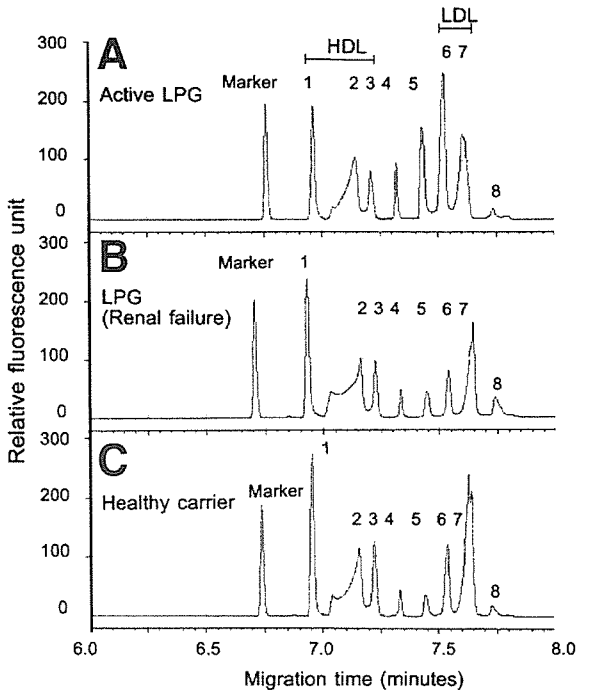

Fig 4. Lipoprotein profiles determined by means of capillary isotachophoresis of whole plasma from patients with (A) the active phase of LPG, (B) renal failure caused by LPG, and (C) a healthy carrier with apoE Sendai. Peaks are fast- (peak 1), intermediate- (peak 2), and slow-migrating (peak 3) high-density lipoprotein (HDL), chylomicron, and remnants (peak 4), VLDL and IDL (peak 5), fast-migrating (electronegative) LDL (peak 6), slow-migrating (native) LDL (peak 7), a minor LDL fraction (peak 8). (A) VLDL/IDL (peak 5) and electronegative LDL fractions (peak 6) are increased. Peak 6 of (B) and (C) are lower than that of (A), but slightly higher than that of a healthy subject (data not shown).

tion of LPG. The interaction of apoE variants and in situ renal factors related to the pathogenesis of LPG are discussed next.

PATHOGENETIC ROLE OF OTHER FACTORS IN LPG

Healthy carriers of apoE variants in families of patients with LPG [17-19] and nonresponders of renal lesions in apoE Sendai–transfected mice[46,47] suggest the involvement of other factors in the induction of LPG. In particular, because lesions are localized to glomeruli, intrinsic glomerular factors may interact with apoE variants and lipoprotein abnormalities for induction of LPG. Moreover, the association with other primary glomerulopathies, eg, IgA nephropathy and membranous nephropathy, sometimes seen in patients with LPG,[17-19] strengthens the likelihood of such a possibility.

Recently, Kanamaru et al[67] showed that chronic graft versus host diseases (GVHD) induced by the transfer of spleen cells from B6.C-H2bm12 mice into Fc receptor (FcR) γ chain (FcRγ)-deficient C57BL/6 mice showed glomerulopathy resembling LPG, with lipoprotein thrombi including apoE. FcRs link the humoral and cellular branches of the immune system and regulate the activation and downmodulation of immune responses.[68] In that experiment, GVHD showed clinical features and renal histopathologic changes of systemic lupus erythematosus, but not of LPG, when FcRγ was expressed normally.[69] Because FcRs require FcRγ for surface expression and signal transduction in mice, the LPG-like lesions in FcRγ-deficient mice indicate that FcR dysfunction is involved in the intraglomerular accumulation of lipoproteins, in contrast to the suppression of autoimmune disease.

Interestingly, nephritic changes similar to lupus were seen in glomeruli without LPG-like lesions in the same mouse. Moreover, LPG appeared to be specific in the GVHD model in FcR$\gamma^{-/-}$ mice because other autoimmune models in FcR$\gamma^{-/-}$ mice did not show such lesions.[70,71]

Because FcRs on macrophages are involved in the recognition and clearance of LDL,[72-74] Kanamaru et al[67] hypothesized that deficiency of FcRs might affect the uptake of modified LDL in a chronically inflamed kidney. They compared the uptake of acetylated LDL by activated peritoneal macrophages in FcR$\gamma^{+/+}$ and FcR$\gamma^{-/-}$ mice. Their results indicated a partial reduction in LDL clearance, which may induce lipoprotein deposition in LPG. Because recent studies showed that FcRs mediate the uptake of immune complex– or CRP-binding LDL into macrophages and induce phagocytosis and atherosclerosis with foam cells,[75-77] FcR dysfunction may lead to the accumulation of lipoproteins without atherogenic changes resembling LPG.

Several groups reported that mesangial cells express FcRs, similar to leukocytes, and mediate glomerular diseases, eg, IgA nephropathy.[78-81] Although this problem is controversial, the function of mesangial FcRs may involve regulating the uptake and clearance of LDL in glomeruli, as discussed regarding macrophages. In various glomerular diseases, immunochemical staining of renal biopsy specimens shows apoB and apoE

deposits in the mesangium, as well as an increase of LDL and scavenger receptors.[82-84] Therefore, nephrophilic apoE variants and modulated LDL may interact with these intrinsic renal mechanisms and contribute to the development of LPG.

Wen et al[85] mentioned that apoE deficiency leading to hyperlipidemia is sufficient for the development of experimental LPG and that apoE Sendai is not a requirement for the development of this injury. Their idea is not applicable to LPG in humans because hyperlipidemia is not always associated with LPG.[15,32] However, it is noteworthy that additional environmental factors inducing hyperlipidemia, eg, high-fat diet, obesity, poor exercise, and nephrotic-range hypoalbuminemia, probably are required for the development of LPG. Recently, it was reported that intensive therapy using hypolipidemic agents can result in clinical remission and histopathologic resolution of LPG.[39,40] In these studies, complete disappearance of lipoprotein thrombi was shown in serial renal biopsy specimens, in addition to significant decreases in serum cholesterol, triglyceride, and apoE concentrations.[39,40] These findings indicate that hyperlipidemia mediated by the environment also is an important factor in the development of LPG, although LPG essentially is based on the genetic abnormality of apoE.

ROLE OF ApoE IN OTHER RENAL DISEASES

There are several reports of familial type III HLP associated with renal lesions. Most patients showed severe glomerulosclerosis with massive foam cells.[2-5] These lesions are the analogies to atherosclerosis, as postulated by Diamond and Karnovsky,[86] because familial type III HLP is characterized by systemic lipidosis with marked hyperlipidemia and atherosclerosis. However, Sakatsume et al[87] reported that lipoprotein thrombi peculiar to LPG also were observed in patients with familial type III HLP caused by the homozygous apoE2 allele. We also observed similar histological characteristics in another patient with familial type III HLP[88] and apoE-deficient mice injected with apoE2-containing virus.[19] Accordingly, apoE2 is presumed to have characteristics partly similar to those of apoE variants with LPG and occasionally shows LPG-like features.

Recently, several studies indicated that the apoE2 allele is a significant risk factor for the development of diabetic nephropathy in patients with diabetes mellitus type 1[89-91] and type 2.[92-96] Conversely, other studies[97-101] argued against the association between apoE polymorphism and diabetic nephropathy, and a few suggested participation of the apoE4 allele.[102,103] At present, the controversy does not seem to be resolved because several other risk factors are involved in the development of diabetic nephropathy. However, it is likely that diabetic nephropathy is influenced by apoE2 through abnormal lipid metabolism because apoE2 may induce hyperlipidemia with the consequent complications, eg, atherosclerosis, glomerulosclerosis, and LPG-like lesions, as mentioned.

Yorioka et al[104] indicated that apoE2 levels correlated with severity of histopathologic damage in patients with IgA nephropathy, and several studies[105,106] suggested that the apoE2 allele could contribute to progression to end-stage renal disease. Therefore, it is possible that the apoE2 allele is a predisposing factor for the development of nephropathy. However, the role of the apoE4 allele is controversial. Some reports support the protection of the apoE4 allele against renal dysfunction,[107-109] but others take the opposite view.[103,110] In addition, it was suggested that the low prevalence of the apoE4 allele in patients with renal failure results from early death caused by the atherogenic character of apoE4.[63,111]

CONCLUSION

LPG is a unique and very important disease in consideration of the relationship between lipids and renal lesions. After the discovery of LPG, we defined clinical and histopathologic characteristics of LPG and described the hypothetical mechanism in 1995.[17] Fortunately, most parts of our hypothesis were confirmed in the last several years by many clinical and experimental studies, as shown in Fig 5. Thus, studies investigating the pathogenesis of LPG have their impact on the analysis of other lipid-related renal diseases because apoE polymorphism is thought to be involved in the development of these diseases. However, to elucidate the precise mechanism(s) of LPG and lipid-related diseases, additional clinical and experimental studies of LPG and apoE are needed.

