increases in LDL-C owing to E4 were 1.9- and 1.4-fold greater in those individuals on a high saturated fatty acid (SAFA)-cholesterol diet (high SAFA/Chol group) compared to those consuming low and middle SAFA-cholesterol diets (low and middle SAFA/Chol groups).[33] In their study, the LDL-C-lowering effect of E2 was 2.4- and 1.6-fold greater in the high SAFA/Chol group compared with the low and middle SAFA/Chol groups, respectively. In the CAD patients, the E2-associated increases in TG were evident only in those on a high sucrose diet.[34] Furthermore, the analyses using polyacrylamide gel electrophoresis and ultracentrifugation revealed that most E7 heterozygotes exhibited elevated remnant lipoproteins irrespective of the presence or absence of hyperlipidaemia. This abnormality was markedly ameliorated by a low-calorie, low-fat and low-cholesterol diet.[27]

Collectively, it appears that the effects of adiponectin on lipoprotein profiles are likely to be masked in groups of obese children who consist of various apoE carriers. In general, serum adiponectin concentration is closely associated with visceral adiposity,[6,7] hypercholesterolaemia,[9–12] hypertriglyceridaemia,[6,9,10,12,35] hypo-α-lipoproteinaemia,[6,9,10,12] hypertension,[11,36] hyperglycaemia and insulin resistance.[6,7,9–11] In obese children, however, studies sometimes found only limited clinical manifestations associated with the low adiponectin concentration. In Japanese obese boys, for example, adiponectin concentration was inversely related to both LDL-C and fasting insulin concentrations; however, no correlations with SBP, DBP, TG and HDL-C were observed.[37] In American obese children and adolescents, adiponectin concentrations were positively associated with the whole body insulin sensitivity index and HDL-C, but no associations were found with LDL-C, TG, SBP, DBP and HOMA-IR.[38] When we analysed all obese children with various apoE phenotypes together, the low adiponectin group was significantly correlated with high HOMA-IR, but not with other metabolic abnormalities. However, when the same analysis was performed in E3/3 children, low adiponectin concentrations were significantly associated with several metabolic parameters including TG, LDL-C, HDL-C, RLP-C, SBP, DBP and HOMA-IR (Table 4; Figures 1 and 2). Therefore, when apoE phenotypes are taken into account, the serum adiponectin concentration appears to have a much greater impact on these parameters than previously reported.

Our findings suggest that some sex differences exist in the effects of adiponectin on metabolic parameters. We found that obese boys in the low adiponectin group had a greater number of abnormalities in lipoprotein profiles and blood pressure than the corresponding obese girls (Table 4; Figures 1 and 2). As both groups had similar values of serum adiponectin and HOMA-IR, the female subjects may have been protected against atherosclerosis by a low susceptibility to metabolic abnormalities induced by insulin resistance. In most cohort studies, menopause decreases HDL-C, but increases LDL-C and TG concentrations.[39] Hormone replacement therapy reduces LDL-C and BP, but increases HDL-C.[40,41] In the obese girls, these favourable effects of oestrogen may obscure the metabolic abnormalities caused by low adiponectin concentration. Further studies are needed to elucidate the specific underlying mechanisms.

We conclude that a low adiponectin concentration is associated with insulin resistance and downstream metabolic abnormalities in obese children, notably when having E3/3. This association seems to be more evident in obese boys than in obese girls. We speculate that the decreased susceptibility of girls to the effects of low adiponectin concentrations may partially account for the low frequency of observed metabolic syndrome and CAD in adult females.

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ORIGINAL ARTICLE

Low-density lipoprotein profile changes during the neonatal period

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Objective: To investigate natural change of low-density lipoprotein (LDL) profile during the neonatal period and the impact of gestational age and birth weight on those changes.

Study Design: We measured lipid composition in LDL fraction, LDL particle size and apolipoprotein B (apoB) concentration at birth, 5 days of age and 1 month of age in 68 healthy neonates that had 37 to 41-week gestational age.

Result: Low-density lipoprotein cholesterol and apoB concentrations increased from birth to 5 days of age, and the concentration persisted at 1 month in breast-fed and mixed-fed infants. However, in formula-fed infants, the concentration decreased at 1 month. At 5 days of age, neonates had larger and more triglyceride (TG)-rich LDL particles than at birth. At 1 month of age, LDL particles were smaller and more cholesterol rich than at 5 days of age. Single regression analyses showed that gestational age had influenced the LDL profile at birth and 5 days of age, while at 1 month milk determined the profile.

Conclusion: The number of LDL particles increased rapidly during the first 5 days of life, and the composition of LDL particles is modulated by TG content throughout the neonatal period. Gestational age and milk, rather than birth weight, determine postnatal changes in LDL profile. *Journal of Perinatology* (2008) 28, 335–340; doi:10.1038/jp.2008.8; published online 13 March 2008

Keywords: LDL particle size; lipid composition; gestational age; postnatal change; formula-feeding

Introduction

Epidemiological studies in humans show that intrauterine growth retardation is an important determinant of cardiovascular risks in later life.[1] Glucose intolerance and hypertension are related to birth size.[2,3] The mechanisms that underlie the relationship between birth size and coronary risks have been investigated in natural and experimental animal models. The results of those studies suggest that glucocorticoids have a key role in intrauterine programming.[4] Furthermore, in animal studies of intrauterine undernutrition, hypercholesterolemia developed in later life.[5,6] In human study,[7] lower birth weight was also associated with higher total cholesterol (TC) concentrations in adult men. However, the link between hypercholesterolemia and birth size in human is controversial. For example, in a study of adolescent twin pairs, genetic, not intrauterine, factors accounted for the relationship between low birth weight and high concentrations of TC, low-density lipoprotein (LDL) cholesterol and apolipoprotein B (apoB).[8] A meta-analysis study[9] suggested that birth weight and blood cholesterol concentration in adolescents are weakly associated and of limited importance for public health.

Studies in adults indicate that not only TC and LDL cholesterol concentrations, but also heterogeneity in LDL density, composition and oxidation, are important determinants of atherosclerosis. Cord blood has a unique lipoprotein profile that is different from adults. Cord TC and LDL cholesterol concentrations are approximately one-third that of adult concentrations, while high-density lipoprotein (HDL) cholesterol and triglyceride (TG) are about one-half that of adults.[9] Furthermore, cord blood has a pattern of lipoprotein subclass heterogeneity that is different from adults. In a study of lipoprotein heterogeneity in cord blood, the concentration of apolipoprotein E-rich HDL cholesterol was about twice that of adults and represented more than 30% of total HDL cholesterol.[10] Kwiterovich *et al.*[11] reported that cord blood of small for their gestational age neonates had an atherogenic lipoprotein profile with TG-rich very low-density lipoprotein and intermediate concentrations of LDL. The group also reported that neonates with elevated cord blood apoC-I-enriched HDL had lower birth weights and younger gestational ages.[12] However, there are many genetic and environmental factors besides birth weight that affect the cord blood lipoprotein profile,[13] and there is limited information linking the cord blood lipoprotein profile to lipoprotein concentrations in later life.[14]

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The aim of the present study was to investigate the natural changes in the lipid profile, especially in LDL, during the neonatal period and explore the impact of gestational age and birth size on postnatal changes in the lipoprotein profile.

Methods

Subjects

A total of 63 healthy (37 male and 26 female) neonates who were born by uneventful vaginal delivery or cesarean operation in the maternity ward of Nihon University Hospital, located in Itabashi-ku, Tokyo, Japan, from September 2004 to March 2005 were included in the study. All of the mothers were healthy, and their pregnancies were without complications. All infants had a 37 to 41-week gestational age. None of the neonates had asphyxia at birth, and all remained healthy throughout the study period based on physical examination. Breast- or formula-feeding was started within the first 12 h. At 5 days, all neonates had mixed feeding every 3 h in the hospital.

Lipoprotein analyses

Serum lipoprotein was collected and analyzed at birth, 5 days of age and 1 month of age. At birth, the umbilical was double clamped, and cord blood was sampled from the umbilical vein. Venous blood was obtained by venipuncture just before feeding at 5 days and 1 month of age. TC and TG concentrations were measured by enzymatic methods. ApoB concentration was measured by turbidimetric immunoassay (Daiichi Chemicals Co., Tokyo, Japan). Serum lipoprotein analyses were performed by high-performance liquid chromatography with gel permeation columns (LipoSEARCH; Skylight-Biotec Inc., Akita, Japan), which measured cholesterol and TG concentration in each lipoprotein fraction and lipoprotein particle size distribution simultaneously.[15] Feeding information (that is, exclusively breast-fed, exclusively formula-fed, mixed-breast and formula-fed) was obtained from each mother 1 month after each child's birth.

Informed consent was obtained from all parents, and the study was approved by the University Ethics Committee (Nihon University, Itabashi Hospital).

Statistical analyses

All statistical analyses were conducted using STATVIEW (version 4.5, Abacus Concepts, Berkeley, CA, USA). Data are reported as mean ± s.e. Differences in measured parameters between birth and 5 days of age, birth and 1 month of age, and 5 days of age and 1 month of age were analyzed with a Mann–Whitney *U*-tests. Significant differences between feeding modes were analyzed with analysis of variance. Simple and multiple regressions were used to assess correlations between variables. To analyze the effect of birth weight, gestational age and milk mode on LDL profile using multiple regression analyses, the three categorized milk subgroups replaced with continuous variables (1, exclusively breast-fed; 2, mixed-fed; 3, exclusively formula-fed). *P*-values <0.05 were considered significant.

