blood glucose and ≥2 metabolic factors was significantly higher than in those with neither high blood glucose nor metabolic risk factors. The risk in nonobese participants with more metabolic factors was also increased.

Although investigating the relation-

Table 4—*Blood glucose category–specific multiple-adjusted HRs and 95% CIs according to number of metabolic factors other than high blood glucose and BMI category–specific multiple-adjusted HRs and 95% CIs according to the number of metabolic factors other than obesity in 2,999 men and 4,220 women aged ≥30 years (NIPPON DATA90, 1990–1999)*

	Number of metabolic factors	*n*	Person-years	Cardiovascular deaths	HR (95% CI)	HR (95% CI)
High blood glucose						
Without	0	1,604	15,740	8	1.00 (—)	
	1	2,600	24,867	63	1.91 (0.91–4.02)	
	2	1,451	13,796	45	1.99 (0.93–4.28)	
	≥3	985	9,522	22	1.61 (0.71–3.67)	
With	0 and 1	249	2,241	9	1.78 (0.68–4.67)	
	2	181	1,638	12	3.67 (1.49–9.03)	
	≥3	149	1,267	12	3.25 (1.31–8.06)	
BMI						
<25 kg/m^2	0	1,604	15,740	8	1.00 (—)	1.00 (—)
	1	2,474	23,576	67	1.98 (0.94–4.17)	2.14 (0.85–5.43)
	2	993	9,282	37	1.95 (0.90–4.25)	2.24 (0.86–5.82)
	≥3	442	4,108	24	2.83 (1.25–6.39)	3.35 (1.25–8.95)
≥25 kg/m^2	0 and 1	833	8,045	15	1.75 (0.73–4.16)	2.12 (0.76–5.89)
	2	551	5,339	10	1.47 (0.57–3.75)	1.78 (0.59–5.19)
	≥3	322	3,080	12	2.37 (0.96–5.89)	2.84 (0.99–8.17)

HRs were estimated by a Cox proportional hazards model adjusted for sex, age, total cholesterol, smoking habits, drinking habits, and physical activity. High blood glucose was defined as nonfasting blood glucose ≥140 mg/dl and/or medication. Metabolic factors were defined as follows: obesity as BMI ≥25 kg/m^2, high blood pressure as systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg and/or medication, high triglycerides as nonfasting triglycerides ≥200 mg/dl and/or medication, low HDL cholesterol as HDL cholesterol ≤40 mg/dl for men or ≤50 mg/dl for women. In the group with high blood glucose, 0 and 1 metabolic factors were combined because we found only two cardiovascular deaths in the group whose number of metabolic factors was 0. †HRs (95% CI) were analyzed for participants with BMI >18.5 kg/m^2. Metabolic factors were defined as follows: high blood pressure as systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg and/or medication, high blood glucose as nonfasting blood glucose ≥140 mg/dl and/or medication, high triglycerides as nonfasting triglycerides ≥200 mg/dl and/or medication, low HDL cholesterol as HDL cholesterol <40 mg/dl for men or ≤50 mg/dl for women. In the group with BMI ≥25 kg/m^2, 0 and 1 metabolic factors were combined because we found no cardiovascular death in the group whose number of metabolic factors was 0.

ship between metabolic factor clustering and CVD mortality is important, prospective studies on the topic are still scarce. On the basis of the NCEP and WHO definitions of metabolic syndrome, several investigators have reported that participants with metabolic syndrome or metabolic factor clustering have a high HR of CVD mortality (20–25). Ford (26) summarized prospective cohort studies and reported that the HRs of CVD mortality were 1.65 [5% CI 1.38–1.99] according to the NCEP definition and 1.93 [1.39–2.67] according to the WHO definition, respectively. This result is consistent with our findings that participants with more metabolic factors have a higher risk of CVD mortality. Our results were also comparable with those of a prospective study in Japan showing that the relative risk of cardiac diseases was 2.23 [1.14–4.34] in participants with >3 metabolic factors compared with that in participants with <3 metabolic factors (27).

The IDF definition requires obesity for diagnosis of metabolic syndrome. These guidelines explain that central (abdominal) obesity is a prerequisite for this diagnosis because it is easy to assess and independently associated with each of the other metabolic syndrome components (8). The IDF guidelines do not essentially require insulin resistance because it is difficult to measure in day-to-day clinical practice (7,8). However, although increased waist circumference is an important component of metabolic syndrome, some individuals with multiple risk factors and an increased risk of CVD mortality have normal waist circumference (28,29). For example, Katzmarzyk et al. (28) reported that waist circumference is a valuable component of metabolic syndrome, but they also raised the concern that the IDF requirement of an increased waist circumference warranted caution because a large proportion of individuals with normal waist circumference also have multiple risk factors and an increased risk of mortality.

We found here that nonobese participants with three or more metabolic factors had significantly higher HRs for CVD death and that their risk was similar to that of obese participants with the corresponding number of metabolic factors. Thus, a proportion of high-risk participants might be overlooked if obesity is a diagnostic requirement for metabolic syndrome. Waist circumference supposedly indicates visceral fat more accurately than BMI in terms of predicting diabetes (30). However, we did not have any information about waist circumference and used BMI as it closely correlates with waist circumference. Furthermore, BMI has been used to diagnose obesity in many epidemiological studies of metabolic syndrome (22,23), indicating that BMI was acceptable for our purposes. However, because of the use of BMI, we might have underestimated the impact of obesity on CVD mortality. A similar study using waist circumference should clarify the relation.

The WHO guidelines indicate that the presence of diabetes, impaired glucose tolerance, or insulin resistance is necessary for a diagnosis of metabolic syndrome because this condition is considered a special classification for those with the potential for diabetes (manifested as impaired glucose tolerance, impaired fasting glucose, or insulin resistance determined using the hyperinsulinemic-euglycemic clamp) (1,7). Here, we also stratified participants according to blood glucose level and found that the HR was higher among those with than among those without high blood glucose. These findings suggest that glucose toler-

ance plays an important role in CVD mortality. Some reports have shown higher HRs with use of the WHO rather than the NCEP definition of metabolic syndrome. This result means that the participants with impaired glucose tolerance have higher HRs, a finding that the present results support (26). However, several participants with clustering of metabolic factors other than impaired glucose tolerance also had an increased risk of CVD mortality.