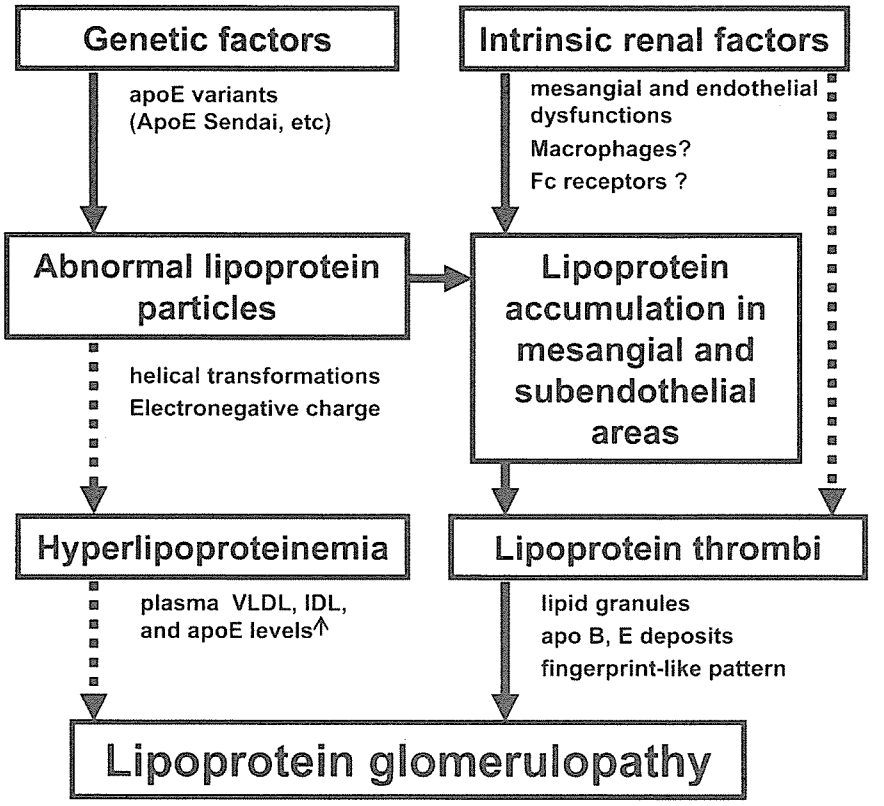

Fig 5. Summary and proposed mechanisms of LPG.

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Combined Cardiovascular Risk Factors and Outcome
— NIPPON DATA80, 1980–1994 —

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Background To examine the prognostic significance of the high-risk group with combined cardiovascular risk factors in the Japanese, we analyzed the relationship between the high-risk group with combined risks and coronary heart disease (CHD) and stroke mortality using the NIPPON DATA80 database.
Methods and Results At baseline in 1980, those of age ≥30 years were randomly selected and 4,144 men and 5,318 women without CHD and/or stroke at baseline were followed for 14 years. The cutoff values for risk components obtained heuristically by Cox analysis were hypertension (systolic ≥130, or diastolic ≥85 mmHg, or on antihypertensive drugs), hypercholesterolemia (total cholesterol ≥200 mg/dl), hyperglycemia (≥130 mg/dl, or self-reported diabetes) and obesity (body mass index ≥27 kg/m^2). Subjects were divided into 3 groups (0, 1–2 and 3–4 risks). Compared with those men in the risk 0 group, the hazard ratios in men in the risk 3–4 for CHD mortality was 8.04 (95% confidence interval: 1.03–62.6), and the stroke mortality was 5.06 (1.53–16.7). In women, no statistically significant difference was found due to a lesser number of events.
Conclusion The high-risk group with combined risk factors is important risk for Japanese men. (*Circ J* 2006; **70:** 960–964)

Key Words: Cohort study; Coronary heart disease; Risk factors; Stroke

Cardiovascular risk factors, such as dyslipidemia, hypertension, hyperglycemia and obesity, are consistent and common but largely undertreated and undercontrolled in many countries[1] although it is known that these risk factors often cluster together[2,3] This clustering is now considered to be the "metabolic syndrome", which is closely related to insulin resistance[3,4] Cutoff values for cardiovascular risk factors have beene derived either empirically or from the results of cross-sectional studies[3,5–7] but ideally, such cutoff values should be derived from the data of longitudinal cohort studies, so that risk factors have prognostic implications. Furthermore, the cutoff values for these risk factors and the prognostic significance of combined risk factors have not yet been reported in Asian populations, where coronary heart disease (CHD) mortality and obesity are relatively rare, but susceptibility to diabetes mellitus has been reported to be higher[8–10] The individual cutoff values should be determined for different populations, so to examine the prognostic significance of the high-risk group with combined risk factors we analyzed the relationship between combined cardiovascular risk factors and CHD and stroke mortality using the database of the National Integrated Project for Prospective Observation of Noncommunicable Diseases and Its Trends in the Aged, 1980 (NIPPON DATA80), which includes more than 10,000 subjects in Japan who were followed for 14 years[11–13]

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Methods

Subjects

The subjects in this cohort were participants in the 1980 National Survey on Circulatory Disorders;[14] the detailed methods of the NIPPON DATA80 have been described previously[13] but are summarized here. A total of 10,546 community-based subjects aged ≥30 years in 300 randomly selected health districts throughout Japan participated in the survey, which consisted of a medical history, physical examinations, blood tests and a self-administered questionnaire on lifestyle. The cohort was followed until 1994[11–13] To clarify the causes of death, we used the National Vital Statistics.

Of the 10,546 subjects, a total of 1,084 were excluded for the following reasons: past history of CHD or stroke (n=166), missing information on the baseline survey (n=48), lost to follow-up (n=870). We analyzed the remaining 9,462 subjects (4,144 men, 5,318 women). Ethical approval for this study was obtained from the Institutional Review Board of Shiga University of Medical Science (No. 12-18, 2000).

Biochemical and Baseline Examinations

The baseline surveys were conducted by public health centers. Systolic and diastolic blood pressures (SBP, DBP)

Table 1 Results of Principal Component Analysis Among 3,820 Men and 4,857 Women Not Taking Medication (NIPPON DATA80: 1980–1994)

	Men		Women
	Factor 1	Factor 2	Factor 1
Variables			
BMI	0.44	0.65	0.50
SBP	0.84	−0.37	0.86
DBP	0.88	−0.18	0.85
Total cholesterol	0.33	0.72	0.44
Glucose	0.24	−0.25	0.31
Total variance	0.37	0.23	0.40
Cumulative variance	0.37	0.60	0.40

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

were measured by trained operators using a standard mercury sphygmomanometer on the subjects' right arm while the subjects were seated and after they had rested for more than 5 min. Height in stockinged feet and weight in light clothing were measured. Body mass index (BMI) was calculated as weight (in kg) divided by the square of height (in m).

A lifestyle survey was carried out using a self-administered questionnaire. Non-fasting blood samples were drawn and centrifuged within 60 min of collection, and then stored at −70°C until analysis. Total cholesterol was analyzed in a sequential auto-analyzer (SMA12/60; Technicon, Tarrytown, NY, USA) at a single laboratory (Osaka Medical Center for Health Science and Promotion), which is a member of the Cholesterol Reference Method Laboratory Network[15] The serum concentration of glucose was measured using the cupric-neocuproline method[16]

Combined Cardiovascular Risk Factors

The previous cutoff values used for the definition of metabolic syndrome[6,7] were not applied in the present study for the following reasons. Waist girth was not measured during the baseline examinations in 1980. The modified WHO definition uses BMI ≥30 kg/m^2 as a criteria for abdominal obesity, but because the average BMI for Japanese adult men and women is around 23 kg/m^2, there are very few subjects whose BMI is more than 30 kg/m^2.[17–20] Non-fasting blood samples were used in the present study, and direct measurement of high density lipoprotein (HDL) cholesterol was not performed. Therefore, we selected 4 components of the combined risk factors, namely, obesity, hyperglycemia, hypercholesterolemia and hypertension. The cutoff value for each component was determined heuristically using the mortality data described below. We considered those who were on antihypertensive medication as having hypertension, and those who with self-reported diabetes mellitus as having hyperglycemia. We then divided the study subjects into 3 groups: risk 0 for subjects who had none of the above components, risk 1–2 for subjects who had 1 or 2 of the components, and risk 3–4 for subjects who had 3 or 4 of the above components.

Statistical Analyses

SAS version 8.02 for WINDOWS (SAS Institute Inc, Cary, NC, USA) was used throughout the analyses. Men and women were analyzed separately. The chi-square test was used to compare dichotomous variables. To compare the means among the 3 groups, one-way analysis of variance was used.