Results

The birth weight in male and female neonates was 3112.6 ± 73.7, 2931.4 ± 67.5 g ($P = 0.1024$), and the birth length was 48.6 ± 0.3, 47.7 ± 0.4 cm ($P = 0.0810$), respectively. No sex difference was demonstrated in gestational age, 38.8 ± 0.2 weeks in male and 38.7 ± 0.3 weeks in female neonates ($P = 0.9086$).

Changes in serum lipids, lipoproteins and apoB

During the first 5 days of life, when all neonates had mixed-fed, serum concentrations of TC and TG increased rapidly. At 1 month of age, serum TC concentration increased further in mixed-fed and breast-fed infants, while serum TG concentration had decreased. At 5 days of age, HDL cholesterol concentration was the same as the cord blood concentration and increased at 1 month. LDL cholesterol concentration increased 2.5-fold from birth to 5 days and persisted at 1 month in mixed-fed. However, LDL cholesterol concentration decreased at 1 month in formula-fed, and increased further in breast-fed. ApoB concentration demonstrated a similar pattern. On the other hand, LDL-TG concentration and LDL particle peak diameter showed a transient increase. At 5 days of age, neonates had more and larger TG-rich LDL particles than at birth and 1 month of age (Table 1).

Correlation of birth weight and gestational age to LDL profile

Single regression analyses showed that gestational age had a marked influence on the LDL profile at birth and at 5 days of age, while birth weight had limited association with the LDL profile during that time. Multiple regression analyses that included birth weight and gestational age as predictors for apoB concentration at 5 days of age demonstrated that birth weight is not a significant predictor (Table 2).

The relationship between gestational age and LDL profile changes during the neonatal period (Figure 1). At birth, LDL cholesterol and apoB concentrations were negatively correlated with gestational age. At 5 days, however, the correlations changed to be positive. Increases in LDL cholesterol and apoB concentrations were associated with gestational age ($r = 0.480$, $P < 0.0001$ and $r = 0.455$, $P = 0.0013$) during the first 5 days of life. The change in LDL peak particle size during the first 5 days was negatively correlated with gestational age ($r = -0.270$, $P = 0.0325$). At 1 month, gestational age had no significant relationship with LDL cholesterol in breast-fed. While in mixed-fed the positive correlation persisted.

Table 1 Change in lipid profile and apoB concentration

Milk-feeding mode	*At birth*	*5 days mixed-fed*	*At birth vs 5 days*	*1 month formula-fed, mixed-fed, breast-fed*	*At birth vs 1 month*	*5 days vs 1 month*
Total cholesterol (mg per 100 ml)	65.1 ± 2.0	116.0 ± 2.5	<0.0001	114.2 ± 5.3	0.0004	0.6214
				128.2 ± 4.5	<0.0001	0.0056
				148.8 ± 5.4	<0.0001	0.0037
Triglyceride (mg per 100 ml)	24.5 ± 1.3	133.4 ± 9.5	<0.0001	59.8 ± 9.4	0.0002	0.0109
				68.9 ± 5.0	<0.0001	<0.0001
				71.5 ± 6.1	<0.0001	0.0059
HDL cholesterol (mg per 100 ml)	37.0 ± 1.3	37.7 ± 1.2	0.5797	60.2 ± 3.8	0.0034	0.0021
				61.9 ± 2.7	<0.0001	<0.0001
				61.6 ± 3.3	<0.0001	<0.0001
LDL cholesterol (mg per 100 ml)	20.8 ± 0.8	49.1 ± 1.6	<0.0001	37.7 ± 2.8	0.0003	0.0205
				47.2 ± 2.4	<0.0001	0.6704
				63.5 ± 2.1	<0.0001	0.0104
LDL triglyceride (mg per 100 ml)	10.1 ± 0.5	33.7 ± 1.8	<0.0001	13.9 ± 0.8	0.0109	0.0012
				17.5 ± 0.9	<0.0001	<0.0001
				21.9 ± 0.6	<0.0001	0.0023
LDL cholesterol/triglyceride ratio	2.3 ± 0.1	1.6 ± 0.1	0.0001	2.7 ± 0.2	0.0527	0.6012
				2.9 ± 0.2	0.0324	<0.0001
				3.2 ± 0.3	0.0195	0.0002
Peak LDL particle diameter (nm)	25.8 ± 0.1	26.3 ± 0.1	<0.0001	25.8 ± 0.2	0.1599	0.0806
				25.8 ± 0.1	0.9679	0.0006
				25.6 ± 0.2	0.0621	0.0159
ApoB (mg per 100 ml)	19.4 ± 1.5	32.1 ± 2.1	<0.0001	23.7 ± 4.1	0.1563	0.0151
				33.3 ± 2.1	<0.0001	0.1892
				39.6 ± 5.0	0.0017	0.6444

Abbreviations: ApoB, apolipoprotein B; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Values are mean ± s.e. Mann–Whitney test.

Relationship between LDL profile and mode of milk-feed at 1 month of age

At 1 month of age, 9 of the subjects were exclusively breast-fed, 35 were breast- and formula-fed, and 16 were exclusively formula-fed. We did not obtain feeding information from three mothers. The three feeding groups did not differ in birth weight or gestational age. Multiple regression analyses including feeding mode, birth weight and gestational age as predictors for LDL cholesterol, LDL-TG and apoB concentrations demonstrated that feeding mode was the only determinant (Table 3).

Discussion

The present study demonstrates that the LDL profile changes rapidly during the neonatal period. LDL cholesterol and apoB concentrations show marked increases from 0 to 5 days of age, and the concentrations persist at 1 month of age in breast- and mixed-fed infants. At 5 days of age, neonates had larger and more TG-rich LDL particles than at birth. At 1 month of age, LDL particles became smaller and more cholesterol rich than at 5 days. These results demonstrate that the number of LDL particles increases rapidly during the first 5 days of life and that the composition of LDL particles is modulated by TG content throughout the neonatal period. The changes in LDL profiles in healthy neonates may represent a natural adaptation process of LDL metabolism for extrauterine life.

Van Biervliet *et al.*[16] measured lipoproteins and apolipoproteins in cord blood and at 7 and 30 days of age. The present results are compatible with their results showing a drastic increase in LDL cholesterol and apoB concentrations during the first week of life

Table 2 Relationship between LDL profile and birth weight, gestational age

	Birth weight		*Gestational age*	
	Coefficient	*P-value*	*Coefficient*	*P-value*
At birth				
LDL cholesterol (mg per 100 ml)	−0.139	0.2765	−0.339	0.0065
LDL triglyceride (mg per 100 ml)	0.201	0.1145	0.312	0.0129
LDL cholesterol/triglyceride ratio	−0.216	0.0887	−0.482	<0.0001
Peak LDL particle diameter (nm)	−0.029	0.8235	0.008	0.9524
ApoB (mg per 100 ml)	−0.096	0.4994	−0.157	0.2665
At 5 days of age				
LDL cholesterol (mg per 100 ml)	0.216	0.0885	0.356	0.0042
LDL triglyceride (mg per 100 ml)	0.140	0.2744	0.473	<0.0001
LDL cholesterol/triglyceride ratio	−0.059	0.6480	−0.384	0.0019
Peak LDL particle diameter (nm)	−0.028	0.8275	−0.336	0.0071
ApoB (mg per 100 ml)	0.320	0.0268	0.476	0.0006
At 1 month of age				
LDL cholesterol (mg per 100 ml)				
Formula-fed	0.279	0.4053	0.276	0.415
Mixed-fed	0.353	0.0348	0.435	0.008
Breast-fed	0.518	0.04	0.044	0.8728
LDL-triglyceride (mg per 100 ml)				
Formula-fed	0.008	0.9806	0.25	0.4582
Mixed-fed	0.457	0.0051	0.348	0.0376
Breast-fed	0.12	0.6582	0.386	0.1396
LDL cholesterol/triglyceride ratio				
Formula-fed	0.341	0.3043	0.587	0.0578
Mixed-fed	−0.025	0.8847	0.087	0.6146
Breast-fed	0.205	0.4472	−0.444	0.0849
Peak LDL particle diameter (nm)				
Formula-fed	0.094	0.7836	0.115	0.7373
Mixed-fed	−0.356	0.033	0.264	0.1203
Breast-fed	0.25	0.3509	−0.193	0.4729
ApoB (mg per 100 ml)				
Formula-fed	0.445	0.269	0.301	0.4683
Mixed-fed	0.26	0.1514	0.449	0.0099
Breast-fed	0.253	0.4267	0.403	0.1944

Abbreviations: ApoB, apolipoprotein B; LDL, low-density lipoprotein.

that persisted through 30 days of age. On the other hand, LDL-TG and LDL peak diameter in neonates has not previously been investigated. Our results demonstrate a rapid, transient increase in LDL-TG concentration and particle size. This is most likely due to a reduction in hepatic lipase activity at birth and 5 days of age.[17] Although cord TG concentration is markedly lower than that in adults, neonate cord LDL is richer in TG.[10] The present study suggests that the influence of hepatic lipase activity on LDL heterogeneity persists during at least the first 5 days of life and is greater in neonates with younger gestational age.