Some limitations other than using BMI should be noted about the present study. First, we used nonfasting blood samples and thus we might have misclassified participants with high blood glucose or hypertriglyceridemia. Second, we did not adjust for socioeconomic status because relevant information was not available. However, all Japanese are covered by the national health insurance program and socioeconomic status does not affect access to treatment. Therefore, the impact of socioeconomic status on our findings should be minimal.

In summary, the CVD risk was obviously higher among individuals with than among those without high blood glucose and multiple metabolic risk factors, suggesting that high blood glucose plays an important role in CVD mortality. Conversely, the prevalence of nonobese participants with several metabolic factors was quite high and their CVD risk was high. Thus, metabolic factors should be carefully considered and appropriately managed even among individuals with a BMI <25.

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REVIEW

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Pathophysiological significance of adiponectin

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Abstract Adipose tissue, which classically has been considered as an energy-storing organ, is now viewed as a massive source of bioactive substances such as leptin, tumor necrosis factor (TNF)-α, and adiponectin. Adiponectin was discovered to be the most abundant adipose-specific transcript. Its function had been unclear, but epidemiological and clinical studies have demonstrated that serum levels of adiponectin are inversely associated with body weight, especially abdominal visceral fat accumulation. In addition, adiponectin was inversely related to cardiovascular risk factors, such as insulin resistance, blood pressure, and low-density lipoprotein (LDL) cholesterol and triglyceride levels, and was positively related to high-density lipoprotein (HDL) cholesterol levels. Moreover, low adiponectin concentration is associated with a high incidence of cardiovascular disease (CVD), diabetes, some kinds of cancer, and other various diseases. These associations suggest the clinical significance of adiponectin, and a number of investigations are now being conducted to clarify the biological functions of adiponectin. Recent studies have revealed that adiponectin exhibits antiinflammatory, antiatherogenic, and antidiabetic properties. In addition, adiponectin has been thought to be a key molecule in "metabolic syndrome," which is an epidemiological target for preventing cardiovascular disease. Various functions of adiponectin may possibly serve to prevent and treat obesity-related diseases and CVD. Furthermore, enhancement of adiponectin secretion or action may become a promising therapeutic target.

Key words Adiponectin · Visceral fat · Adipocytokine · Cardiovascular disease · Metabolic syndrome

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Introduction: the discovery of adiponectin

Obesity, in particular, abdominal visceral fat accumulation, is an important risk factor for hyperlipidemia, diabetes mellitus, hypertension, cardiovascular disease (CVD), and some kinds of cancer. However, the molecular mechanism underlying these linkages had not been previously elucidated. We investigated the characteristics of adipose tissue by analyzing the gene expression profile in visceral and subcutaneous fat in the human complementary DNA project.[1] Of approximately 1000 independent clones, 60% of the whole genes were already identified as known human genes. The remaining 40% of genes were novel genes.[2] Although adipose tissue was considered as an energy-storing organ, we found unexpectedly high frequencies of the genes encoding secretory proteins.[3] In subcutaneous adipose tissue, approximately 20% of all known genes were the genes encoding secretory protein (Fig. 1).[2,3] Furthermore, its frequency reached approximately 30% in visceral adipose tissue. In addition, leptin and tumor necrosis factor (TNF)-α had been well recognized as bioactive substances from adipose tissues that regulate the functions of other organs. We named these adipose tissue-derived bioactive substances adipocytokines,[3] although some of them are not cytokines according to the classical category.

We identified the gene that expressed most abundantly and specifically in adipose tissue in 1996.[1] The molecule encoded by this gene, adipose most abundant gene transcript-1 (apM-1), possesses a signal peptide and collagen-like motif (Fig. 2).[1] We termed this matrix-like protein adiponectin. Adiponectin was independently isolated from human plasma as gelatin-binding protein-28.[4] The mouse homologue of adiponectin was cloned as ACRP30 and AdipoQ at the same time.[5,6] However, the significance of this novel molecule was unclear. Then, we developed a method for the measurement of plasma adiponectin levels using an enzyme-linked immunosorbent assay.[7] Measurement of plasma adiponectin revealed the clinical significance of adiponectin.

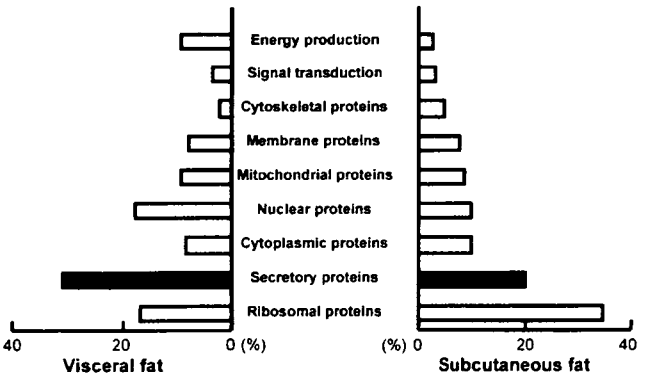

Fig. 1. The high frequency of genes for secretory proteins in adipose tissue. Although adipose tissue has been considered to be an energy-storing organ, an unexpectedly high frequency of the genes encoding secretory proteins is demonstrated. In subcutaneous adipose tissue, approximately 20% of all known genes are the genes encoding secretory protein. Furthermore, its frequency reaches approximately 30% in visceral adipose tissue. [Reproduced with permission from Maeda et al.[7] (1997) Gene 190:227–235; Funahashi et al.[2] (1999) Intern Med 38:202–206]

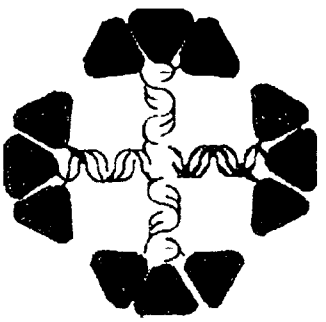

Fig. 2. Structure of adiponectin. This 244-amino-acid protein contains a signal sequence, and a collagen-like domain at the N-terminus and a C1q-like globular domain at the C-terminus. Some units of the trimer of adiponectin are bound in a bouquet-like formation in plasma. [Reproduced with permission from Matsuzawa et al.[10a] (2004) Arterioscler Thromb Vasc Biol 24:29–33]