Table 2 Results of Heuristic Analysis in Men to Obtain the Cutoff Values for Risk Components Among 4,144 Men (NIPPON DATA80: 1980–1994)

	Risk 3–4 (%)	CHD HR (p)	Stroke HR (p)
BMI (kg/m^2)			
26	*15*	*6.94 (0.064)*	*4.77 (0.010)*
27	*13*	*8.04 (0.046)*	*5.06 (0.008)*
28	*11.5*	*9.17 (0.035)*	*5.26 (0.007)*
BG (mg/dl)			
120	*17.5*	*6.12 (0.081)*	*4.43 (0.042)*
130	*13*	*8.04 (0.046)*	*5.06 (0.008)*
140	*10*	*7.11 (0.068)*	*4.33 (0.007)*
TC (mg/dl)			
180	*20*	*2.39 (0.144)*	*4.80 (0.131)*
200	*13*	*8.04 (0.046)*	*5.06 (0.008)*
220	*7.8*	*10.29 (0.028)*	*5.24 (0.008)*
SBP/DBP (mmHg)			
120/80	*14.9*	*>50 (0.986)*	*4.56 (0.137)*
130/85	*13*	*8.04 (0.046)*	*5.06 (0.008)*
140/90	*10.3*	*3.20 (0.053)*	*4.51 (0.002)*

Fixing the values of 3 components, value of the 4th component was varied categorically and the Cox analyses were performed. Hazard ratios (HR) with p values for CHD and stroke mortality were compared. The risk 0 group was used as the reference group. The Cox analyses were repeated until the lowest cutoff value for each component that had a prognostic significance for CHD and/or stroke mortality was obtained. The obtained cutoff values were BMI=27 kg/m^2, BG=130 mg/dl, TC=200 mg/dl, SBP/DBP=130/85 mmHg for men. Risk 3–4= subjects who had 3 or 4 of the risk (risks are: hypertension: SBP ≥130 mmHg, or DBP ≥85 mmHg, or on anti-hypertensive drugs; hypercholesterolemia: TC ≥200 mg/dl; hyperglycemia: BG ≥130 mg/dl or self-reported diabetes mellitus; obesity: BMI ≥27 kg/m^2).
CHD, coronary heart disease; BG, blood glucose; TC, total cholesterol. Other abbreviations see in Table 1.

Principal component analysis was conducted using the FACTOR procedure of SAS in order to examine clustering. Subjects who were taking antihypertensive, antidiabetic or cholesterol-lowering medications were excluded from this principal component analysis. The number of components to be retained was based on eigenvalue criteria (≥1.0). The resulting factor pattern was interpreted using factor loadings of ≥0.40.

The multivariate-adjusted hazard ratios for CHD, and stroke mortality were calculated using the Cox proportional hazard model, including age, cigarette smoking (currently smoking or not), and alcohol intake (drinkers or non-drinkers) as covariates. The risk 0 group was used as the reference group. The cutoff values for each of the 4 components were determined heuristically using the Cox analyses on CHD and stroke mortality. Namely, fixing the values of 3 components, the value of the 4th component was categorically varied and the Cox analyses were performed. The Cox analyses were repeated until we found the lowest cutoff value for each component that had prognostic significance for CHD and/or stroke mortality. The entered values for each component were BMI: from 25 to 30 kg/m^2 with 1 kg/m^2 increments; blood glucose: from 100 to 160 mg/dl with 10 mg/dl increments; total cholesterol: from 180 to 260 mg/dl with 20 mg/dl increments; SBP/DBP: 120/80, 130/85 and 140/90 mmHg. Because most of the analyses in women did not yield cutoff values that had prognostic significance, the cutoff values for men were applied in the analyses of women.

Hazard ratios for the association of CHD and stroke mortality with the component conditions were analyzed by the Cox proportional hazard model as above. Tests of linear

Table 3 Baseline Characteristics According to Risk Group Among 4,144 Men and 5,318 Women (NIPPON DATA80: 1980–1994)

	Risk 0	Risk 1–2	Risk 3–4	p (χ^2 or ANOVA)
Men (N=4,144) (% prevalence)	655 (15.8)	2,950 (71.2)	539 (13.0)	
Age (years)	43.7±10.8	51.4±13.2	52.8±13.0	<0.0001
BMI (kg/m^2)	21.4±2.3	22.3±2.7	24.8±3.3	<0.0001
SBP (mmHg)	117±8	141±20	149±18	<0.0001
DBP (mmHg)	73±7	85±12	90±12	<0.0001
BG (mg/dl)	112±12	130±37	157±50	<0.0001
TC (mg/dl)	168±19	184±31	221±28	<0.0001
Smoking (%)	65.5	64.2	55.3	0.0002
Drinking (%)	73.0	75.2	73.5	0.405
Women (N=5,318) (% prevalence)	1,124 (21.1)	3,303 (62.1)	891 (16.8)	
Age (years)	41.5±9.8	52.0±13.1	58.3±11.5	<0.0001
BMI (kg/m^2)	21.3±2.3	22.7±3.0	25.6±4.0	<0.0001
SBP (mmHg)	115±8	134±20	151±20	<0.0001
DBP (mmHg)	71±8	81±11	87±12	<0.0001
BG (mg/dl)	112±11	128±31	154±46	<0.0001
TC (mg/dl)	167±20	190±32	223±29	<0.0001
Smoking (%)	10.0	8.6	8.2	0.291
Drinking (%)	24.8	18.7	17.7	<0.0001

Data are % or mean ± SD.
ANOVA, analysis of variance. Other abbreviations see in Tables 1,2.

Table 4 Hazard Ratios of CHD and Stroke Mortality According to Risk Group Among 4,144 Men and 5,318 Women (NIPPON DATA80: 1980–1994)

	Men				Women			
	Risk 0	Risk 1–2	Risk 3–4	Trend p	Risk 0	Risk 1–2	Risk 3–4	Trend p
Subgroup N	655	2,950	539		1,124	3,303	891	
CHD, N	1	33	8		3	31	10	
/1,000 PY	0.1	0.9	1.2		0.1	0.4	1.7	
HR*	1	3.51	8.04	0.0002	1	1.04	0.75	0.66
		(0.47–26.1)	(1.03–62.6)			(0.31–3.46)	(0.20–2.78)	
Stroke, N	3	85	30		6	71	31	
/1,000 PY	0.3	2.2	4.4		0.3	0.9	5.1	
HR*	1	2.64	5.06	<0.0001	1	1.24	1.27	0.53
		(0.83–8.39)	(1.53–16.7)			(0.53–2.88)	(0.52–3.08)	

Hazard ratios (95% confidence interval) are shown.
HR: age, cigarette smoking and alcohol intake were entered as covariates for multivariate analyses.*
/1,000 PY, per 1,000 person-years. Other abbreviations see in Table 2.

trends across risk groups were conducted by assigning an ordinal value to each number risk (0 to 4) and modeling this as a continuous variable in separate Cox proportional hazard models.

All p values were two-tailed, and $p<0.05$ was considered significant. Data are presented as means ± SDs unless stated otherwise.

Results

Principal Components

The results of principal component analysis of the risk factors are shown in Table 1. In men, a 2-component solution explained 60% of the common variance in the data set. The component has large positive loadings (≥0.40) for 3 of the 5 risk factor components, and the second has large positive loadings for 2 of the 5 risk factor components. One component, BMI, shows overlap. In women, a one-component solution explained 40% of the common variance in the data set. The component has large positive loadings for 4 of the 5 risk factors. In both men and women, loadings for glucose were not large.

Cutoff Values for Risk Components

The obtained categorical cutoff values for the risk components by heuristic analyses were BMI: 27 kg/m^2, blood glucose = 130 mg/dl, total cholesterol = 200 mg/dl, and SBP/DBP = 130/85 mmHg for men. Table 2 shows the results of heuristic analyses in men to obtain the cutoff values for the risk components. Only the results of 3 representative categorical values for each component are shown. The prevalence of the risk 3–4 group in %, hazard ratios and p values for CHD and stroke mortality are shown. It can be seen that selecting the cutoff values for the 4 components satisfied prognostic significance for both CHD and stroke mortality in men.

We did not have appropriate cut-offs for the women; therefore, we used the same cut-offs as for the men.

Baseline Characteristics

The baseline characteristics for men and women in each risk group are shown in Table 3. Age was significantly greater in the higher risk groups for men and women. Smoking was less in the higher risk groups for men and drinking was less in the higher risk groups for women. BMI, SBP, DBP, blood glucose and total cholesterol were significantly higher in the higher risk groups by definition.

If the high risk group is defined here as those who have 3 or 4 risk components, its prevalence was 13.0% for men and 16.8% for women.

Combined Risks and Outcome: Multivariate Cox Analyses

Case number, unadjusted mortality per 1,000 person-years, and hazard ratios of CHD, and stroke mortality by multivariate Cox analyses adjusted for age, smoking and drinking are shown in Table 4 for both men and women. In men, for those in the risk 3–4 group, the hazard ratio of CHD was 8.04 and that of stroke was 5.06, in comparison with the risk 0 group. Both trends were significant (trend $p<0.0001$ and 0.0002). Men in the risk 1–2 group carried intermediate risks for CHD and stroke.

However, in women, no significant trend was noted for CHD or stroke mortality, probably because of lower mortality.