The present results also demonstrate the effect of gestational age, but not birth weight, on the LDL profile. In human fetuses, increases in hepatic LDL receptor activity were positively correlated with gestational age and were negatively correlated with LDL cholesterol concentration.[18] Our finding that LDL cholesterol concentration was negatively correlated with gestational age in cord blood may be partly explained by LDL receptor activity. Furthermore, in fetuses, the major organ to utilize cholesterol is the adrenal gland, and adrenal gland development may affect LDL cholesterol concentration.[19] Therefore, the LDL profile of cord blood may represent maturational status, particularly of the liver and adrenal gland, rather than nutritional status. In the present study, the influence of gestational age on LDL profile was seen at 5 days of age. LDL cholesterol, LDL-TG and apoB concentrations were positively correlated with gestational age at 5 days, and were lower in infants with younger gestational age. As suggested by Toth *et al*.[20] TC metabolism in LDL, most likely representing cholesterol synthesis, may develop more slowly in infants with younger gestational age compared to those with older gestational age.

Milk source is a determinant of the LDL profile in infancy.[21] Both TC and LDL cholesterol concentrations are higher in breast-fed infants compared to formula-fed infants.[22,23] Therefore, in the present study, we investigated the relationship between LDL profile and gestational age, birth weight and feeding mode at 1 month, and found that only feeding mode was a significant predictor for LDL cholesterol, LDL-TG and apoB concentrations. However, in single regression, LDL cholesterol concentration tended to be lower in younger gestational age in mixed- and formula-fed infants. It was previously reported that the effects of milk source and early cholesterol intake on TC and LDL cholesterol concentrations do not persist at 12 months.[24] In a large meta-analysis study, however, TC concentrations are lower in adults that were breast-fed.[23] Long-term changes in cholesterol metabolism may be based on early nutritional status. A possible mechanism was reported that early cholesterol exposure suppressed endogenous cholesterol synthesis even in later life.[25] The present study suggested that infants with younger gestational age did not have enough cholesterol exposure in neonatal period, when they were given formula milk. Additional longitudinal studies are necessary to determine whether the effect of gestational age on LDL profile persists into adulthood or changes with age, as observed in TC concentrations in breast-fed adults. Furthermore, mother's diets and the subsequent effect of fatty acid composition in breast milk should be also investigated to assess neonatal nutritional status.

To summarize, the LDL profile changes rapidly during the neonatal period. LDL particles increase in number during the first 5 days of life, and the composition of LDL particles is modulated by TG content throughout the neonatal period. Gestational age and

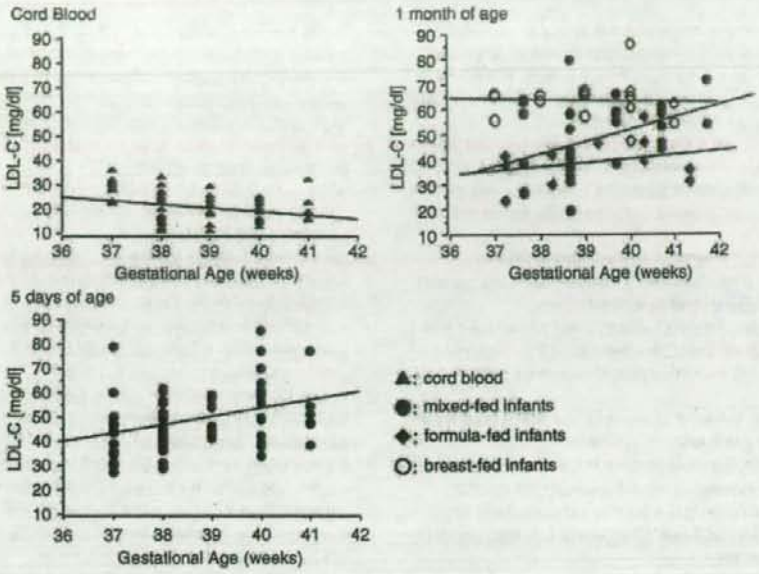

Figure 1 Relationship between low-density lipoprotein (LDL) cholesterol concentrations and gestational age. At birth, LDL cholesterol concentrations were negatively correlated with gestational age. At 5 days, however, the correlations changed to be positive. At 1 month in mixed-fed infants, the positive correlation persisted.

Table 3 Multiple regression analyses of the relationship between LDL profile and mode of milk-feed, birth weight, and gestational age

	β	*S.e.*	P-*value*
LDL cholesterol (mg per 100 ml*)*	*($r^2 = 0.460$, P<0.0001)*		
Gestational age	1.674	1.321	0.2101
Birth weight	0.007	0.004	0.0831
Mode of milk-feed	−13.819	2.217	<0.0001
LDL triglyceride (mg per 100 ml*)*	*($r^2 = 0.294$, P = 0.0001)*		
Gestational age	0.911	0.583	0.1234
Birth weight	0.001	0.002	0.4634
Mode of milk-feed	−4.098	0.978	<0.0001
ApoB (mg per 100 ml*)*	*($r^2 = 0.244$, P = 0.0036)*		
Gestational age	2.918	1.563	0.0681
Birth weight	0.004	0.005	0.4213
Mode of milk-feed	−6.828	2.797	0.0184

Abbreviations: ApoB, apolipoprotein B; LDL, low-density lipoprotein.

milk, rather than birth weight, determined postnatal changes in the LDL profile. The increase in LDL cholesterol during the neonatal period developed more slowly in infants with younger gestational age. The long-term effects of gestational age on the LDL profile should be evaluated in longitudinal studies.

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Circ J 2008; 72: 1594–1597

Association Between the Number of Cardiovascular Risk Factors and Each Risk Factor Level in Elementary School Children

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Background Little is known regarding the association between numbers of cardiovascular (CV) risk factors and the level of each risk factor in elementary school children based on a longitudinal study.
Methods and Results A descriptive study of 319 obese children aged 6–11 years who participated in a screening program for comorbidity of obesity between 2003 and 2005, and who participated in consecutive years thereafter, was performed. Abdominal obesity, hypertension, dyslipidemia (low high-density lipoprotein-cholesterol levels and/or high triglyceride levels), and raised fasting glucose levels were used as the CV risk factors. Metabolic syndrome and each CV risk factor were defined using the criteria newly established by a Task Force financed by the Health and Labour Science Research in Japan. An increase in the total number of CV risk factors implied a worsening of each CV risk factor level over a 1-year interval, and vice versa. Abdominal obesity in males and insulin resistance in females were prevalent in children who were at elementary school level.
Conclusions We should assess not only obesity but all CV risk factor levels, because a cluster of risk factors implies a worsening of the individual risk factor levels in children as young as those in elementary school. (*Circ J* 2008; **72:** 1594–1597)

Key Words: Longitudinal studies; Metabolic syndrome; Risk factors

Obesity accompanies a clustering of cardiovascular (CV) risk factors including abdominal obesity, impaired glucose tolerance, hypertension and dyslipidemia, which has been termed metabolic syndrome[1,2] Clustering of CV risk factors is strongly associated with an increase in CV events in adults[3,4] It is well known that an improvement or worsening of obesity is strongly associated with an improvement or worsening of other CV risk factors, such as impaired glucose tolerance, hypertension, and dyslipidemia in children, adolescents, and adults by cross-sectional analyses[3–10] However, little is known regarding the association between clustering of CV risk factors and the level of each CV risk factor in children based on a longitudinal study.

The aim of the present study was to determine whether a change in the total number of CV risk factors was associated with a change in the individual risk factor levels over a 1-year period in elementary school children.

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Methods

Subjects

Subjects were elementary school children, aged 6–11 years old, who participated in a screening program for comorbidity of obesity conducted between 2003 and 2005 by the Kagoshima City Board of Education and the Kagoshima City Medical Association, Japan. The program is held once a year during summer holidays. The program components are described elsewhere[11] Briefly, school nurses screened all children in elementary schools every year for children who have a percent relative bodyweight (%RBW) of ≥+35%. The %RBW was calculated as: (individual bodyweight)/(age-, sex-, and height-specific bodyweight from a reference population)×100[12] Overweight is defined when a child has a %RBW of ≥20%, however, children with a %RBW of ≥+35% were screened because of financial limitations. Children screened could visit their family doctors if they chose to. If the family doctor determined that the student's weight should be treated, the student could choose to attend the treatment program. The number of participating children in the screening program was 419 in 2003, 504 in 2004, and 444 in 2005. Inclusion criteria were those children who participated in the screening program for 2 consecutive years, and those who did not participate in the treatment program. Children who were obese might have been screened every year, and children who underwent screening visited family doctors if they or their parents chose to do so, every year. The data of the first and second visits were used in the present study. We obtained permission to use and analyze these data from the Ethics Committee of the National Hospital Organization, Kagoshima Medical Center under the condition that confidentiality regarding all