Molecular characteristics of adiponectin

Structure

The gene encoding adiponectin is located on chromosome 3q27, and recent genome-wide scans have mapped a diabetes susceptibility locus to this chromosome.[8–10] This 244-amino-acid protein contains a signal sequence, and a collagen-like domain at the N-terminus and a C1q-like globular domain at the C-terminus (see Fig. 2).[1] The globular domain has sequence homology to collagens VIII and X and complement factor C1q. The crystal structure is similar to that of the TNF family, which has identical folding topologies and similar trimer interfaces.[11] Some units of the trimer of adiponectin are bound up in a bouquet-like formation.[12] Two major oligomeric forms of adiponectin, a hexamer and a 400-kDa high molecular weight (HMW) complex, exist in plasma. The HMW form of adiponectin has been shown to be more active than low molecular weight forms.[13] Hydroxylation and glycosylation of the lysine residues within the collagenous domain of adiponectin are critically involved in regulating the formation of its HMW oligomeric complex.[14]

Full-length adiponectin protein is proteolytically cleaved, with a smaller form, including the globular domain, although in very small amounts.[15] It is reported that the globular domain of adiponectin exhibits more extensive biological activity than the full-length form. However, further studies are needed to clarify the biologically active form of adiponectin and the relative abundance of the different cleavage products in plasma under physiological conditions.

Localization

Adiponectin messenger RNA is exclusively expressed in adipose tissue of humans[1,4] and experimental animals.[5,16] Recent evidence indicated that adiponectin can be produced by organs other than adipose tissue, such as bone marrow,[17] bone-forming cells,[18] fetal tissue,[19] myocytes, cardiomyocytes,[20] and salivary gland epithelial cells,[21] but the major source of plasma adiponectin in adults is the adipocyte. Plasma levels of adiponectin usually range from 3 to 30 μg/ml in adults, whereas adiponectin levels in umbilical venous blood from human fetuses was about 30 μg/ml.[19] Adiponectin was detected in several fetal tissues at mid- and late gestation (from 14 to 36 weeks) but not in the placenta. Adiponectin was detected in human fetal tissues of mesodermic origin, such as brown and white adipocytes, skeletal muscle fibers of diaphragm and iliopsoas, smooth muscle cells of small intestine and arterial walls, perineurium and renal capsule, and tissues of ectodermal origin, such as epidermis and ocular lens. The distribution of adiponectin detection in nonadipose tissues showed a general decline during the progression of gestation.[19] It should be noted that these results do not necessarily demonstrate the production of adiponectin in nonadipose tissues but represent the existence of adiponectin that may adhere to the tissue. In mouse embryos, production of adiponectin was demonstrated in brown adipose tissues (BAT) and surrounding immature tissues using immunohistochemical staining and in situ hybridization.[22] This interspecies difference of fetal localization of adiponectin may be attributed to the small amount of BAT in humans. These studies suggest the adiponectin may have a role during fetal development.

Secretion

Recent studies established the concept that adipose tissue is not only a fuel storage depot but also a critical endocrine tissue secreting a variety of bioactive adipocytokines into the circulation. Despite the importance of adipocytokines in metabolism, the mechanism of secretion from adipocytes remains poorly elucidated. Cellular localization of adiponectin in the steady state is predominantly in the Golgi apparatus or trans-Golgi network (TGN).[23–25] Treatment of 3T3-L1

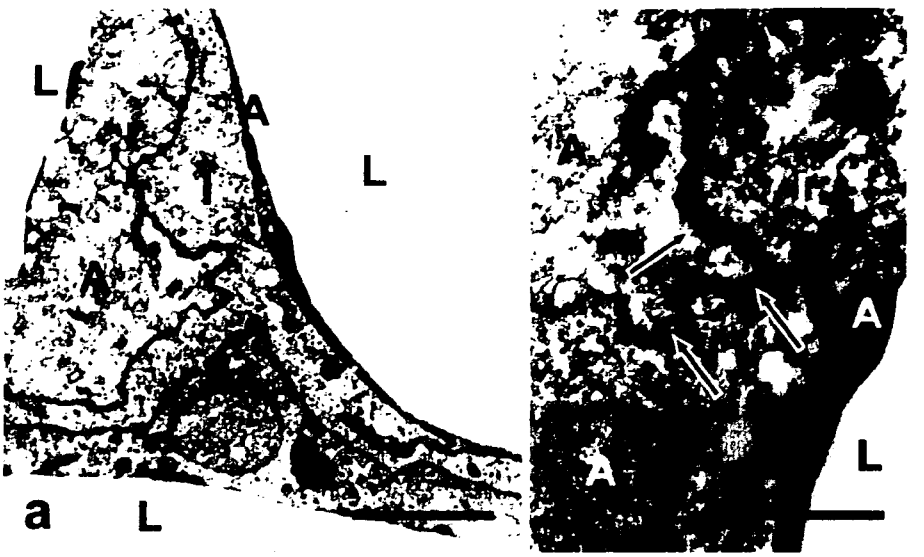

Fig. 3. Caveolae of adipocytes. Three human adipocytes are demonstrated in the *left panel* (a). Many caveolae on the cell membrane (*arrows*) are found at a higher magnification (b). Most studies of function of caveolae have focused on endocytosis and signal transduction. Further morphological studies in adipocytes as secretory cells may reveal a new secretory mechanism of adipocytokines including adiponectin. *A*, adipocytes; *I*, interstitial tissue; *L*, lipid droplet. *Bars* a 2μm; b 1μm

adipocytes with brefeldinA (BFA), an inhibitor of the post-Golgi trafficking pathway, severely inhibited the secretion of adiponectin.[24,25] There should be secretory pathways of adiponectin from Golgi/TGN to the cell surface. Moreover, fractional analyses demonstrated that the adiponectin fraction overlapped with transferrin receptor-positive membranes, indicating that secretory pathways of adiponectin involve the transferrin receptor-positive endosomal system.[25] Generally, some secretory cargo can traffic to the cell surface via the endosomal system.[26,27] The traffic in the endosomal system is controlled by a variety of small molecular weight GTPases of both the Rab and ADP ribosylation factor (Arf) classes.[28-31] Rab11 has been involved in insulin-dependent trafficking in adipocytes.[32] Arf6 controls cell phosphatidylinositol-(4,5)-bisphosphate levels in the plasma membrane and may be involved in regulated exocytosis.[33] In addition, Arf6 may play a role in the recycling of endosomal components with the plasma membrane.[34,35] In 3T3-L1 adipocytes, it has been demonstrated that Rab11 and Arf6 are important mediators of constitutive and insulin-stimulated secretion of adiponectin.[25]