Discussion

This prospective population-based cohort study in Japan reports an association of the high-risk group with CHD and stroke mortality. The previously proposed cut-off values for cardiovascular risk factors cannot be applied to non-Western populations because, for instance, the average BMI and waist circumference for Asians are smaller.[5–9,17–20] Several studies in Asians report that for the definition of obesity in Asians the cutoff value for BMI is 23 kg/m^2 and for waist circumference is 90 cm for men and 80 cm for women.[20,21]

In the present study, we selected hypertension, hypercholesterolemia, hyperglycemia and obesity as the components of the combined cardiovascular risk, and the cutoff values for each of these were determined heuristically using Cox analyses of CHD and stroke mortality. By the present definition, the prevalence of the high-risk group with 3 or more risk factors was 13.0% for Japanese men and 16.8% for women in 1980. Although CHD mortality in Japan is relatively low in comparison with that of the Western population, the impact of the combined risk factors on CHD mortality in men was significant, with a multivariate adjusted hazard ratio of 8.04.

The lack of prognostic significance of the combined cardiovascular risk in women in the present study is probably due to a lower incidence of CHD and stroke compared with men.

Study Limitations

The method of obtaining the categorical cutoff values for the risk components and the method of evaluating the prognostic significance of the newly obtained diagnostic criteria of the combined risks were the same, namely Cox analyses. This may appear to be a circular tautology. However, the second Cox analysis was applied merely to show the magnitude of the prognostic significance of the criteria. Applying these criteria to a different population or to the same study with a longer follow-up may be needed in the future to verify this method. Another method of obtaining the cutoff values may be to apply the recursive partitioning method.[26,27] This method may be quite valuable in handling variables that are independent of each other, such as handling gene expression data for tumor and cell classification,[27] but may not be useful when the variables are confounded by each other, such as blood pressure, BMI, total cholesterol concentrations, blood glucose and age, as in the present study. In fact, trial use of this method for the present data resulted in impractical cutoff values, with one variable having different values that appeared at more than 2 ranches of the tree.

The results of principal component analysis in this study suggest clustering of 4 of the 5 components (BMI, SBP, DBP, total cholesterol and non-fasting glucose) except for 1 component, non-fasting glucose. This may be due to the fact that we did not have fasting glucose data. We also need more variables, such as HDL-cholesterol, triglyceride concentrations, an index of insulin resistance, and inflammation markers, to examine the clustering and to find the primary unifying underlying abnormality of the clustered risk factors, as performed in recent studies.[28,29]

Non-fasting blood samples were used in the present study, and direct measurement of HDL-cholesterol was not performed. Therefore, we did not have measurements for fasting blood glucose, triglycerides or HDL-cholesterol, which are other important components of metabolic syndrome. Furthermore, waist circumference measurements will be required in future studies.

We used mortality data as endpoints, which might have led to misclassification of the causes of death. However, it has been reported that the death-certificate diagnosis of stroke and cancer in Japan is quite accurate,[30,31] although it has also been reported that most cases of sudden cardiac death tend to be described on Japanese death certificates as "coronary heart disease", "heart failure" or "unknown cause".[32,33] Furthermore, mortality statistics for coronary heart disease by the end of 1994 may have been underestimated using ICD9, since deaths coded as "heart failure" may hide certain coronary events.[32–35]

Conclusion

Cutoff values for cardiovascular risk factors have been obtained and the defined high-risk group with combined risk factors is important risk for Japanese men.

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Serum C-Reactive Protein and Its Relation to Cardiovascular Risk Factors and Adipocytokines in Japanese Children

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Background: C-Reactive protein (CRP) is an independent risk factor for atherosclerotic coronary heart diseases (ACHD) in adults. To help prevent ACHD, it may be useful to understand risk factors during childhood.

Objective: The objective of this study was to investigate serum CRP and its relation to other risk factors for ACHD and adipocytokines (adiponectin, IL-6, and TNF-α) in Japanese children.

Methods: CRP, conventional risk factors for ACHD, and adipocytokines were determined in 568 children (340 boys and 228 girls, aged 7–10 yr). Serum concentrations of adipocytokines were measured by sandwich ELISA.

Results: Children with high CRP concentrations (highest tertile) had higher body mass index (BMI) SD scores, insulin, insulin resistance, uric acid, and adipocytokines and had more atherogenic lipoprotein profiles than other children. However, after being corrected by BMI SD, only high-density lipoprotein cholesterol, apolipoprotein A-I, IL-6, and TNF-α for boys and high-density lipoprotein cholesterol, apolipoprotein B, uric acid, IL-6, and TNF-α for girls were significantly correlated with CRP. IL-6 was the strongest predictive variable for CRP and accounted for 26.2 and 27.7% of the variability in serum concentrations of CRP in boys and girls, respectively. Serum concentrations of IL-6 were partly dependent on BMI SD and TNF-α in both boys and girls.

Conclusion: Although serum concentrations of CRP are partly regulated by adipocytokines and conventional risk factors for ACHD, high CRP levels were associated with atherogenic profiles of cardiovascular risk factors in children. Our findings suggest that it may be important to control body weight to prevent an increase in serum CRP in children. **(*J Clin Endocrinol Metab* 91: 2133–2137, 2006)**

C-REACTIVE PROTEIN (CRP) increases nonspecifically in inflammatory disorders. The recent development of a highly sensitive assay for serum CRP concentrations has led to the unexpected finding that elevation of CRP levels within the normal range is associated with an increased risk for atherosclerotic coronary heart diseases (ACHD) in apparently healthy subjects (1–3). Although the underlying mechanism by which CRP contributes to the development of ACHD is not yet clear, Sternik *et al.* (4) reported in an *in vitro* study that CRP induces vasorelaxation independent of the endothelium. In addition, it has been reported that CRP induced apoptosis in human coronary vascular smooth muscle cells (5). However, van den Berg *et al.* (6) recently reported that vasorelaxation induced by CRP may be an artifact caused by the reagent used in their experiment. CRP is correlated with many conventional risk factors for ACHD, such as insulin resistance, obesity, high-density lipoprotein cholesterol (HDL-C), *etc.* (7, 8). Because most of these epidemiological studies were performed in adults, ACHD risk factors acquired later in life, such as smoking, alcohol use, *etc.*, may affect these relationships. In contrast to adults, children rarely drink alcohol or smoke and usually exercise regularly at school. Thus, environmental factors that affect the relationship between CRP and risk factors may have less of an effect in children than in adults. It seems reasonable to consider the relationship between serum CRP and risk factors for ACHD in schoolchildren. Several studies in children are currently available (9–11). Most of these have indicated that adiposity is the major determinant of CRP levels in children and have speculated that cytokines secreted from adipocytes may be responsible for the relationship between serum concentrations of CRP and adiposity. In the present study we investigated serum CRP and factors that influence serum CRP in children to better understand the roles of various risk factors in the development of atherosclerosis.

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Abbreviations: ACHD, Atherosclerotic coronary heart disease; apo, apolipoprotein; BMI, body mass index; CRP, C-reactive protein; HDL-C, high-density lipoprotein cholesterol; HOMA-R, homeostasis model approximation index; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

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Subjects and Methods

Subjects

The present study was approved by the review board of University of the Ryukyus. Informed consent was obtained from the parents of all children. We studied 568 Japanese children (340 boys and 228 girls), aged 7–10 yr, who underwent screening and had been enrolled in a care program for lifestyle-related diseases since 2002 in Okinawa, Japan (Table 1). Sex maturity stages in the children we studied were equal to or less than Tanner stage 2. The subjects were not patients who visited our hospital. Body mass index (BMI) was calculated as weight (kilograms)/height (meters)^2. BMI SD scores adjusted for age and sex were obtained based on data for Japanese schoolchildren provided by the Ministry of Education, Culture, Sports, Science, and Technology (Murata, M., unpublished observations). None of the children studied were receiving therapy for weight reduction or drugs that affected lipid metabolism. None had a smoking habit. Venous blood was drawn after an overnight fast.

TABLE 1. Clinical and chemical data

	Boys	Girls
No. of subjects	340	228
Median age (range) (yr)	9.2 (6.5–10.4)	9.2 (6.5–10.4)
BMI SD	1.43 ± 1.25 (−1.77–5.06)	1.25 ± 1.29 (−2.04–5.45)
CRP (mg/liter)	1.08 ± 1.50 (0.05–9.34)	0.82 ± 1.28 (0.05–8.82)[d]
Glucose (mg/dl)	91 ± 6 (70–111)	90 ± 7 (48–121)
Insulin (μU/ml)	10.4 ± 9.0 (1.0–54.6)	12.7 ± 10.5 (1.0–65.3)
HOMA-R	2.43 ± 2.33 (0.19–12.40)	2.86 ± 2.48 (0.12–14.03)
TC (mg/dl)	184 ± 29 (118–282)	177 ± 27 (123–281)[b]
TG (mg/dl)	84 ± 70 (20–272)	91 ± 95 (20–367)
LDL-C (mg/dl)	109 ± 26 (51–215)	103 ± 26 (38–193)[b]
HDL-C (mg/dl)	59 ± 12 (31–97)	57 ± 11 (28–92)[a]
ApoA-I (mg/dl)	140 ± 18 (96–198)	132 ± 17 (91–178)[d]
ApoB (mg/dl)	79 ± 17 (35–140)	76 ± 17 (38–161)
Adiponectin (μg/ml)	8.4 ± 3.8 (0.2–22.7)	8.3 ± 4.2 (0.9–24.5)
Uric acid (mg/dl)	4.8 ± 1.0 (2.4–8.4)	4.7 ± 0.9 (1.8–7.0)
IL-6 (pg/ml)	2.24 ± 2.27 (0.24–19.49)	1.99 ± 0.69 (0.19–18.92)
TNF-α (pg/ml)	1.19 ± 0.73 (0.17–6.42)	1.03 ± 0.69 (0.04–4.11)[c]

Values are expressed as mean ± SD (range) unless specified otherwise. To convert glucose to mmol/liter, divide by 18. To convert TC, LDL-C, and HDL-C to mmol/liter, multiply by 0.0259. To convert TG to mmol/liter, multiply by 0.0113.
[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.
[d] $P < 0.0001$.