Table 1 Characteristics of the Subjects

	Boys (n=213)			Girls (n=106)			Gender difference	
	First visit	Second visit	p value*	First visit	Second visit	p value†	First visit	Second visit
Age (years)	9.0±1.3	10.0±1.3	<0.0001	8.6±1.3	9.6±1.3	<0.0001	0.01	0.006
Height (cm)	134±8	140±9	<0.0001	132±9	138±9	<0.0001	0.02	0.04
Weight (kg)	43.8±8.6	49.4±9.3	<0.0001	42.2±9.9	47.8±10.6	<0.0001	0.16	0.17
Body mass index	24.0±2.1	24.8±2.1	<0.0001	23.9±2.5	24.7±2.6	<0.0001	0.71	0.66
RBW (%)	43±10	43±12	0.93	45±12	45±12	0.99	0.09	0.12
Waist (cm)	77±8	80±7	<0.0001	75±8	77±7	<0.0001	0.04	0.006
Waist/height ratio	0.57±0.04	0.57±0.04	0.93	0.57±0.04	0.56±0.04	0.08	0.31	0.01
Systolic BP (mmHg)	111±11	110±12	0.38	108±12	109±11	0.65	0.09	0.44
Diastolic BP (mmHg)	63±9	62±10	0.48	62±9	62±10	0.90	0.38	0.83
HDL-C (mg/dl)	57±11	57±12	0.43	55±10	55±11	0.85	0.116	0.07
Triglycerides (mg/dl)	97 (89–105)	107 (99–115)	0.01	101 (92–111)	112 (100–125)	0.0497	0.52	0.47
FBG (mg/dl)	87±6	86±6	0.13	84±6	85±7	0.13	0.0004	0.20
Insulin (μU/ml)	11.1 (10.3–11.9)	12.6 (11.7–13.5)	0.001	12.8 (11.5–14.1)	16.6 (14.9–18.2)	<0.0001	0.02	<0.0001
HOMA-IR	2.4 (2.2–2.6)	2.7 (2.5–2.9)	0.004	2.7 (2.4–3.0)	3.5 (3.2–3.9)	<0.0001	0.09	<0.0001
Number of CV risks	1.5±0.7	1.6±0.7	0.07	1.6±0.7	1.6±0.7	0.47	0.50	0.83
Metabolic syndrome	14 (6%)	18 (8%)	0.58	11 (10%)	10 (9%)	>0.99	0.27	0.83

RBW, relative body weight; BP, blood pressure; HDL-C, high-density lipoprotein-cholesterol; FBG, fasting blood glucose; HOMA-IR, homeostasis model assessment of insulin resistance; CV, cardiovascular.

**Statistical significance levels between the first and second visits in boys.*

†Statistical significance levels between the first and second visits in girls.

The data for triglycerides, insulin, and HOMA-IR levels are expressed as the mean and 95% confidence interval in parentheses because the data were skewed.

personal data would be maintained.

Physical and Blood Biochemical Parameters

We measured height, weight, waist circumference, systolic and diastolic blood pressures (BPs), high-density lipoprotein (HDL)-cholesterol levels, triglyceride levels, fasting glucose levels, and fasting insulin levels. The height, weight, and waist circumference of the children were measured at each individual's doctor's clinic. Height was measured to the nearest 0.1 cm and weight was measured to the nearest 0.1 kg. Body mass index (BMI) was calculated as: (weight in kg)/(height in m)^2. BP was measured 3 times and the lowest value was used. Waist circumference was measured to the nearest 0.1 cm at the umbilical level.

Blood samples were collected in the morning after an overnight fast at each clinic and examined at the Laboratory Center of the Kagoshima City Medical Association. Serum cholesterol levels were determined by the cholesterol oxidase-peroxidase method. Triglycerides were determined by the glycerol kinase-glycerol-3-phosphate oxidase-peroxidase method. Insulin concentrations were measured by a chemiluminescence immunological assay. The homeostasis model assessment of insulin resistance (HOMA-IR)[13] was used as a surrogate marker of insulin resistance and was calculated as follows: [fasting insulin (μU/ml)]×[fasting glucose (mg/dl)]/405.

Definition of Individual CV Risk Factors and Metabolic Syndrome

Metabolic syndrome was defined using the newly established criteria created by a Task Force, which was financed by the Health and Labour Science Research Grants in Japan[14] The criteria set out that a person should be diagnosed as having metabolic syndrome when there is abdominal obesity plus any 2 of the following 3 individual factors: dyslipidemia (raised triglyceride levels and/or reduced HDL-cholesterol levels), hypertension, and raised fasting blood glucose. Each CV risk factor was defined by the same criteria: abdominal obesity (waist circumference ≥75 cm and/or waist/height ratio ≥0.5 for elementary school children), elevated BPs (systolic BP ≥125 mmHg and/or diastolic BP ≥70 mmHg), low HDL-cholesterol levels (<40 mg/dl), high fasting serum triglyceride levels (≥120 mg/dl), and high fasting serum glucose levels (≥100 mg/dl). The total number of CV risk factors in a subject was evaluated at the first and second visits.

Statistical Analysis

Variables that were not normally distributed were log transformed. Statistical significance of the mean values between groups was based on the Mann–Whitney test or the Wilcoxon's signed-rank test, and that for prevalence of metabolic syndrome was based on Fisher's exact probability test. To determine whether clustering of CV risk factors was associated with a worsening of the individual risk factor levels, multivariate regression analysis was performed with adjustment for age and gender. Here, the changes in the levels of CV risk factors between the 2 visits were used as dependent variables, and the change in the total number of CV risk factors, age, and gender were used as independent variables. All statistical analyses were performed using SPSS 15.0J® software (Tokyo, Japan). A level of $p<0.05$ was considered statistically significant.

Results

The final number of subjects in the study was 319 obese children, consisting of 213 boys and 106 girls. Characteristics of the subjects are shown in Table 1. The mean values of waist circumference and fasting blood glucose in boys were higher than those in girls. The mean value of fasting insulin in girls was higher than that in boys. Over the 1-year interval, the mean values of waist circumference, and triglyceride, insulin, and HOMA-IR levels had significantly worsened; however, %RBW was not significantly increased. Gender difference was present in some CV risk factors. Boys showed a significantly higher waist circumference level than girls at both visits (Table 1). In contrast, girls had significantly higher fasting insulin and HOMA-IR levels than boys.

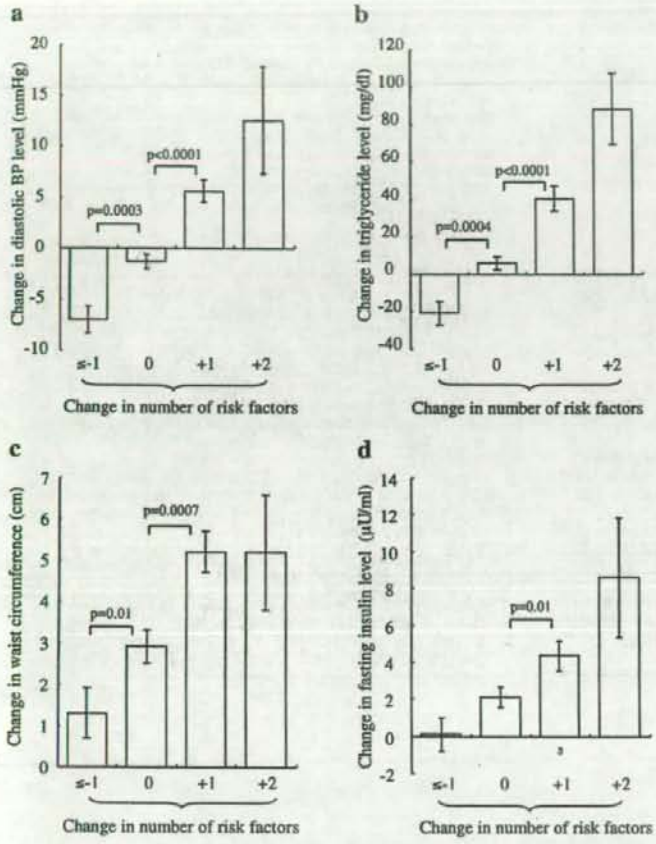

Fig 1. Association between the change in numbers of total risk factors from the first to the second visits and the change in diastolic blood pressure (BP) level (a), triglyceride level (b), waist circumference (c), and fasting insulin level (d). Numbers of subjects with a change in the total number of cardiovascular risk factors of ≤−1, 0, 1, and 2 were 74, 162, 73, and 10 children, respectively. Each bar shows the mean value and the standard error of the mean. As the change in numbers of risk factors increases, the individual risk factor level worsens, and vice versa.

The numbers of subjects with a change in the total number of CV risk factors of ≤−1, 0, 1, and 2 were 74, 162, 73, and 10 children, respectively. Change in the total number of CV risk factors was independently associated with changes in several risk factor levels between visits by multivariate regression analysis after adjusting for age and gender, in the following order; diastolic BP (t value and p value, 8.31, p<0.0001, respectively), triglycerides (6.48 and p<0.0001), systolic BP (5.95, p<0.0001), waist circumference (5.88, p<0.0001), fasting insulin (2.95, 0.003), HDL-cholesterol (−2.52, p=0.01), %RBW (2.31, p=0.02), and HOMA-IR levels (2.30, p=0.02). Fig 1 clearly shows that an increase in the total numbers of CV risk factors is associated with a worsening of each risk factor level, and vice versa.

Discussion

The present study showed that an increase in the total number of CV risk factors was associated with an increase in the levels of CV risk factors over a 1-year interval, and vice versa. Gender differences were present in CV risk factor levels at both first and second visits.