Regulated exocytosis in adipocytes has been investigated by insulin-stimulated secretion of peptides and recycling of vesicles containing GLUT4 to the cell surface.[24] A confocal microscopic study demonstrated that the subcellular distributions of adiponectin and GLUT4 are distinct and non-overlapping,[25] although some molecules have been shown to regulate both adiponectin and GLUT4, implying the existence of common trafficking pathways between adiponectin and GLUT4. One molecule is the GGA1 (for Golgi localizing γ-adaptin ear homology domain ARF-binding protein) protein, which is a monomeric clathrin adaptor that mediates sorting at the TGN of specific cargo in an Arf-dependent manner. Inhibition of GGA1 blocks both traffic of the GLUT4 to its insulin-sensitive intracellular compartment and secretion of adiponectin (but not leptin).[24] It is also speculated that GGA proteins regulate selective cargo formation at the TGN and that insulin may act via a distal (post-TGN) compartment.[24] Another molecule is a kind of v-SNARE. It is suggested that the v-SNARE Vti1a is likely regulating a common and early step in the trafficking of both adiponectin and GLUT4 in 3T3-L1 adipocytes.[36]

There are many caveolae in the cell membrane of adipocytes[37] (Fig. 3). Most studies on the functions of caveolae have focused on endocytosis and signal transduction. Regarding secretion, cholesterol efflux mediated by caveolae or rafts has been investigated. Further morphological studies in adipocytes as secretory cells may reveal a new secretory mechanism of adipocytokines, including adiponectin.

Receptor

Two adiponectin receptors were identified in 2003.[38] AdipoR1 is a receptor for globular adiponectin that is abundantly expressed in skeletal muscle. AdipoR2 is a receptor for full-length adiponectin that is mainly expressed in the liver. Expression of AdipoR1 and -R2 was also detected in the hypothalamus, and increased AdipoR2 expression was found in the paraventricular nucleus (PVN), which may be involved in energy regulation.[39] These molecules are distantly related to the family of seven-transmembrane-spanning G protein-coupled receptors. They have an inverted topology with the N-terminus intracellular and the extracellular portion being small, as distinct from members of this class of receptors that bind peptide hormones.[38,40] Further studies are needed to elucidate the physiological role and the signal transduction pathways of these receptors.

T-cadherin has been demonstrated as a receptor for hexameric and HMW adiponectin but not for trimeric or globular adiponectin.[40] T-cadherin is a glycosylphosphatidylinositol (GPI)-anchored extracellular protein. Tissue distribution of T-cadherin is widespread in cardiovascular system, nervous system, and muscle. T-cadherin is involved in signal transduction in addition to cell–cell ad

hesion.[41,42] However, T-cadherin is not highly expressed in the hepatocyte, which is one of the major targets of adiponectin.[13,43]

The plasma concentration of adiponectin is much higher than that of common cytokines. Therefore, some physiological roles of adiponectin may not be mediated by receptors. Adiponectin may have an important regulatory function that involves low-affinity macromolecular interactions.

Clinical significance of adiponectin

Obesity

Many studies have been showed relationships between plasma adiponectin levels and a variety of diseases. First of all, it was demonstrated that plasma adiponectin levels were inversely correlated with body mass index (BMI).[7] In addition, reduction of body weight increased plasma adiponectin levels.[44] Conversely, leptin is another adipose tissue-specific secretory protein that increased with BMI.[45] The negative correlation between adiponectin levels and visceral adiposity becomes more apparent than that between adiponectin levels and subcutaneous adiposity.[46,47] The mechanism of reduction in plasma adiponectin levels in subjects with visceral fat accumulation has not been elucidated. For one explanation, expression of TNF-α, which is a potent inhibitor of adiponectin promoter activity,[48] increases along with the accumulation of visceral fat.

Cardiovascular disease

The significance of adiponectin is that this protein shows lower levels in patients with ischemic heart disease.[49] In end-stage renal disease, subjects with low adiponectin levels died of cardiac events more frequently during 4 years of observation.[50] These data suggest that hypoadiponectinemia may be a novel and important risk factor of atherosclerosis.[49] Moreover, a prospective study demonstrated that men with high adiponectin levels were at lower risk of myocardial infarction (MI) than those with medium to low levels.[51] This association was independent of traditional cardiovascular risk factors such as hypertension or diabetes.[51] Cross-sectional studies have also demonstrated a relationship between adiponectin levels and CVD.[49,52-54] The risk of CVD was twofold higher in the lowest versus the highest quartiles of adiponectin levels, after adjusting for other risk factors including diabetes, dyslipidemia, hypertension, smoking, and BMI.[49] Low adiponectin levels were also associated with increased carotid atherosclerosis as well as CVD.[53]

Vulnerability of coronary plaque is known to be a very important issue in acute coronary syndrome (ACS). Plasma concentrations of adiponectin in patients with ACS were significantly lower than those in patients with stable angina pectoris. Multiple logistic regression analysis revealed independent correlation of hypoadiponectinemia with the development of ACS.[55] In addition, plasma adiponectin levels are significantly associated with coronary lesion complexity in men with CAD.[56] These data suggest that adiponectin plays a significant role in plaque stability.

Diabetes

Another main clinical significance of adiponectin has been confirmed in diabetes.