Laboratory measurements

The serum CRP concentration was measured by a highly sensitive immunoturbidimetric assay with the use of reagents and calibrators from Dade Behring Marbura GmbH (Marburg, Germany; the lower limit of detection for the serum CRP concentration was 0.05 mg/liter). IL-6 and TNF-α were measured by ELISAs (R&D Systems, Inc., Minneapolis, MN). The serum adiponectin concentration was measured by sandwich ELISA (Otsuka Pharmaceutical Co., Ltd., Tokushima City, Japan). Serum insulin was measured by a two-step sandwich ELISA (SRL, Inc., Hachioji, Japan). Routine chemical methods were used to determine the serum concentrations of total cholesterol (TC), HDL-C, triglycerides (TG), uric acid, and glucose. Low-density lipoprotein-cholesterol (LDL-C) was calculated as TC − HDL-C − TG/5. Apolipoproteins (apoA-I and apoB) were measured by the turbidity immunoassay method (12). Insulin resistance was calculated using the homeostasis model approximation index (HOMA-R) (13). This equation, which is based on both fasting glucose and insulin, correlates well with insulin dynamics, as measured by the hyperinsulinemic clamp and the iv glucose tolerance test (13).

Statistical evaluation

For statistical analysis, serum concentrations of CRP below the limit of detection were assigned a value of 0.05 mg/liter (lower limit of detection). Gender-related differences were determined by the Mann-Whitney *U* test. Differences in parameters among subjects with low, middle, and high CRP concentrations (tertiles) were determined by the Kruskal-Wallis test. Parameters in these three groups were compared with Scheffé's multiple comparison test. The distributions of HOMA-R and levels of CRP, insulin, TG, IL-6, and TNF-α were markedly skewed. Thus, these parameters were normalized by log transformation. Pearson and partial correlation coefficients were computed to assess the associations between CRP and various parameters. A stepwise multiple regression analysis was performed by entering the independent variable with the highest partial correlation coefficient at each step until no variable remained with an F value of 4 or greater. Group differences or correlations with $P < 0.05$ were considered statistically significant. All statistical analysis was performed using StatView J-5.0 software (SAS Institute, Inc., Cary, NC).

TABLE 2. Clinical and chemical data on boys with different CRP levels

	Low (n = 113)	*P* value	Middle (n = 114)	*P* value	High (n = 113)
CRP (mg/liter)	0.14 ± 0.07	<0.0001	0.52 ± 0.18	<0.0001	2.55 ± 1.82[d]
Age (yr)	9.4 ± 1.2	ns	9.7 ± 0.9	<0.05	10.0 ± 0.3[d]
BMI SD	0.71 ± 0.10	<0.0001	1.49 ± 1.17	<0.05	2.09 ± 1.15[d]
Glucose (mg/dl)	90 ± 6	ns	90 ± 7	ns	92 ± 6
Insulin (μU/ml)	7.7 ± 5.2	<0.05	10.9 ± 10.7	ns	13.3 ± 10.9[d]
HOMA-R	1.7 ± 1.2	<0.05	2.5 ± 2.7	ns	3.0 ± 2.6[d]
TC (mg/dl)	183 ± 28	ns	183 ± 28	ns	186 ± 30
TG (mg/dl)	75 ± 48	ns	83 ± 51	ns	95 ± 99
LDL-C (mg/dl)	105 ± 26	ns	108 ± 27	ns	113 ± 26[a]
HDL-C (mg/dl)	64 ± 13	<0.01	59 ± 11	<0.05	55 ± 11[d]
ApoA-I (mg/dl)	144 ± 19	ns	139 ± 19	ns	135 ± 19[c]
ApoB (mg/dl)	76 ± 18	<0.05	78 ± 17	ns	83 ± 17[b]
Adiponectin (μg/ml)	9.3 ± 3.8	ns	8.4 ± 4.1	ns	7.5 ± 3.2[b]
Uric acid (mg/dl)	4.4 ± 0.9	<0.01	4.8 ± 1.1	ns	5.2 ± 0.9[c]
IL-6 (pg/ml)	1.33 ± 0.89	<0.01	2.23 ± 2.39	<0.01	3.16 ± 2.69[d]
TNF-α (pg/ml)	1.04 ± 0.62	ns	1.16 ± 0.62	ns	1.36 ± 0.89[b]

Values are expressed as mean ± SD. ns, Not significant. To convert glucose to mmol/liter, divide by 18. To convert TC, LDL-C, and HDL-C to mmol/liter, multiply by 0.0259. To convert TG to mmol/liter, multiply by 0.0113.
[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$; [d] $P < 0.0001$, significantly different from low.

TABLE 3. Clinical and chemical data on girls with different CRP levels

	Low (n = 76)	*P* value	Middle (n = 76)	*P* value	High (n = 76)
CRP (mg/liter)	0.09 ± 0.03	<0.0001	0.35 ± 0.17	<0.0001	2.06 ± 1.63[d]
Age (yr)	9.5 ± 1.1	ns	9.5 ± 1.1	ns	9.8 ± 0.7
BMI SD	0.80 ± 1.17	ns	1.09 ± 1.10	<0.001	1.87 ± 1.35[d]
Glucose (mg/dl)	90 ± 6	ns	89 ± 10	ns	90 ± 5
Insulin (μU/ml)	10.6 ± 7.6	ns	10.8 ± 10.8	<0.01	16.7 ± 11.7[c]
HOMA-R	2.4 ± 1.8	ns	2.4 ± 2.6	<0.01	3.8 ± 2.7[b]
TC (mg/dl)	172 ± 23	ns	175 ± 21	ns	184 ± 33
TG (mg/dl)	75 ± 40	ns	85 ± 63	ns	96 ± 50[a]
LDL-C (mg/dl)	99 ± 21	ns	101 ± 20	ns	108 ± 34
HDL-C (mg/dl)	60 ± 10	ns	58 ± 11	<0.05	54 ± 11[b]
ApoA-I (mg/dl)	133 ± 15	ns	132 ± 17	ns	130 ± 18
ApoB (mg/dl)	72 ± 15	ns	74 ± 13	<0.001	84 ± 21[d]
Adiponectin (μg/ml)	8.7 ± 4.1	ns	8.5 ± 4.7	ns	7.7 ± 3.7
Uric acid (mg/dl)	4.4 ± 0.9	ns	4.6 ± 0.9	<0.05	5.1 ± 1.0[c]
IL-6 (pg/ml)	1.25 ± 0.65	<0.01	2.03 ± 2.20	<0.05	2.68 ± 1.44[d]
TNF-α (pg/ml)	0.97 ± 0.66	ns	1.01 ± 0.69	ns	1.10 ± 0.71

Values are expressed as mean ± SD. ns, Not significant. To convert glucose to mmol/liter, divide by 18. To convert TC, LDL-C, and HDL-C to mmol/liter, multiply by 0.0259. To convert TG to mmol/liter, multiply by 0.0113.
[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$; [d] $P < 0.0001$, significantly different from low.

Results

As shown in Table 1, gender-related differences were found in several parameters (CRP, TC, LDL-C, HDL-C, apoA-I, and TNF-α). Thus, we separated the data for boys and girls in the following analysis. To understand the relation between CRP and lipids and other parameters, subjects were divided into tertiles based on CRP concentrations (low, lowest tertile; middle, intermediate tertile; high, highest tertile; Tables 2 and 3). In boys, there were significant graded relationships among the three groups of CRP concentrations and all parameters except for glucose, TC, and TG. For parameters other than BMI SD, HDL-C, and IL-6, significant differences were found in one or two combinations (between low and high and/or between low and middle or between middle and high; Table 2). Table 3 shows the findings in girls. In contrast to the findings in boys, significant differences were not found in LDL-C, adiponectin, and TNF-α, but were found in TG.