It is well known that the presence of obesity is strongly associated with the worsening of other CV risk factors; hypertension, dyslipidemia, and impaired glucose tolerance[5,9,10] From a cross-sectional analysis, Retnakaran et al reported that the levels of CV risk factors (BMI, waist circumference, systolic BP, fasting insulin levels, and HOMA-IR levels) increased with an increasing number of total CV risk factors in 236 children aged 10–19 years[8] From a case-control study among 122,051 workers with a mean age of 50 years old, Nakamura et al reported that the odds ratio for the presence of ischemic heart disease significantly increased with an increasing number of total CV risk factors (obesity, hypertension, hyperglycemia, and hypercholesterolemia).[4] However, little is known about the association between the total number of CV risk factors and the level of each CV risk factor in children based on a longitudinal study. The present study showed that an increase in the total number of CV risk factors implied a worsening of each CV risk factor level over a 1-year interval, and vice versa.

The present study showed gender differences in the presence of CV risk factors. A significantly higher waist circumference level in boys and significantly higher fasting insulin and HOMA-IR levels in girls indicated that abdominal obesity in males and insulin resistance in females were prevalent in children who were at elementary school level, which has also been shown in other studies[15–18]

The approach used in the present study had limitations. The present study included a larger percentage of boys than

girls. Recent increases in the prevalence of obesity during elementary school years have been shown in boys but not in girls in Japan[1] indicating that a focus on boys is justified. The reason for this rapid increase in the prevalence of obesity in boys needs further investigation.

In conclusion, we should assess not only obesity, but all CV risk factor levels, because a cluster of risk factors implies a worsening of individual risk factor levels in children as young as those in elementary school.

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Circ J 2008; **72:** 1874–1878

Adipokines and the Prediction of the Accumulation of Cardiovascular Risk Factors or the Presence of Metabolic Syndrome in Elementary School Children

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Background Information is limited about how adipokines predict the accumulation of cardiovascular (CV) risk factors or the presence of metabolic syndrome (MS) in children.

Methods and Results The subjects were 321 children (200 boys and 121 girls; 109 normal and 212 obese) aged 6–12 years. Obesity was defined as a body mass index of ≥ the 95th percentile for age and sex. MS was defined by using the newly established Task Force criteria. The levels of the adipokines–adiponectin, leptin, ghrelin, high sensitive C-reactive protein (CRP) and resistin–were measured. Regression analyses revealed that high leptin levels were predictive of the accumulation of CV risk factors in normal weight, obese, and entire (normal weight and obese) group of subjects. High CRP in the normal weight group and low adiponectin in the obese and the entire groups were also independently predictive of the accumulation of risk factors. A high leptin level was solely predictive of the presence of MS in obese and entire groups.

Conclusions Leptin was the most sensitive marker for predicting the accumulation of CV risk factors and the presence of MS in elementary school children. Primary prevention is important because both leptin and adiponectin levels abruptly worsened when children obtained any 1 risk factor. (*Circ J* 2008; **72:** 1874–1878)

Key Words: Adipokines; Cardiovascular risk factors; Metabolic syndrome

Obesity is a serious problem among young people and adults[1–3] Obesity accompanies a clustering of cardiovascular (CV) risk factors, including abdominal obesity, impaired glucose tolerance, hypertension and dyslipidemia, which is collectively recognized as metabolic syndrome (MS)[4]

Pathophysiology of the MS remains a subject of continuing controversy[5] Adipokines, biologically active proteins produced by endocrine organs including leptin, adiponectin, tumor necrosis factor-α and resistin, are associated with the development of MS[5–11] Inflammatory markers such as C-reactive protein (CRP), interleukin-6, and tumor necrosis factor-α have been found to be associated with MS[6,7,11–13] Ghrelin, a somatotropic and orexigenic hormone, has been recognized as an important regulator of energy metabolism[7,14,15] Several reports have supported these findings in children and adolescents with obesity and/or the MS;[8–10,12,14,15] however, information is limited about the association between the accumulation of the CV risk factors or the development of the MS and several adipokines by multivariate regression analysis in pediatric populations that include both normal weight and obese subjects.

The present study aimed to identify the adipokines that are predictive of the accumulation of CV risk factors or the presence of MS in elementary school children.

Methods

Subjects

The subjects were 2 cohorts of elementary school children. One cohort included 229 children (150 boys and 79 girls, 20 normal weight and 209 obese children) aged 6–12 years old who participated in a program to screen obesity-related CV risk factors in Kagoshima City, Kagoshima, Japan (Kagoshima Study) in 2006. The components of the screening program have been described elsewhere[16] In brief, school nurses screen children (from 6 to 12 years old) in elementary schools in April every year to select children with a percent relative body weight ≥+35%[17] Children who undergo screening can visit family doctors if they or their parents so choose. Each year, informed consent is obtained from the children's parents. The program was financed by the Kagoshima City Medical Association and the Board of Education in Kagoshima City.

The other cohort included 92 volunteers (50 boys and 42 girls, 89 normal weight and 3 obese children) aged 6–12 years old from an elementary school in Kirishima City, Kagoshima, Japan (Kirishima Study), who participated in a project to establish the criteria for MS in Japanese children in 2007. The program was announced through the local Board of Education. Elementary school children visited their school if they or their parents chose to participate during a summer holiday. The project was financed by Health and Labour Science Research Grants [Comprehensive Research on Cardiovascular Diseases (17160501)]. The components

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of the project were the same as those in the Kagoshima Study.

We obtained permission to use and analyze these data from the ethics committees of the National Hospital Organization Kagoshima Medical Center under the condition that confidentiality regarding all personal data would be maintained, and that all subjects gave their written informed consent.

Physical and Blood Biochemical Parameters

The parameters examined included height, weight, waist circumference, systolic and diastolic blood pressures (BPs), high-density lipoprotein (HDL)-cholesterol, triglycerides, fasting glucose, and fasting insulin.

The heights, weights and waist circumferences of the children were measured at each home doctor's clinic in the Kagoshima Study or by nurses at the elementary school in the Kirishima Study. Height was measured without socks and shoes, and weight was measured while the children wore only underclothing. Height was measured to the nearest 0.1 cm, and weight to the nearest 0.1 kg. Body mass index (BMI) was calculated as (weight in kg)/(height in m)^2. A BMI SD score was calculated using the 1990 reference data for BMI in Japan![8] BP was measured 3 times and the mean value of the last 2 measurements was used. Waist circumference was measured to the nearest 0.1 cm at the umbilical level.

Blood samples were collected in the morning after an overnight fast at each clinic (in the Kagoshima Study) or at the elementary school (in the Kirishima Study) and examined at the Laboratory Center of the Kagoshima City Medical Association Hospital in the Kagoshima Study or at a commercial laboratory (SRL Inc, Tokyo, Japan) in the Kirishima Study. Serum cholesterol was determined by the cholesterol oxidase-peroxidase method. Triglycerides were determined by the glycerol kinase-glycerol-3-phosphate oxidase-peroxidase method. Insulin concentrations were measured by chemiluminescence immunological assay. The homeostasis model assessment (HOMA) of insulin resistance (HOMA-IR) was calculated as follows: [fasting insulin (μU/ml)]×[fasting glucose (mg/dl)]/405.

Among predictors of MS, adiponectin, leptin, desacyl ghrelin, high sensitivity CRP (hs-CRP), and resistin were measured at the laboratory (SRL Inc, Tokyo, Japan); these 5 predictors are hereafter collectively called adipokines. Adipokines were measured using a Human Adiponectin ELISA kit® (Otsuka Pharmaceutical Inc, Tokyo, Japan), a Human Leptin RIA kit® (Linco Research Inc, St Charles, MO, USA), a Desacyl Ghrelin ELISA kit® (Mitsubishi Kagaku Iatron Inc, Tokyo, Japan), N-Latex CRP II® (Dade Behring Inc, Marburg, Germany), and a Human Resistin ELISA kit® (BioVender Laboratory Medicine, Modrice, Czech Republic).

Definition of Obesity, Individual CV Risk Factors and MS

Obesity was defined as a BMI of ≥ the 95th percentile for age and sex based on Japanese reference data in 1990 (Table 1)![8] MS was defined using the newly established criteria by a Task Force financed by Health and Labour Science Research Grants in Japan![9] The criteria set out that a person can be diagnosed as having MS when there is abdominal obesity plus any 2 of 3 individual factors: dyslipidemia (raised triglyceride level and/or reduced HDL-cholesterol), hypertension, and raised fasting plasma glucose. Each CV risk factor was defined by the same criteria:

Table 1 Definition of Obesity Based on BMI Reference Data for Japanese People From 1990 (95th Percentile for Age and Sex)

Age (years)	*Male*	*Female*
6	*18.75*	*18.80*
7	*19.22*	*19.20*
8	*20.71*	*20.19*
9	*21.94*	*21.22*
10	*22.83*	*23.17*
11	*22.63*	*22.83*
12	*24.56*	*24.06*

BMI, body mass index.

abdominal obesity (waist circumference ≥75 cm and/or waist/height ratio ≥0.5 for elementary school children), elevated BPs (systolic BP ≥120 mmHg and/or diastolic BP ≥70 mmHg), low HDL-cholesterol levels (<40 mg/dl), high fasting serum triglyceride levels (≥120 mg/dl), and high fasting serum glucose levels (≥100 mg/dl).