We found that subjects with type 2 diabetes had lower adiponectin levels than control subjects.[52] It was also revealed that plasma adiponectin levels were lower in Pima Indians, a unique cohort with a high prevalence of obesity and diabetes.[57] In this cohort, a longitudinal study showed that the individuals with high adiponectin levels were less likely to develop type 2 diabetes than those with low adiponectin levels.[57] Additionally, a high adiponectin level was a more protective factor against development of diabetes than small waist circumference, fasting glucose, 2-h glucose, or fasting insulin levels.[57] Other studies in different populations including the Japanese have also suggested that low adiponectin levels are predictive of future development of insulin resistance and diabetes,[58-61] and that a high adiponectin level is strongly associated with a lower risk of impaired glucose metabolism and type 2 diabetes, particularly in women.[62] In obese and diabetic monkeys, plasma adiponectin levels decreased before the onset of diabetes, in parallel with the decrease of insulin sensitivity.[16] This result supports the findings observed in humans and demonstrates the significance of adiponectin in the development of diabetes.

Hypertension and dyslipidemia

Recent studies have shown the significance of low adiponectin levels in hypertensive patients. A case-control study showed low adiponectin levels in hypertensive patients and a significant negative correlation between plasma adiponectin concentration and mean, systolic, and diastolic blood pressure.[63] Multiple regression analysis also indicated that hypoadiponectinemia was an independent risk factor for hypertension in a study of 758 hypertensive and normotensive men.[64] In addition, a significant relationship between hypertension and adiponectin levels was found in men but not in women.[65] The significance of adiponectin in hypertension has been confirmed in a mouse model. In obese mice, adenovirus-delivered adiponectin significantly decreased blood pressure. This study suggests that hypoadiponectinemia contributes to the development of obesity-related hypertension, at least in part, directly, in addition to its effect via insulin resistance.[66]

It was well documented that adiponectin is associated with dyslipidemia.[46,67-71] Plasma adiponectin levels positively correlated with HDL-C and negatively correlated with triglycerides and apolipoprotein (Apo) B-100 and were not appreciably altered after adjusting for obesity-associated variables.[67] A recent study demonstrated a direct effect of adiponectin in hepatocytes. HMW adiponectin reduced the

hepatic release of ApoB and ApoE, whereas ABCA1 function and ApoA-I secretion were not influenced. In addition, HMW adiponectin reduced hepatic nuclear factor 4-α (HNF4-α) and HNF4-α-regulated genes such as the ApoB gene.[72] This mechanism may explain the association between hyperlipidemia and low adiponectin levels in the portal vein accompanied by visceral fat accumulation.

Metabolic syndrome

Metabolic syndrome is defined by a cluster of pathological conditions that include abdominal obesity, dyslipidemia, hypertension, and hyperglycemia. Metabolic syndrome has been well recognized as a promising target to prevent CVD. Low adiponectin levels have shown an association with the metabolic syndrome in healthy middle-aged subjects.[69] A prospective cohort study in elderly Koreans confirmed the significance of adiponectin.[73] In a study of 661 Japanese adults, the number of components of the metabolic syndrome increased along with the decrease of plasma adiponectin concentration.[74]

We found four types of missense mutation of adiponectin. In these mutations, I164T mutation was accompanied by remarkable hypoadiponectinemia. We have found nine subjects with the I164T mutation; eight of the nine exhibited hypertension or hyperlipidemia and all nine were accompanied by impaired glucose metabolism, including impaired glucose tolerance (IGT) or diabetes mellitus.[75] These results suggest that genetic hypoadiponectinemia may be part of the genetic background of metabolic syndrome. Now, adiponectin is viewed as a key molecule in metabolic syndrome.

Inflammation

It is well established that inflammatory markers are predictive of CVD. Inflammation is now considered as an important pathological basis for both CVD and metabolic syndrome. Some studies found an inverse association between adiponectin and inflammatory markers such as TNF-α, interleukin 6, and C-reactive protein in normal subjects and patients with CVD or metabolic syndrome.[76-80] We found that expression of CRP was detected in adipose tissue and that expression of adiponectin is inversely associated with that of CRP.[77] We also demonstrated that hypertrophied mesenteric adipose tissue produced and secreted adiponectin in Crohn's disease.[81] We think that mesenteric adipocytes may act as immunoregulating cells in the case of intestinal inflammation via adiponectin production. Another study found that synovial fluid and plasma levels of adiponectin were significantly higher in rheumatoid arthritis (RA) than in control subjects. Adiponectin levels were negatively associated with the leukocyte count in RA synovial fluid, implying an antiinflammatory effect of adiponectin in RA synovial fluid.[82] These data indicate that adiponectin may be induced compensatorily in response to local inflammation. Increased adiponectin may prevent fibrosis in these inflammatory lesions.

Cancer and other diseases

Obesity is a significant risk factor for the development of several cancers,[83] but the mechanisms underlying this relationship remain to be fully elucidated. Adiponectin is likely to play an important role in the development and progression of some obesity-related malignancies. Recent studies showed that plasma adiponectin levels are inversely associated with the risk of cancers associated with obesity and insulin resistance.[84] Low adiponectin levels have been associated with endometrial cancer,[85-87] breast cancer, especially in postmenopausal women,[88,89] colorectal cancer,[90,91] gastric cancer,[92] prostate cancer,[93] and leukemia.[94] Low plasma adiponectin levels may be a novel risk factor for cancer. The mechanism of anticarcinogenic effects of adiponectin has been unclear. However, in vitro, recombinant adiponectin potently inhibited endothelial cell proliferation and migration and induced caspase-mediated endothelial cell apoptosis.[95]

Adiponectin will be of note not only in studies of metabolic disorders but also in investigation of various diseases. For example, increasing levels of adiponectin are associated with a decrease in bone mineral density.[96] It finding suggests that adiponectin may play a role in bone metabolism and may prevent osteoporosis.

Biological functions of adiponectin

Antiatherosclerotic effect

We demonstrated that circulating adiponectin can enter into vascular walls. Immunohistochemical examination demonstrated that adiponectin is not present in untreated normal vascular walls of the rat carotid artery. On the other hand, positive immunohistochemical stain was detected in the balloon-injured vascular walls[97] (Fig. 4). In addition, adiponectin has been shown to bind subendothelial collagen, such as collagen V, VIII, and X. We think that endothelial injury in the process of atherogenesis may lead to entry of adiponectin into the subendothelial space, following the binding of adiponectin to these collagens. Adiponectin has beneficial effects on vascular cells including endothelial cells, macrophages, and smooth muscle cells, and may play a protective role against atherogenesis.