Tables 4 and 5 show Pearson and partial correlations between logCRP and the other parameters studied. In boys, logCRP was correlated with all parameters listed except TC ($P = 0.000–0.010$). After being corrected for BMI SD, logCRP was positively correlated with logIL-6 and logTNF-α and was inversely correlated with HDL-C and apoA-I ($P = 0.000–0.049$; Table 4). In girls, logCRP was positively correlated with BMI SD, age, logInsulin, logHOMA-R, logTG, ApoB, uric acid, and logIL-6 and was inversely correlated with HDL-C ($P = 0.000–0.016$). After being corrected for BMI SD, logCRP was positively correlated with apoB, uric acid, logIL-6, and logTNF-α ($P = 0.000–0.031$) and was inversely correlated with HDL-C (Table 5). Because each of these parameters can potentially contribute directly to the regulation of CRP, we performed a stepwise multiple regression analysis with logCRP as the dependent variable and the other parameters listed in Table 4 (HOMA-R was excluded because it was value calculated) as independent variables. In boys, BMI SD had the most significant association with logCRP and accounted for 24.3% of the variability in logCRP. HDL-C had additional effects (2.4%; Table 6, model 1, logIL-6 and TNF-α were excluded). When logIL-6 and logTNF-α were included

TABLE 4. Log CRP and variables in boys

	Simple correlation		Partial correlation	
	r[a]	*P*	r[b]	*P*
BMI SD	0.494	0.000		
Age	0.289	0.000	0.045	0.409
Glucose	0.121	0.026	0.050	0.359
Log insulin	0.364	0.000	0.054	0.322
Log HOMA-R	0.362	0.000	0.053	0.331
TC	0.049	0.369	0.010	0.854
Log TG	0.142	0.009	0.051	0.349
LDL-C	0.140	0.010	0.078	0.152
HDL-C	−0.327	0.000	−0.180	0.001
ApoA-I	−0.216	0.000	−0.107	0.049
ApoB	0.182	0.001	0.093	0.087
Adiponectin	−0.223	0.000	−0.012	0.826
Uric acid	0.323	0.000	0.098	0.072
Log IL-6	0.502	0.000	0.403	0.000
Log TNF-α	0.199	0.002	0.218	0.000

[a] Pearson correlation coefficient.
[b] Variables corrected by BMI SD.

TABLE 5. Log CRP and variables in girls

	Simple correlation		Partial correlation	
	r[a]	*P*	r[b]	*P*
BMI SD	0.364	0.000		
Age	0.160	0.016	−0.041	0.540
Glucose	0.031	0.647	−0.059	0.539
Log insulin	0.219	0.001	−0.081	0.224
Log HOMA-R	0.209	0.002	−0.082	0.219
TC	0.108	0.104	0.088	0.187
Log TG	0.181	0.005	0.106	0.112
LDL-C	0.092	0.169	0.059	0.377
HDL-C	−0.189	0.004	−0.195	0.003
ApoA-I	−0.049	0.464	−0.004	0.952
ApoB	0.211	0.001	0.151	0.023
Adiponectin	−0.065	0.331	0.090	0.178
Uric acid	0.257	0.000	0.131	0.049
Log IL-6	0.509	0.000	0.453	0.000
Log TNF-α	0.128	0.098	0.167	0.031

[a] Pearson correlation coefficient.
[b] Variables corrected by BMI SD.

TABLE 6. Stepwise multiple regression models for predicting Log CRP

	Independent parameters	r	r^2
Boys			
Model 1			
Step 1	BMI SD	0.492	0.243
Step 2	BMI SD, HDL-C	0.516	0.267
Model 2			
Step 1	Log IL-6	0.514	0.262
Step 2	Log IL-6, BMI SD	0.599	0.359
Step 3	Log IL-6, BMI SD, HDL-C	0.618	0.382
Step 4	Log IL-6, BMI SD, HDL-C, Log TNF-α	0.627	0.393
Girls			
Model 1			
Step 1	BMI SD	0.363	0.132
Step 2	BMI SD, ApoB	0.389	0.151
Model 2			
Step 1	Log IL-6	0.526	0.277
Step 2	Log IL-6, BMI SD	0.560	0.313
Step 3	Log IL-6, BMI SD, Log TG	0.578	0.335

Model 1, Log IL-6 and Log TNF-α were excluded. Model 2, All parameters were included. For all steps, $P < 0.0001$.

in the model (Table 6, model 2), logIL-6 had the most significant association with logCRP and accounted for 26.2% of the variability in logCRP. BMI SD, HDL-C, and logTNF-α had additional effects (9.7, 2.3, and 1.1%, respectively). In girls, BMI SD had the most significant association with logCRP and accounted for 13.2% of the variability in logCRP. ApoB had an additional effect (1.9%, respectively; Table 6, model 1). When logIL-6 and logTNF-α were included in the model (Table 6, model 2), as in boys, logIL-6 had the most significant association with logCRP and accounted for 27.7% of the variability in logCRP. BMI SD and logTG had additional effects (3.6 and 2.2%, respectively).

To determine the relationship between IL-6 and the other parameters listed in Table 4, we performed a stepwise multiple regression analysis with logIL-6 as the dependent variable and the other parameters as independent variables. BMI SD was most significantly associated with IL-6 and accounted for 14.0 and 15.4% of the variabilities in IL-6 in boys and girls, respectively ($r^2 = 0.140$ and 0.154; $P < 0.0001$). TNF-α and age in boys and TNF-α in girls had additional effects (5.4% in boys and 9.2% in girls).

Discussion

In the present study, we have shown that: 1) boys with high serum concentrations of CRP have more atherogenic clinical and chemical profiles than other children (high levels of BMI SD, insulin, HOMA-R, LDL-C, apoB, uric acid, IL-6, and TNF-α, and low levels of HDL-C, apoA-I, and adiponectin); 2) girls with high serum concentrations of CRP show high levels of BMI SD, insulin, HOMA-R, TC, TG, apoB, uric acid, and IL-6, and low levels of HDL-C; 3) IL-6 had the most significant association with serum concentrations of CRP in children; and 4) BMI SD in children was the most powerful predictor of serum IL-6 concentrations.

In adults, serum concentrations of CRP are increased in subjects with obesity, insulin resistance, hypertension, and/or metabolic syndrome (1–3, 7, 8, 14, 15). These conditions are all well-known risk factors for ACHD. Two recent studies have suggested that serum concentrations of CRP were significant predictors of ACHD even after adjusting for conventional risk factors for ACHD, including serum lipid levels, smoking status, and BMI (3, 14). To date, there have been several large-scale studies of CRP levels in schoolchildren (9–11). Cook *et al.* (9) reported that serum concentrations of CRP were associated with BMI, heart rate, systolic blood pressure, fibrinogen, and HDL-C, but not with other lipid parameters. Ford (11) showed that serum concentrations of CRP were associated with BMI, systolic blood pressure, and TG, but not with glycosylated hemoglobin or glucose. Wu *et al.* (10) reported that BMI, TG, and HDL-C were associated with serum concentrations of CRP in schoolchildren in Taiwan. A common finding among these reports is that BMI was the most powerful predictor of serum concentrations of CRP in schoolchildren. In contrast to our study, adiponectin, IL-6, TNF-α, and uric acid were not determined in these previous reports. When we removed cytokines and uric acid from our statistical analysis, in agreement with previous reports, BMI SD was the most powerful predictor of CRP in our children. After being corrected for BMI SD, age, HOMA-R, TG, LDL-C, apoB, and adiponectin in boys and age, HOMA-R, and TG in girls were no longer correlated with CRP. When we added uric acid to the statistical analysis, it was correlated with CRP in both boys and girls. However, a significant correlation was only found in girls after being corrected for BMI SD. Based on a recent report, human vascular smooth muscle cells and human umbilical vein endothelial cells are also sources of CRP production (16). CRP mRNA expression in human vascular smooth muscle cells, and human umbilical vein endothelial cells and the release of CRP into cell culture medium were both up-regulated by uric acid (16). Although additional studies are needed, our data for girls suggest that vascular cell damage induced by uric acid may begin in childhood.

With respect to cytokines, IL-6 and TNF-α themselves have been reported to be risk factors for ACHD and type 2 diabetes mellitus, even after adjusting for BMI (17, 18). To date, IL-6 and TNF-α are believed to mediate the relationship between BMI and CRP in children, because IL-6 and TNF-α are the main inducers of the hepatic production of CRP and are expressed in and secreted from adipose tissue (19–21). To the best of our knowledge, no previous epidemiological evidence is available on the relation between CRP and these cytokines in children. In the present study IL-6 was the most significant predictor of CRP in both boys and girls. TNF-α was not a significant predictor of CRP in girls. BMI SD was the most powerful predictor of serum IL-6 in children. Mohamed-Ali *et al.* (22) reported that in humans, although IL-6 and TNF-α were both expressed in sc adipose tissues, only IL-6 was released from sc adipose tissues. However, in adults, serum concentrations of these cytokines were closely related to obesity, particularly central obesity (21). These findings suggest that sc fat may be responsible for the production of IL-6 in children, and the accumulation of visceral fat may be less evident in girls than in boys.

In conclusion, although serum concentrations of CRP are partly regulated by adipocytokines and conventional risk factors for ACHD, high CRP levels were associated with atherogenic profiles of cardiovascular risk factors in children. IL-6 was the most powerful predictor of serum CRP in chil-

dren. BMI SD in both boys and girls was the most significant predictor of IL-6. These findings suggest that it may be important to control body weight to prevent an increase in serum CRP in children.