Statistical Analysis

Variables that were not normally distributed were log transformed. The statistical significance of differences in mean values between groups was assessed using the Mann–Whitney test; that for prevalence of MS was based on Fisher's exact probability test. To identify adipokines that are predictive of the accumulation of CV risk factors in normal weight, obese, and the entire group of subjects, multivariate regression analysis was performed using the number of CV risk factors as a dependent variable, and adipokine levels, age and gender as independent variables, using SPSS 15.0J software (Tokyo, Japan). To identify adipokines predictive of the presence of MS in obese and the entire group of subjects, logistic regression analysis was performed using the presence or absence of MS as a dependent variable, and adipokine levels, age and gender as independent variables. The logistic regression analysis was not performed in the normal weight subjects because of a very small number of the subjects with the MS (3 out of 109 subjects, 2.8 %). A level of $p<0.05$ was considered statistically significant.

Results

Gender differences were present in waist circumference, waist/height ration, and systolic BP in both normal weight and obese groups, in fasting blood glucose and leptin in the normal weight group, and in adiponectin, hs-CRP and the number of CV risk factors in the obese group; however, the BMI SD score was similar between genders in each group (Table 2). Differences in the mean values of the following variables between normal weight and overweight groups were strongly significant: waist circumference, waist/height ratio, systolic and diastolic BPs, triglycerides, fasting insulin, HOMA-IR, adiponectin, leptin, hs-CRP, and the number of CV risk factors (Table 2). The prevalence of MS was 2.8 and 7.1% in normal weight and obese children, respectively (Table 2).

Multivariate regression analyses revealed that high leptin was independently predictive of the accumulation of CV risk factors in normal weight, obese, and the entire group of subjects (Table 3). High hs-CRP levels in normal weight subjects and low adiponectin levels in obese and the entire group of subjects were also independently predictive of the accumulation of CV risk factors. Logistic regression analy-

Table 2 Characteristics of Normal and Obese Children

Subjects	Normal weight		Obese		Entire group	
	Male	Female	Male	Female	Male	Female
N	62	47	138	74	200	121
Age (years)	9.7±1.6	9.3±1.7	9.7±1.5	9.5±1.6	9.7±1.5	9.5±1.6
Height (cm)	134±11	132±11	139±10[a]	136±11	138±11	134±11
Weight (kg)	33.1±10.4	29.6±8.4	48.6±10.1[d]	45.2±10.2*,[d]	43.8±12.5	39.1±12.2[†]
BMI (kg/m²)	17.8±3.2	16.6±2.5*	24.8±2.2[d]	24.1±2.4*,[d]	22.6±4.1	21.2±4.4[‡]
BMI SD score	0.2±1.1	−0.2±0.9	3.2±1.0[d]	3.2±1.2[d]	2.3±1.7	1.9±2.0
Waist (cm)	61.8±10.7	56.8±8.0[†]	80.2±7.3[d]	76.5±7.8[†,d]	74.5±12.0	68.9±12.4[‡]
Waist/height ratio	0.46±0.06	0.43±0.05[†]	0.58±0.04[d]	0.56±0.04*,[d]	0.54±0.07	0.51±0.08[†]
SBP (mmHg)	101±10	97±9*	109±11[d]	107±13*,[d]	106±11	103±12[†]
DBP (mmHg)	56±8	54±9	61±10[d]	59±10[c]	60±10	57±10[*]
HDL-C (mg/dl)	55±12	54±10	55±11	55±9	55±11	54±9
TG[¶] (mg/dl)	80 (68–91)	69 (57–80)	118[d] (106–129)	113[d] (92–135)	106 (97–115)	96 (82–111)
FBG (mg/dl)	90±7	88±7*	86±6[d]	8±7[a]	87±7	86±7
Fasting insulin[¶] (μU/ml)	6.0 (4.8–7.1)	6.3 (5.0–7.6)	13.0[d] (12–14)	13.9[d] (12–15)	10.8 (9.8–12)	10.9 (9.7–17.1)
HOMA-IR[¶]	1.3 (1.1–1.6)	1.4 (1.1–1.8)	2.8[d] (2.5–3.1)	3.0[d] (2.6–3.3)	2.3 (2.1–2.6)	2.4 (2.1–2.6)
Adiponectin (μg/ml)	10.8±3.3	12.3±4.5	8.3±3.1[d]	9.4±3.2*,[c]	9.1±3.4	10.5±4.0[†]
Leptin[¶] (ng/ml)	4.9 (3.8–6.0)	4.3* (3.2–5.5)	18.1[d] (16.7–19.4)	18.9[d] (17.2–20.7)	14.0 (12.7–15.3)	13.3 (11.6–15.0)
Ghrelin[¶] (fmol/ml)	46 (35–58)	58 (35–81)	59 (49–70)	59 (43–75)	55 (47–63)	59 (46–72)
hs-CRP[¶] (ng/ml)	265 (142–389)	276 (120–433)	1,795[d] (1,288–2,301)	1,119[†,d] (730–1,508)	1,321 (957–1,684)	792[‡] (538–1,046)
Resistin[¶] (ng/ml)	4.3 (3.7–4.9)	4.7 (4.1–5.2)	4.7 (4.2–5.2)	4.3 (3.8–4.8)	4.6 (4.2–5.0)	4.4 (4.0–4.8)
No. CV risks	0.6±0.8	0.4±0.5	1.6±0.6[d]	1.4±0.7*,[d]	1.3±0.8	1.0±0.8[†]
Metabolic syndrome	2 (3.2%)	1 (2.1%)	10 (7.2%)[a]	5 (6.8%)	12 (6.0%)	19 (5.0%)

SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein-cholesterol; TG, triglycerides; FBG, fasting blood glucose; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high sensitivity C-reactive protein; CV, cardiovascular. Other abbreviation see in Table 1.
*The statistical significance of differences between genders in each parameter is shown using the following asterisks: *p<0.05, †p<0.01, ‡p<0.001.*
The statistical significance of differences between the normal and obese groups is expressed as follows: [a]p<0.05, [b]p<0.01, [c]p<0.001, [d]p<0.0001.
¶Data for TG, fasting insulin, HOMA-IR, leptin, ghrelin, hs-CRP, and resistin are expressed as the means with the 95% confidence intervals (CIs) in parentheses because these levels were skewed.

Table 3 Association Between the Accumulation of CV Risk Factors and Adipokine Levels by Multivariate Regression Analysis

Subjects	Normal weight		Obese		Entire group	
	t value	p value	t value	p value	t value	p value
No. subjects	109		212		321	
Age	−0.19	0.85	−0.02	0.99	−0.28	0.78
Gender	−1.21	0.23	−0.64	0.10	−2.07	0.04
Adiponectin	−0.89	0.38	−2.60	0.01	−2.77	0.006
Ln (Leptin)	5.41	<0.0001	2.51	0.013	10.2	<0.0001
Ln (Ghrelin)	0.11	0.91	0.70	0.48	0.95	0.34
Ln (hs-CRP)	2.04	0.04	−0.23	0.82	1.35	0.18
Ln (Resistin)	0.12	0.90	1.24	0.22	0.79	0.43

Ln, natural logarithm. Other abbreviations see in Table 2.

Table 4 Association Between the Presence of Metabolic Syndrome and Adipokine Levels by Logistic Regression Analysis

	t value	p value	OR	95%CI
Obese subjects				
Age	−1.26	0.21	0.78	0.53–1.15
Gender	−0.33	0.75	0.82	0.25–2.68
Adiponectin	−1.15	0.25	0.90	0.74–1.08
Ln (Leptin)	1.98	0.048	4.25	1.01–17.8
Ln (Ghrelin)	0.41	0.68	1.12	0.65–1.95
Ln (hs-CRP)	−0.93	0.35	0.78	0.46–1.32
Ln (Resistin)	1.36	0.17	2.19	0.71–6.77
Entire group of subjects				
Age	−0.90	0.37	0.85	0.60–1.21
Gender	−0.47	0.64	0.77	0.26–2.31
Adiponectin	−1.32	0.19	0.89	0.75–1.06
Ln (Leptin)	2.93	0.003	4.78	1.68–13.6
Ln (Ghrelin)	0.96	0.34	1.28	0.78–2.10
Ln (hs-CRP)	−1.46	0.14	0.69	0.42–1.13
Ln (Resistin)	1.84	0.07	2.67	0.94–7.59

OR, odds ratio. Other abbreviations see in Tables 2,3.

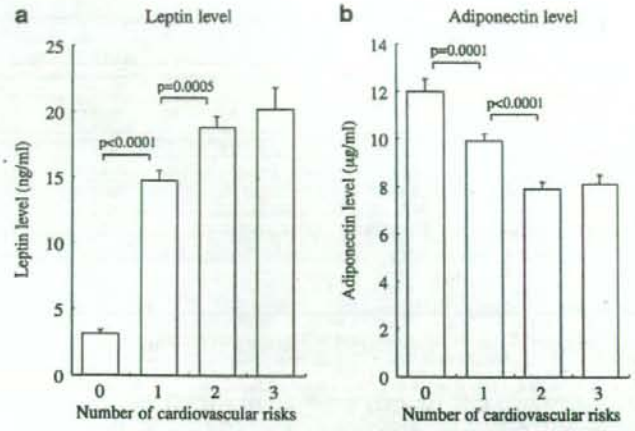

Fig 1. Serum leptin (a) and adiponectin (b) levels among children classified on the basis of the number of cardiovascular (CV) risk factors. Each bar shows the mean value and standard error of the mean. The maximum number of CV risk factors of the subjects was 3 in the present study. Leptin and adiponectin levels worsened abruptly when children developed any 1 CV risk.

ses showed that a high leptin level was predictive of the presence of MS in obese and the entire group of subjects (Table 4).