Regarding endothelial cells, decreased adiponectin effect enhances endothelial dysfunction.[98] Plasma adiponectin levels were inversely associated with endothelium-dependent vasodilation in both diabetes patients and controls.[99] Nitric oxide (NO), a potent vasodilator, mediates the effect of adiponectin on endothelial cells.[100,101] Adiponectin ameliorates oxidized LDL (oxLDL)-induced suppression of endothelial NO synthase (eNOS) activity. Adiponectin stimulates production of NO through phosphatidylinositol 3-kinase (PI3K)-dependent pathways involving phosphorylation of eNOS by AMP-activated protein kinase (AMPK).[100] Furthermore, endothelium-dependent vasodilation is significantly reduced in adiponectin knockout (KO) mice.[102]

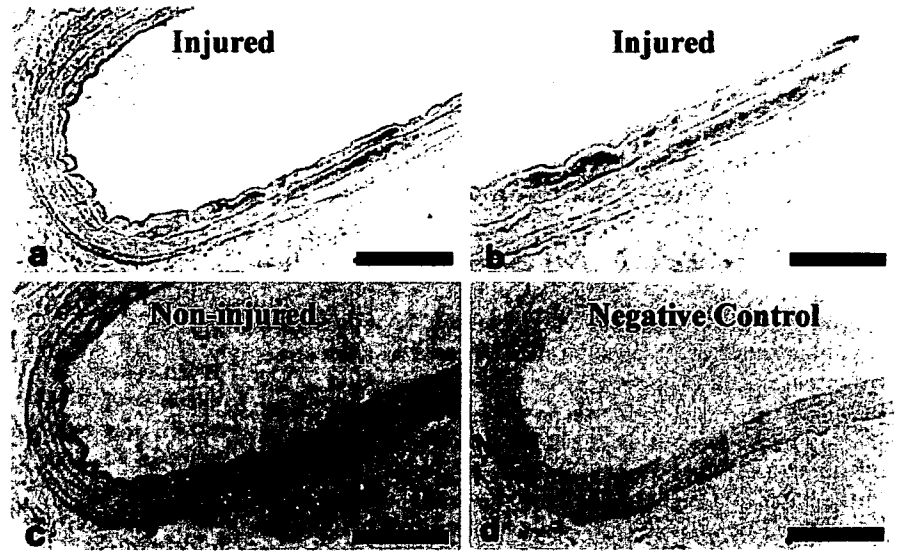

Fig. 4. Localization of adiponectin in an injured artery. Immunohistochemical examination demonstrates the existence of adiponectin in a balloon-injured rat carotid artery (a). At high magnification, adiponectin is detected in subendothelial spaces and media (b). In contrast, adiponectin is not detected in a noninjured artery (c). d Control staining with nonimmune immunoglobulin (Ig). *Bars* a, c, d 100μm; b 50μm. [Reproduced with permission from Okamoto et al.[97] (2000) Horm Metab Res 32:47–50]

Adhesion molecules expressing on the endothelial surface have a critical role in infiltration of macrophages into a vascular wall. The stimulated expression of adhesion molecules by TNF-α was markedly inhibited by the presence of adiponectin dose-dependently.[54] Regulation of adhesion molecules is involved in inflammatory signals, as described next.

In macrophages, adiponectin inhibits the expression of the scavenger receptor class A-1 (SR-A), resulting in markedly decreased uptake of oxLDL and inhibition of foam cell formation.[103] Foam macrophages produce many kinds of matrix metalloproteinases (MMP), which induce rupture of atherosclerotic plaques, causing CVD. Adiponectin increased tissue inhibitor of MMP (TIMP) expression and secretion in human macrophages via induction of IL-10.[104] Consequently, adiponectin may have a role in preventing plaque rupture.

In smooth muscle cells, adiponectin inhibits proliferation and migration. This inhibition was shown to be attributable to binding competition to platelet-derived growth factor (PDGF)-BB receptor of adiponectin and the inhibition of signal transduction through extracellular signal-related kinase (ERK).[105] Adiponectin also inhibited proliferation and migration of smooth muscle cells stimulated by heparin-binding epidermal growth factor (EGF)-like growth factor (HB-EGF). These findings are summarized in Fig. 5. Many offensive factors including oxidized LDL, inflammatory stimuli, and oxidative stress can induce vascular injuries. At that time, circulating adiponectin may accumulate at the injured arteries and protect against the development of atherogenic vascular changes.[106]

Moreover, we investigated platelet thrombus formation in adiponectin KO mice, which showed an accelerated thrombus formation on carotid arterial injury with a He-Ne laser.[107] This study reveals a new role of adiponectin as an endogenous antithrombotic factor. Apart from atherosclerosis, adiponectin may prevent CVD in aspects of cardiac hypertrophy and cardiac ischemic injury. It has been demonstrated in animal models that adiponectin protects against myocardial ischemia-reperfusion injury through both AMPK-dependent antiapoptotic effects and cyclooxygenase (COX)-2-dependent antiinflammatory effects.[108] Adiponectin inhibits hypertrophic signals in the myocardium through activation of AMPK signaling.[109]

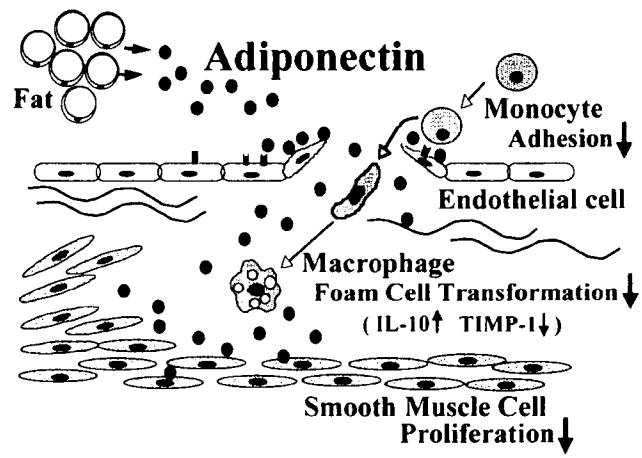

Fig. 5. Antiatherogenic effects of adiponectin. [Reproduced with permission from Matsuzawa et al.[106] (2004) Arterioscler Thromb Vasc Biol 24:29–33]