Acknowledgments

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Original Article

Birthweight and risk factors for cardiovascular diseases in Japanese schoolchildren

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Abstract

Background: Low birthweight (LBW) is associated with an increased risk for atherosclerotic coronary heart disease (ACHD) later in life. However, little information is currently available on the relationship between birthweight (BW) and risk factors for ACHD in children.

Methods: The relationship between BW and risk factors for ACHD was evaluated in 330 Japanese children (187 boys and 143 girls) aged between 7 and 12 years, who underwent screening for lifestyle-related diseases in Okinawa, Japan. Routine chemical methods were used to determine the serum concentrations of lipids, apolipoproteins, uric acid and glucose. Serum insulin and adiponectin were measured by sandwich enzyme-linked immunosorbent assay.

Results: BW was significantly correlated with serum concentrations of adiponectin ($r = 0.163$, $P = 0.003$) and uric acid ($r = -0.166$. $P = 0.003$), but not with insulin, lipids or apolipoproteins. These correlations were still significant even after adjusting for age, gender and body mass index (BMI) percentile (BW and adiponectin, $r = 0.239$, $P = 0.000$; BW and uric acid, $r = -0.247$, $P = 0.000$). In addition, BW was correlated with high-density lipoprotein-cholesterol (HDL-C) only after adjusting for age, gender and BMI percentile ($r = 0.117$, $P = 0.034$). In a stepwise multiple regression analysis, BW was a significant predictive variable for adiponectin and uric acid. However, weight velocity (weight gain/year) was a stronger predictive variable than BW for both adiponectin and uric acid. BW was not a significant predictive variable for HDL-C. Adiponectin was the strongest predictive variable for HDL-C.

Conclusion: BW is related to serum concentrations of adiponectin and uric acid. However, weight velocity was a stronger determinant of serum adiponectin and uric acid levels than BW in Japanese schoolchildren. Thus, it may be important to control weight gain to prevent the development of ACHD in children, especially in children with LBW.

Key words adiponectin, atherosclerosis, birthweight, BMI, insulin resistance, weight velocity.

Several epidemiological studies have indicated that low birthweight (LBW) is associated with an increased prevalence of, and mortality due to, atherosclerotic coronary heart disease (ACHD) later in life.[1–3] Fetal nutrition and early postnatal growth are thought to contribute to this association.[1,4] The initial stage of atherosclerosis begins in childhood and progresses from fatty streaks to raised lesions in adolescence and young adulthood.[5,6] This process is accelerated in children with risk factors for ACHD.[7] Thus, it seems reasonable to consider the relationship between birthweight (BW) and risk factors for ACHD in children. To date, most studies have been performed in adults and have indicated that there are significant relationships between BW and risk factors for ACHD including blood pressure, cholesterol, and insulin sensitivity.[8–10] However, conventional risk factors for ACHD acquired later in life such as smoking, obesity, diabetes mellitus and so on may affect these relationships. In contrast, children rarely drink alcohol or smoke, and usually exercise regularly at school. Thus, environmental factors that affect the relationship between BW and risk factors may have less of an effect in children than in adults. Several reports are now available in children. However, the relations between BW and risk factors for ACHD are still controversial.[11–21] While some studies have reported a significant relationship, others have reported no association between BW and risk factors for ACHD.

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In the present study we investigated the relationships between BW and conventional risk factors for ACHD in Japanese schoolchildren. In addition, we studied the relationships between BW and adiponectin and uric acid. Adiponectin is a so-called adipocytokine that is secreted from adipocytes. In recent reports, adiponectin levels have been shown to be lower in the presence of prevalent coronary artery diseases.[22,23] Higher adiponectin levels were associated with a lower risk of myocardial infarction in the Health Professionals Follow-up Study.[24] This relationship can be only partly explained by differences in blood lipids and is independent of inflammation and glycemic status.[24] In the case of uric acid, many reports have demonstrated that uric acid is not an independent risk factor for ACHD.[25,26] However, recent studies have shown that serum uric acid levels are positively associated with hypertension, inflammation and ACHD mediated by endothelial dysfunction and pathologic vascular remodeling.[27] The aim of the present study was to clarify the effect of BW on conventional and newly identified risk factors for ACHD in Japanese schoolchildren.

Methods

Subjects

Informed consent was obtained from the parents of all of the children. The present study was approved by the Review Board of the University of the Ryukyus. We studied 330 Japanese children (187 boys and 143 girls) aged between 7 and 12 years, who underwent screening for lifestyle-related diseases in Okinawa, Japan. Body mass index (BMI) was calculated as weight [kg]/height^2 [m^2]. BMI percentiles were obtained based on data regarding BMI percentiles for Japanese children.[28] The mother provided BW based on the *Maternal and Child Health Handbook*. None of the children studied was receiving therapy for weight reduction or drugs to affect lipid metabolism. Venous blood was drawn after an overnight fast.

Laboratory measurements

Routine chemical methods were used to determine the serum concentrations of total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), triglycerides (TG), uric acid, glucose, and electrolytes. Serum insulin was measured by two-step sandwich enzyme-linked immunosorbent assay (ELISA; SRL, Hachioji, Japan). Low-density lipoprotein-cholesterol (LDL-C) was calculated as TC – HDL-C – TG/5. Apolipoproteins (A-I and B) were measured by the turbidity immunoassay method.[29] The serum adiponectin concentration was measured by sandwich ELISA (Otsuka Pharmaceutical, Tokusima, Japan). LDL-size was evaluated by electrophoresis in non-denaturing polyacrylamide gradient gels on precast Multigel-LP (2–15%; Daiichi Pure Chemical, Tokyo, Japan), as described previously.[30] Insulin resistance and insulin sensitivity were calculated using the homeostasis model approximation index (HOMA2-IR) and the quantitative insulin-sensitivity check index (QUICKI).[31,32] These equations, both based on fasting glucose and insulin, correlate well with insulin dynamics as measured on the hyperinsulinemic clamp and the i.v. glucose tolerance test. Weight velocity (WV) between birth and current age was calculated as kg gained per year.

Statistical evaluation

Differences in parameters among subjects in three groups (BW: small, middle, large) were determined using the Kruskal–Wallis test. Parameters in these three groups were compared with Scheffe's multiple comparison test. Age and gender were adjusted for by an analysis of covariance (ancova). Pearson and partial correlation coefficients were computed to assess the associations between BW and other parameters. A stepwise multiple regression analysis was performed by entering the independent variable with the highest partial correlation coefficient at each step, until no variable remained with $F > 4$. Group differences or correlations with $P < 0.05$ were considered to be statistically significant. All statistical analysis was performed using Stat View J-5.0 software (SAS Institute, Cary, NC, USA).

Results

The BW in the present children ranged from 1520 to 4704 g, and 8.4% of the BW were <2500 g. To understand the relation between BW and lipids and other parameters, subjects were divided into three groups based on BW percentiles (small, <10th; middle, 10th–90th; large, >90th). As shown in Table 1, no difference was found in BMI percentiles, lipids, apolipoproteins, insulin resistance (HOMA2-IR) or insulin sensitivity (QUICKI). Significant graded relationships between these three groups were found in adiponectin and uric acid. However, significant differences in adiponectin were found only between small and middle BW groups. In the case of uric acid, significant differences were found between the small and large and between the middle and large BW groups.

As shown in Table 2, BW was correlated with adiponectin ($r = 0.163$, $P = 0.003$) and inversely correlated with uric acid ($r = -0.166$, $P = 0.003$). Significant correlations were not found in other parameters listed in Table 2. After being corrected for age, gender and BMI percentile, significant correlations were still found in both adiponectin and uric acid (adiponectin, $r = 0.239$, $P = 0.000$; uric acid, $r = -0.247$,

Table 1 Clinical and chemical data on children adjusted for age and gender (mean ± SEM)

	Small <10th Percentile	(*P*)	Middle 10th–90th Percentile	(*P*)	Large >90th Percentile
n	33		264		33
Birthweight (kg)	2.21 ± 0.05	(<0.0001)	3.16 ± 0.02	(<0.0001)	3.94 ± 0.04***
BMI percentile	67.4 ± 6.2	(n.s.)	63.8 ± 2.0	(n.s.)	74.8 ± 4.3
Weight velocity (kg/year)	3.85 ± 0.23	(n.s.)	3.54 ± 0.07	(n.s.)	3.81 ± 0.20
Adiponectin (μg/mL)	8.0 ± 0.5	(n.s.)	9.2 ± 0.2	(n.s.)	10.4 ± 0.7*
Glucose (mg/dL)†	91 ± 1	(n.s.)	91 ± 1	(n.s.)	92 ± 1
Insulin (μU/mL)	10.0 ± 0.4	(n.s.)	9.2 ± 0.5	(n.s.)	9.9 ± 1.7
HOMA2-IR	1.3 ± 0.2	(n.s.)	1.2 ± 0.1	(n.s.)	1.3 ± 0.2
QUICKI	0.37 ± 0.01	(n.s.)	0.38 ± 0.01	(n.s.)	0.37 ± 0.01
TC (mg/dL)‡	175 ± 5	(n.s.)	176 ± 2	(n.s.)	183 ± 5
TG (mg/dL)§	82 ± 7	(n.s.)	74 ± 3	(n.s.)	79 ± 9
LDL-C (mg/dL)‡	103 ± 4	(n.s.)	102 ± 2	(n.s.)	104 ± 4
HDL-C (mg/dL)‡	58 ± 2	(n.s.)	62 ± 1	(n.s.)	64 ± 2
ApoA-I (mg/dL)	132 ± 3	(n.s.)	141 ± 4	(n.s.)	140 ± 3
ApoB (mg/dL)	76 ± 3	(n.s.)	73 ± 1	(n.s.)	75 ± 3
Uric acid (mg/dL)	5.2 ± 0.2	(<0.01)	4.6 ± 0.1	(n.s.)	4.4 ± 0.1**
LDL-size (nm)	26.9 ± 0.2	(n.s.)	27.2 ± 0.1	(n.s.)	27.2 ± 0.2