Leptin levels were greatly increased when children of normal weight status developed 1 CV risk factor (Fig 1a). The difference in leptin level between subjects with no risk factors and 1 risk factor was highest among neighboring subgroups. Adiponectin levels were also decreased significantly when boys developed any 1 CV risk factor (Fig 1b).

Discussion

The present study showed that high leptin was independently predictive of the accumulation of CV risk factors in normal weight, obese, and the entire group of subjects. High CRP in normal weight subjects and low adiponectin in obese and the entire group of subjects were also independently predictive of the accumulation of CV risk factors. A high leptin level was predictive of the presence of MS in obese and the entire group of subjects.

The levels of adipokines have been widely investigated in several conditions in both adult and pediatric populations to compare individuals with or without MS or diabetes mellitus, subjects showing evidence of weight change, and groups of lean and obese subjects[6–15] Among the many studies involving children and adolescents in which measurements of a few cytokines were included, some reports have discussed the identification of predictive adipokines by regression analysis. Gilardini et al recently reported an association between the presence of MS and several biomarkers, including adiponectin, interleukin-18, plasminogen activator inhibitor 1, CRP, uric acid and fibrinogen![10] These authors showed that a low adiponectin level was independently associated with the presence of MS in Caucasian obese children and adolescents with a mean age of 14 years. Liu et al reported that a low adiponectin level is independently associated with the presence of MS in adults ≥18 years of age[5] In the present study, both high leptin and low adiponectin levels were independent predictors of the accumulation of CV risk factors. A high leptin level also emerged as the one significant predictor of the presence of MS. One of the reasons for the importance of leptin levels in the present study might be because of the inclusion of normal-weight children. The serum leptin level is known to be high in both children and adults with obesity and/or MS[5,7,8] Studies that include only subjects with obesity and/or MS might diminish the importance of the leptin level. Analyses separately performed in the normal weight and obese subjects in the present study (Table 3) might support this hypothesis. The present study shows for the first time that both high leptin and low adiponectin levels are independently predictive of the accumulation of CV risk factors, and that high leptin is solely predictive of the presence of the MS in elementary school children.

Leptin and adiponectin levels worsened abruptly when children developed any 1 CV risk factor (Fig 1). Among adult individuals with a given BMI, elevated levels of glucose- and lipid-related factors are more likely to be present in Asians and Aboriginals compared with Europeans[20] Obesity-associated disorders arise in mildly and moderately obese adults in Japan?[21] An abrupt worsening of CV risk factors emerged from mild obesity in Japanese elementary school children[22] These data indicate that primary prevention of the development of CV risk factors is extremely important among some ethnic groups.

The inflammatory process has been reported to be associated with the development of the MS![11–13] The hs-CRP level was significantly elevated in subjects with MS both in youth[12] aged 12–17 years and in adults![1,13] The present study showed that a high hs-CRP level was an independent predictor of the accumulation of the CV risk factors in normal-weight children aged 6–12 years.

Gender difference was present in some levels of the entire group in the present study. Many studies have also reported a gender difference in the levels of waist circumference![10,12] systolic BP![13,23] adiponectin![10,24] hs-CRP[13,25] and the prevalence of the MS[26] in both pediatric and adult populations. The present study could not provide clear reason(s) for this rapid appearance of gender difference in children as young as those in elementary school and therefore this needs further investigation in the future.

The approach used in the present study had some limitations. We combined 2 cohorts: participants in a program to screen obesity-related CV risk factors and a group of healthy volunteers in a related program. However, both cohorts were residents of the same prefecture. Future studies should include both normal weight and obese children from the same cohort. Another limitation was the lack of age- and

ethnicity-adjusted values for individual CV risk factors for Japanese children from data of relatively large cohorts. Projects to establish criteria for defining MS in Japanese children started 3 years ago; thus, age- and ethnicity-adjusted values for Japanese children and adolescents will be presented in the near future.

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Glucocorticoid Regulation of the Promoter of 11β-Hydroxysteroid Dehydrogenase Type 1 Is Indirect and Requires CCAAT/Enhancer-Binding Protein-β

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11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) converts inert 11keto-glucocorticoids to active 11β-hydroxy forms, thereby amplifying intracellular glucocorticoid action. Up-regulation of 11β-HSD1 in adipose tissue and liver is of pathogenic importance in metabolic syndrome. However, the mechanisms controlling 11β-HSD1 transcription are poorly understood. Glucocorticoids themselves potently increase 11β-HSD1 expression in many cells, providing a potential feed-forward system to pathology. We have investigated the molecular mechanisms by which glucocorticoids regulate transcription of 11β-HSD1, exploiting an A549 cell model system in which endogenous 11β-HSD1 is expressed and is induced by dexamethasone. We show that glucocorticoid induction of 11β-HSD1 is indirect and requires new protein synthesis. A glucocorticoid-responsive region maps to between −196 and −88 with respect to the transcription start site. This region contains two binding sites for CCAAT/enhancer-binding protein (C/EBP) that together are essential for the glucocorticoid response and that bind predominantly C/EBPβ, with C/EBPδ present in a minority of the complexes. Both C/EBPβ and C/EBPδ are rapidly induced by glucocorticoids in A549 cells, but small interfering RNA-mediated knockdown shows that only C/EBPβ reduction attenuates the glucocorticoid induction of 11β-HSD1. Chromatin immunoprecipitation studies demonstrated increased binding of C/EBPβ to the 11β-HSD1 promoter in A549 cells after glucocorticoid treatment. A similar mechanism may apply in adipose tissue *in vivo* where increased C/EBPβ mRNA levels after glucocorticoid treatment were associated with increased 11β-HSD1 expression. C/EBPβ is a key mediator of metabolic and inflammatory signaling. Positive regulation of 11β-HSD1 by C/EBPβ may link amplification of glucocorticoid action with metabolic and inflammatory pathways and may represent an endogenous innate host-defense mechanism. (*Molecular Endocrinology* 22: 2049–2060, 2008)

11β-HYDROXYSTEROID DEHYDROGENASE type 1 (11β-HSD1) catalyzes the reduction of intrinsically inert 11-keto corticosteroids (cortisone and 11-dehydrocorticosterone), regenerating active glucocorticoids (cortisol and corticosterone) and increasing intracellular glucocorticoid action in cells in which it is expressed (1). In contrast, 11β-HSD2, expressed in aldosterone-target tissues such as distal nephron and colon, is a high-affinity 11β-dehydrogenase that catalyzes rapid inactivation of glucocorticoids and confers mineralocorticoid specificity upon the mineralocorticoid receptor. Both 11β-HSD isozymes, the products of distinct genes, are crucial controls of tissue glucocorticoid action.

Chronic glucocorticoid excess causes Cushing's syndrome (obesity, hypertension, insulin resistance/type 2 diabetes, dyslipidemia, and heart disease) (2), yet plasma cortisol levels are normal in idiopathic obesity. However, in obesity/metabolic syndrome, expression and activity of 11β-HSD1 are strikingly up-regulated in adipose tissue (3–13), increasing tissue glucocorticoid levels and possibly explaining the paradoxical similarity between common metabolic syndrome/obesity spectrum disorders and rare Cushing's syndrome (14). Understanding the basis of this tissue-specific regulation is of substantial interest.

Glucocorticoids themselves up-regulate 11β-HSD1 mRNA levels in a variety of primary cells *in vitro* (15–19), including in human adipocytes (8) and preadipocytes (20).

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Abbreviations: C/EBPα, CCAAT/enhancer binding protein-α; ChIP, chromatin immunoprecipitation; GR, glucocorticoid receptor; GRE, glucocorticoid response element; 11β-HSD1, 11β-hydroxysteroid dehydrogenase type 1; LAP, liver-enriched activator protein; LIP, liver-enriched inhibitor protein; siRNA, small interfering RNA.

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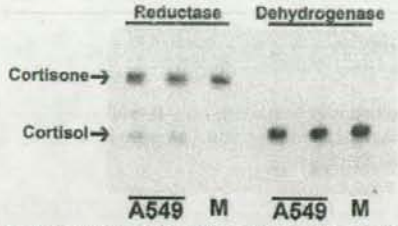

Fig. 1. Endogenous 11β-HSD1 Is Expressed in A549 Cells and Shows Exclusively Reductase Activity

Representative image of thin-layer chromatography plate showing 11keto-reductase activity (conversion of 200 nM [^{3}H]cortisone to cortisol) but no 11β-dehydrogenase activity (conversion of 200 nM [^{3}H]cortisol to cortisone) in intact A549 cells. *Arrows* indicate cortisone and cortisol. M indicates medium with [^{3}H]steroid alone (no cells).