Antidiabetic effect

In addition to antiatherogenic effects, adiponectin is known as an insulin-sensitizing protein. Intravenous administration of adiponectin lowered hepatic glucose production by reducing the expression of enzymes involved with gluconeogenesis.[110] Treatment with adiponectin, particularly the globular domain of adiponectin, improves insulin sensitivity in animal models of insulin resistance.[43,110–114] Adiponectin is likely to improve insulin sensitivity by stimulating glucose

utilization and fatty acid oxidation through the phosphorylation and activation of AMPK in both skeletal muscle and liver.[111,115] We also demonstrated the antidiabetic effect of adiponectin using adiponectin KO mice. These mice showed no specific phenotype with a normal diet. However, a high-fat and high-sucrose diet induced marked elevation of plasma glucose and plasma insulin levels and marked insulin resistance in these mice.[116] The adiponectin KO mouse may be considered as a model animal of overnutrition-induced diabetes. The supplementation of adiponectin clearly improved this insulin resistance.

As for adiponectin receptors, overexpression of AdipoR1 and -R2 in diabetic mouse liver increased AMPK activation and peroxisome proliferator-activated receptor (PPAR)-α signaling pathway, respectively. AdipoR1- and -R2-deficient mice showed insulin resistance. These data suggest involvement of the adiponectin receptor with insulin resistance in vivo.[117] In vitro, adiponectin binds to the adiponectin receptor on the cell membrane, activating AMPK pathways. Activation of the AMPK signaling pathway reduces serine phosphorylation of IRS proteins, leading to enhanced IRS tyrosine phosphorylation and insulin signaling.[118]

Antiinflammatory effect

Inflammation has been recognized as an important pathological basis in the development of atherosclerosis. Several studies have indicated that adiponectin exhibits antiinflammatory properties in atherogenesis.[76,80,101,119,120] The mechanism of antiinflammatory effects on the endothelial cells has been investigated. Nuclear transcription factor, NF-κB, induces the expression of cytokines and adhesion molecules in the inflammatory process. Adiponectin suppressed TNF-α-induced NF-κB activation without affecting other TNF-α-mediated phosphorylation signals.[120] Moreover, treatment with adenovirus-mediated adiponectin reversed the increased levels of adipose expression of TNF-α and plasma TNF-α in adiponectin KO mice.[116] Besides TNF-α, oxidative stress potently induces inflammatory reactions. Adiponectin also inhibits oxLDL-induced cell proliferation and suppress cellular superoxide generation.[101] A recent study demonstrated that adiponectin promotes the clearance of early apoptotic cells by macrophages through a receptor-dependent pathway involving calreticulin.[121] This function of adiponectin is similar to surfactant proteins and C1q, which serve an antiinflammatory function by promoting the clearance of apoptotic cell debris.[122] In addition, oligomerization of adiponectin seems to be important in the activation of NF-κB signaling pathway.[123]

Adiponectin alters inflammatory reactions in various pathogeneses other than atherogenesis. Adiponectin is protective against chemical-induced colitis in mice, probably because of the inhibition of chemokine production in intestinal epithelial cells and the subsequent inflammatory responses, including infiltration of macrophages and release of proinflammatory cytokines.[124] Adiponectin prevents carbon tetrachloride-induced liver fibrosis by reducing transforming growth factor (TGF) expression in hepatic satellite cells.[125] Adiponectin suppresses TNF-α and induces IL-10 production by Kupffer cells of liver in response to lipopolysaccharide (LPS) stimulation.[126] Continuous infusion of adiponectin attenuates allergic airway inflammation and airway hyperresponsiveness in mice.[127] In bone marrow, adiponectin is an important negative regulator in hematopoiesis and immune systems. Adiponectin blocks fat cell formation in bone marrow cultures by the induction of COX-2 and prostaglandins (PGs) in preadipocytes.[17] A recent study found another mechanism in antiinflammatory effects of adiponectin. Both recombinant and native adiponectin directly bound LPS derived from three different bacteria in the acidic site of inflammation.[128]

Clinical application of adiponectin in the future

Clinical applications of adiponectin have been conducted in animal models for preventing CVD. Adiponectin-deficient mice show excess neointimal thickening in mechanically injured arteries[129,130] (Fig. 6). In adiponectin KO mice, neointimal proliferation is attenuated by adenovirus-mediated adiponectin administration through reducing

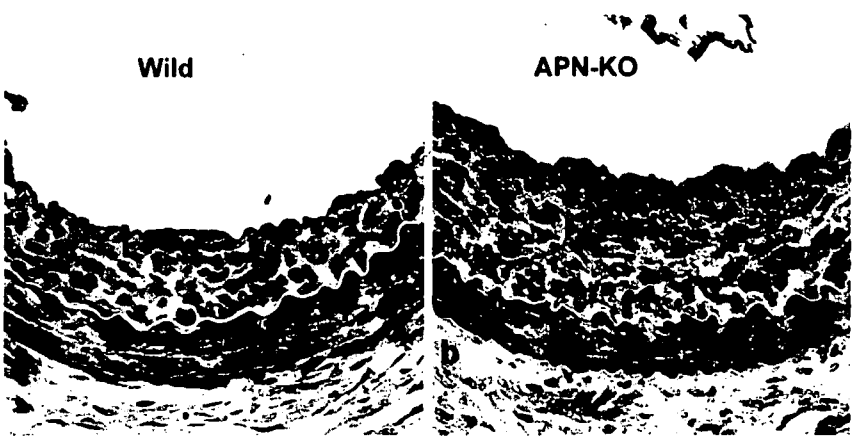

Fig. 6. Adiponectin deficiency exacerbates neointimal thickening after balloon injury. Immunostaining for smooth muscle actin shows neointimal thickening in a femoral artery of a wild-type mouse after mechanical injuries (a). An adiponectin-deficient mouse (*APN-KO*) shows excess neointimal thickening by increase of smooth muscle cells (b). In addition, neointimal thickening in adiponectin-deficient mice is attenuated by adenovirus-mediated adiponectin administration. *Arrows* indicate internal elastic lamina. *I*, intima; *M*, media. [Reproduced with permission from Matsuda et al.[131] (2002) J Biol Chem 277:37487–37491]