Apo, apolipoprotein; BMI, body mass index; HDL-C, high-density lipoprotein-cholesterol; HOMA2-IR, homeostasis model approximation index; LDL-C, low-density lipoprotein-cholesterol; QUICKI, quantitative insulin-sensitivity check index; TC, total cholesterol; TG, triglyceride.†To convert to mmol/L, divide by 18; ‡to convert to mmol/L, multiply by 0.0259; §]to convert to mmol/L, multiply by 0.0113. *$P < 0.05$; **$P < 0.01$; ***$P < 0.0001$; significantly different from small.

$P = 0.000$). In addition, HDL-C was correlated with BW only after being corrected for age, gender and BMI percentile. Serum concentrations of adiponectin, uric acid and HDL-C are associated with many risk factors for ACHD. Thus, to understand the contribution of BW to serum concentrations of adiponectin, uric acid and HDL-C in schoolchildren, we performed a stepwise multiple regression analysis with adiponectin, uric acid and HDL-C as dependent variables and the other parameters as independent variables. As shown in Table 3, BW was a significant predictive variable for adiponectin and uric acid but not for HDL-C. However, WV (weight gain/year) was a stronger predictive variable than BW for both adiponectin and uric acid. HDL-C was also a stronger predictive variable than BW for adiponectin. Adiponectin was the strongest predictive variable for HDL-C. Table 4 shows the correlations between WV and several parameters. WV was positively correlated with age, gender, BMI percentile and uric acid, and inversely associated with adiponectin. After being corrected for age, gender and BMI percentile, the correlations of WV with adiponectin and uric acid were still significant (partial correlation in Table 4).

Table 2 Birthweight and variables

	Simple correlation		Partial correlation	
	r†	*P*	r‡	*P*
Age	0.034	0.539		
Gender	−0.070	0.207		
BMI percentile	0.084	0.128		
Weight velocity	0.028	0.614	−0.077	0.163
Adiponectin	0.163	0.003	0.239	0.000
Glucose	0.057	0.308	0.057	0.303
Log insulin	0.020	0.722	−0.093	0.092
Log HOMA2-IR	0.020	0.712	−0.083	0.133
QUICKI	−0.023	0.672	0.081	0.143
TC	0.013	0.814	0.003	0.957
Log TG	−0.074	0.181	-0.098	0.076
LDL-C	−0.009	0.876	−0.029	0.600
HDL-C	0.073	0.189	0.117	0.034
ApoA-I	0.038	0.498	0.050	0.366
ApoB	−0.035	0.529	−0.061	0.270
Uric acid	−0.166	0.003	−0.247	0.000
LDL-size	0.022	0.693	0.068	0.219

Apo, apolipoprotein; BMI, body mass index; HDL-C, high-density lipoprotein-cholesterol; HOMA2-IR, homeostasis model approximation index; LDL-C, low-density lipoprotein-cholesterol; QUICKI, quantitative insulin-sensitivity check index; TC, total cholesterol; TG, triglyceride.†Pearson correlation coefficient; ‡variables corrected for age, gender and BMI percentile.

Discussion

In the present study, serum concentrations of adiponectin, uric acid and HDL-C, but not insulin, lipids (except HDL-C) or apolipoproteins were correlated with BW. A significant correlation was not recognized between BW and BMI percentile.

Table 3 Stepwise multiple regression analysis of correlates of adiponectin, uric acid and HDL-C

	Independent parameters	r	r^2
Adiponectin			
Step 1	WV	0.511	0.261
Step 2	WV, HDL-C	0.568	0.323
Step 3	WV, HDL-C, BW	0.590	0.348
Step 4	WV, HDL-C, BW, ApoB	0.605	0.368
Step 5	WV, HDL-C, BW, ApoB, BMI percentile	0.614	0.378
Steps 1–5: $P < 0.0001$			
Uric acid			
Step 1	WV	0.537	0.288
Step 2	WV, BW	0.566	0.321
Step 3	WV, BW, ApoB	0.592	0.350
Step 4	WV, BW, ApoB, LDL-C	0.619	0.384
Steps 1–4: $P < 0.0001$			
HDL-C			
Step 1	Adiponectin	0.415	0.172
Step 2	Adiponectin, log TG	0.502	0.252
Step 3	Adiponectin, log TG, TC	0.605	0.366
Steps 1–3: $P < 0.0001$			

Apo, apolipoprotein; BMI, body mass index; BW, birthweight; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; TC, total cholesterol; TG, triglyceride; WV, weight velocity.

Table 4 Weight velocity and variables

	Simple correlation		Partial correlation	
	$r^\dagger$	P	$r^\ddagger$	P
Age	0.236	0.000		
Gender	0.161	0.004		
BMI percentile	0.835	0.000		
Adiponectin	−0.512	0.000	−0.188	0.001
Uric acid	0.542	0.000	0.243	0.000

†Pearson correlation coefficient; ‡variables corrected for age, gender and BMI percentile.

BMI, body mass index.

Birthweight and adiponectin

Clinically, serum concentrations of adiponectin are decreased in subjects with obesity, type 2 diabetes or coronary heart disease.[22,33] Similar to the results in adults, serum concentrations of adiponectin in obese children are inversely correlated with body fat and, as a result, serum adiponectin levels are significantly lower than those in non-obese children.[34,35] In the present study we confirmed these findings in Japanese schoolchildren (T. Ohta, unpubl. data, 2005: BMI percentile >95th, 6.6 ± 0.3 μg/mL; <95th, 10.2 ± 0.2 μg/mL). Regarding the relationship between adiponectin and BW, Cianfarani *et al.* reported that serum adiponectin concentrations were reduced in children who were born small for gestational age (SGA).[36] In contrast, Lopez-Bermejo *et al.* reported opposite results: serum adiponectin concentrations were higher in SGA children.[37] In the present study most of the children studied were not SGA. BW was positively correlated with serum concentrations of adiponectin. Thus, the present data extend the finding of Cianfarani *et al.* to non-SGA children. Although BW was an independent predictor for adiponectin, it had a much weaker contribution than WV in schoolchildren. This suggests that postnatal weight gain may affect the function of adipocytes more than fetal growth *in utero*.

Birthweight and uric acid

Low birthweight correlates with impaired renal development and a reduced number of nephrons at birth.[38–40] Based on an animal study, a reduction in the number of nephrons results in an increase in proximal reabsorption.[41] Uric acid reabsorption is linked to proximal sodium reabsorption.[42] These serial findings might explain the inverse relationship between BW and uric acid. Most recently, Feig *et al.* reported that the serum uric acid level correlates inversely with BW in adolescents with essential hypertension.[41] In the present study we measured blood pressure in only 140 children (data not shown) and hypertensive children were not identified. Feig *et al.* did not provide any data on subjects with normal blood pressure. Because the present children were younger than the children in the Feig *et al.* study, the present data may suggest that children with LBW might have a risk for future development of hypertension. However, in the present study the most powerful predictor of the serum uric acid level was WV. As with adiponectin, postnatal weight gain seems to be more important than fetal growth *in utero* in the control of uric acid levels. To date, many studies have demonstrated that uric acid is not an independent risk factor for ACHD.[25,26] Conventional risk factors for ACHD such as LDL-C and apolipoprotein B influence serum uric acid levels (Table 3). Thus, further studies are needed to clarify the effect of uric acid on the future development of ACHD.

Birthweight and lipids and apolipoproteins

Based on reports in adults, BW shows only a weak or no association with lipids and apolipoproteins.[9,21] Similar to the results in adults, weak inverse relationships between BW and lipids (TC and LDL-C) have been reported in 8-year-old Indian children.[11] Mean values of BW, TC and LDL-C in Indian children were lower than those in the present children (BW, 2.7–2.8 vs 3.14–3.15 kg; TC, 131 vs 172–183 mg/dL; LDL-C, 77 vs 96–110 mg/dL). Mortaz *et al.* reported that BW is not related to TC, TG, LDL-C, HDL-C, apolipoprotein A-I or apolipoprotein B in children (BW, <1850 g) aged 8–12 years.[12] The age distribution and lipid values of the present subjects were similar to the Mortaz *et al.* subjects and, as in their