Glucocorticoids also increase 11β-HSD1 mRNA levels *in vivo* (21–25), although the *in vivo* regulation is tissue specific and complex (21, 23–25). Nevertheless, 11β-HSD1 expression in mouse adipose tissue is markedly increased by corticosterone (25). The underlying mechanisms remain unknown. Most regulators of 11β-HSD1 expression are likely to act indirectly, and the only direct regulators of 11β-HSD1 gene transcription described to date comprise members of the C/EBP family of transcription factors (26–28). The 11β-HSD1 gene is transcribed from two promoters, P1 and P2 (27). Transcription in liver, brain, and adipose tissue is predominantly from P2 and is dependent upon the transcription factor CCAAT/enhancer-binding protein (C/EBPα) (26, 27). Transcription from P1 is C/EBPα independent (27). Other members of the C/EBP family also regulate hepatic 11β-HSD1 transcription (26), although their role in 11β-HSD1 regulation in other tissues remains to be determined.

Few cell lines express endogenous 11β-HSD1. Most, including HepG2 hepatoma cells, previously used to characterize the 11β-HSD1 promoter (26, 27), contain only trace amounts of endogenous 11β-HSD1 mRNA and show minimal 11β-HSD1 promoter activity in the absence of cotransfected activators (26, 27). However, the P2 promoter of 11β-HSD1 shows robust activity in transfected A549 lung epithelial cells (27), which are also glucocorticoid responsive (29, 30). Here we have investigated the critical mechanisms by which glucocorticoids themselves regulate endogenous 11β-HSD1 transcription, exploiting the A549 cell model.

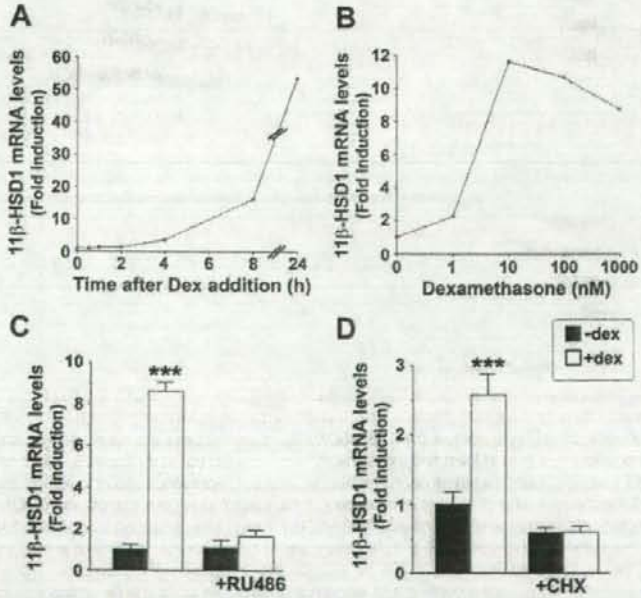

Fig. 2. Glucocorticoid Regulation of 11β-HSD1 in A549 Cells Is Indirect and Dependent upon New Protein Synthesis

Real-time PCR measurement of 11β-HSD1 mRNA levels, expressed as fold induction, relative to levels in vehicle-treated cells. A, 11β-HSD1 mRNA levels were increased within 4 h of addition of 10^{-6} M dexamethasone to A549 cells and were maximal at 24 h. B, Dexamethasone (Dex, 24 h) increased endogenous 11β-HSD1 mRNA levels in a dose-dependent manner in A549 cells. C, The GR antagonist RU38486 (RU486, 10^{-5} M), added 30 min before 10^{-6} M dexamethasone, blocked the induction of 11β-HSD1 mRNA. D, Dexamethasone induction of 11β-HSD1 mRNA in A549 cells was blocked by cycloheximide (CHX, 10^{-5} M) added 30 min before 10^{-6} M dexamethasone (4 h). Data are mean ± SEM of at least three independent mRNA samples. ***, $P < 0.0001$.

RESULTS

A549 Cells Express Endogenous 11β-HSD1, Which Acts Exclusively as a Reductase

The robust activity of the 11β-HSD1 P2 promoter in A549 cells (27) suggested they may express the endogenous gene. Assay of intact A549 cells demonstrated endogenous 11β-HSD enzyme, acting as an 11keto-reductase (conversion of cortisone to cortisol; 0.23 pmol/h·mg protein), with no 11β-dehydrogenase activity detectable (Fig. 1), consistent with activity due to 11β-HSD1 but not 11β-HSD2.

Glucocorticoid Induction of 11β-HSD1 mRNA in A549 Cells Is Indirect and Requires New Protein Synthesis

Addition of dexamethasone to A549 cells increased 11β-HSD1 mRNA levels within 4 h, with maximal in-

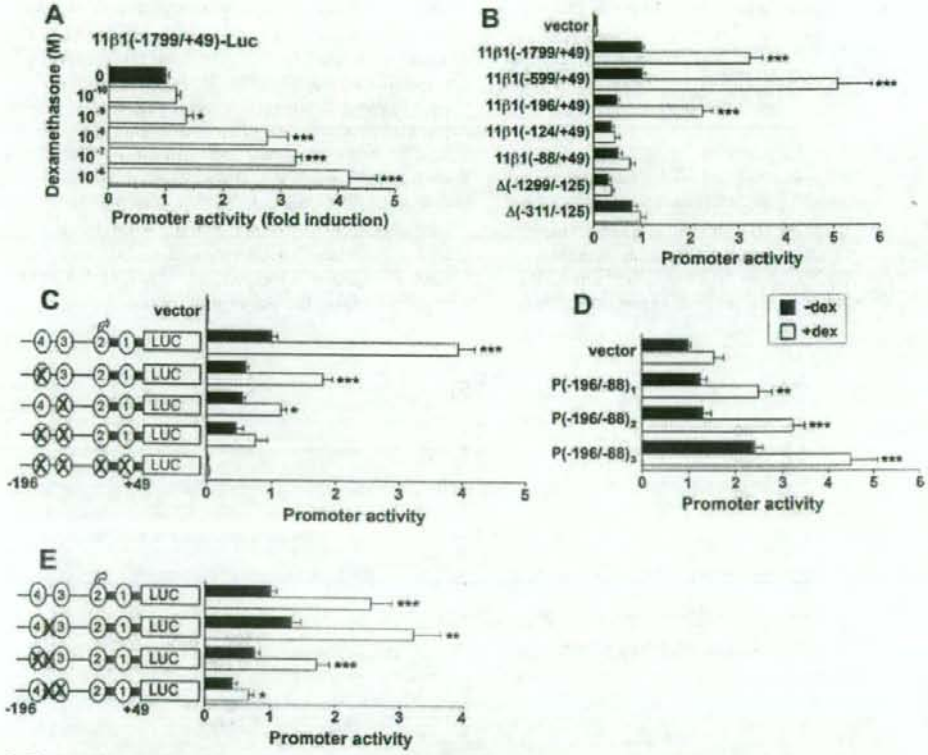

Fig. 3. Dexamethasone Induction of the P2 Promoter of 11β-HSD1 Requires C/EBP-Binding Sites FP3 and FP4

A, In transiently transfected A549 cells, dexamethasone dose-dependently increased activity of p11β1(−1799/+49)-LUC, a luciferase reporter construct encoding −1799 to +49 of the 11β-HSD1 P2 promoter. Data are expressed as fold induction relative to vehicle-treated cells and are mean ± SEM of at least two experiments, each carried out in triplicate. B, Effect of 5′ deletion or internal deletion of the 11β-HSD1 P2 promoter upon dexamethasone response. Data are expressed relative to vehicle-treated p11β1(−1799/+49) (arbitrarily set to 1) and are mean ± SEM of at least three experiments, each carried out in triplicate. C, Effect of mutation of FP3 and/or FP4 upon the glucocorticoid response of the proximal 11β-HSD1 P2 promoter, encoded by p11β1(−196/+49)-LUC. Data are expressed relative to vehicle-treated p11β1(−196/+49)-LUC (arbitrarily set to 1) and are mean ± SEM of at least three experiments, each carried out in triplicate. D, The fragment encoding −196 to −88 of the 11β-HSD1 P2 promoter in one [P(−196/−88)$_1$], two [P(−196/−88)$_2$], or three [P(−196/−88)$_3$] copies confers a glucocorticoid response upon the heterologous promoter present in the vector pGL2-promoter. Data are expressed relative to vehicle-treated vector (arbitrarily set to 1) and are mean ± SEM of at least three experiments, each carried out in triplicate. E, No effect of mutation of the putative GRE (indicated between FP3 and FP4 by an X) upon the glucocorticoid response of the proximal 11β-HSD1 P2 promoter, encoded by p11β1(−196/+49)-LUC, either alone or in combination with either FP3 or FP4 mutation. Data are expressed relative to vehicle-treated p11β1(−196/+49)-LUC (arbitrarily set to 1) and are mean ± SEM of at least five experiments, each carried out in triplicate. In each transfection, 1×10^5 cells were seeded per well in six-well plates and transfected with 250 ng test plasmid and 250 ng pRSV-lacZ (internal control). Promoter activity is expressed as luciferase/β-galactosidase (internal control) activity. *Black bars* represent activity in vehicle-treated cells; *white bars* represent activity in dexamethasone-treated cells (10^{-6} M unless stated otherwise). *, $P < 0.05$; **, $P < 0.001$; ***, $P < 0.0001$.