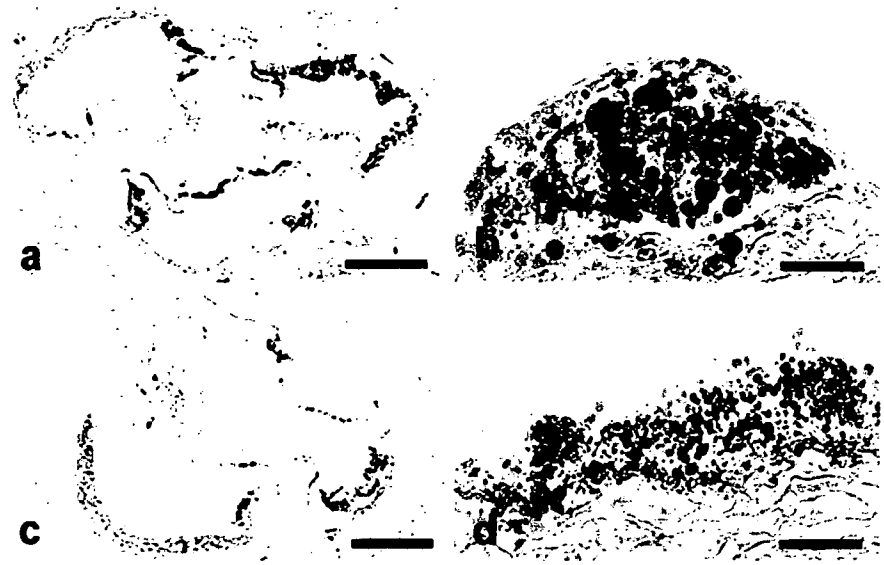

Fig. 7. Adiponectin reduced atherosclerotic plaques of apolipoprotein E-deficient mice. In aortic sinus of an apolipoprotein E-deficient mouse, oil red-O staining shows many atherosclerotic plaques (a). At higher magnification, many large lipid droplets are seen in a plaque (b). Administration of adenovirus-mediated adiponectin in this mouse reduces the area of the plaque in the aortic sinus (c) and the size of the lipid droplets in a plaque (d). *Bars* a, c 500μm; b, d 100μm. [Reproduced with permission from Okamoto et al.[119] (2002) Circulation 106:2767–2770]

proliferation of smooth muscle cells.[130] This result suggests that enhancement of adiponectin may prevent arterial restenosis after balloon angioplasty.[77] Besides smooth muscle cells, foam macrophages are the main components in human atherosclerotic lesions. An apolipoprotein E-deficient mouse is a well-established model of atherosclerosis containing foam cells. We treated these mice with adenovirus-mediated adiponectin. Two weeks after the injection, the plaque area in the aortic sinus was inhibited compared with control mice.[119] The lipid droplets in the lesion of adiponectin-treated mice became smaller compared with nontreated mice (Fig. 7).

Immunohistochemical analyses demonstrated that the adenovirus-mediated adiponectin was localized in the fatty streak lesions. These studies suggest that increase of plasma adiponectin should be a useful target in preventing CVD.

PPARγ-dependent pathways are important targets to induce the expression of adiponectin. PPARγ agonists, the thiazolidinediones, increase plasma adiponectin levels in humans.[48,131–133] Treatment with troglitazone induced plasma adiponectin levels in mildly overweight subjects with glucose intolerance by approximately threefold,[48] and also did so in diabetic patients and in both lean and obese nondiabetic subjects.[131] In a randomized, placebo-controlled study in patients with diabetes, administration of rosiglitazone increased plasma adiponectin levels more than twofold.[132] In addition, pioglitazone increased the ratio of HMW adiponectin/total levels and was related to the hepatic insulin-sensitizing effects.[133] In mice, PPARγ agonists including troglitazone, rosiglitazone, and pioglitazone significantly increased plasma adiponectin levels without affecting body weight.[48] PPARγ agonists induced adiponectin mRNA expression in the adipose tissues of obese mice and enhanced the mRNA expression and secretion of adiponectin in a dose- and time-dependent manner in cultured 3T3-L1 adipocytes.[48] In human and mouse adipose tissue, pioglitazone induces the secretion of HMW adiponectin, but not the secretion of low molecular adiponectin.[134]

As well as PPARγ agonists, the fibrates are also useful to increase adiponectin levels. Significant increase of serum adiponectin was observed in bezafibrate-treated subjects compared with a placebo group.[135] Bezafibrate and fenofibrate significantly elevated adiponectin levels in wild-type mice and 3T3-L1 adipocytes. We also demonstrated that the activation mechanism of adiponectin promoter by fibrates is partly through a PPAR-responsive element (PPRE).[135] In addition, AdipoR2 is induced by both PPARγ and PPARγ in human macrophages.[136]

Analysis of adiponectin promoter is another approach to upregulate the expression of adiponectin. We identified two responsive elements in the human adiponectin promoter region. One is a functional PPAR-responsive element (PPRE), and another is a orphan nuclear receptor, liver receptor homolog-1 (LRH-1)-responsive element (LRH-RE). LRH-1 amplified PPAR-γ-induced transactivation of adiponectin promoter.[137] Our results indicate that PPAR-γ and LRH-1 play significant roles in activation of the adiponectin gene via the PPRE and the LRH-RE in its promoter.

Conclusion

Adiponectin was discovered as an adipose-specific protein. Visceral fat accumulation reduces plasma adiponectin levels. Decreased levels of plasma adiponectin are associated with various kinds of disease. Now, the clinical significance of adiponectin in obesity-related disease has been established. In addition, adiponectin has been shown to have direct effects on obesity-related disease and atherogenesis. Therefore, adiponectin has been recognized as a key molecule in metabolic syndrome.

Various functions of adiponectin provide possibilities to prevent and treat obesity-related diseases and CVD. Furthermore, enhancement of adiponectin secretion or action may become a promising therapeutic target.

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