Serum adipocyte fatty acid–binding protein, retinol-binding protein 4, and adiponectin concentrations in relation to the development of the metabolic syndrome in Korean boys: a 3-y prospective cohort study[1–5]

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ABSTRACT

Background: Adipocyte fatty acid–binding protein (A-FABP), retinol-binding protein 4 (RBP4), and adiponectin have been associated with insulin resistance and the metabolic syndrome in adults.

Objective: We evaluated the association of A-FABP, RBP4, and adiponectin with the metabolic syndrome in Korean boys.

Design: In this prospective cohort study, 159 boys participated in a school-based health examination and were followed up after 3 y. The metabolic syndrome in children was defined by using the pediatric adaptation of the National Cholesterol Education Program criteria.

Results: Compared with normal-weight participants, overweight children had significantly higher A-FABP (23.6 ± 8.2 compared with 12.8 ± 5.1 μg/L, $P < 0.001$) and RBP4 (69.3 ± 17.1 compared with 59.7 ± 15.3 μg/mL, $P = 0.001$) concentrations and significantly lower adiponectin concentrations (11.5 ± 5.4 compared with 18.1 ± 8.4 μg/mL, $P < 0.001$). Baseline A-FABP concentrations were significantly higher in children who developed the metabolic syndrome than in those who did not, whereas adiponectin concentrations were significantly lower. Baseline RBP4 concentrations were not significantly different between the 2 groups. Multiple logistic regression analysis showed that only A-FABP was an independent predictor of the development of the metabolic syndrome after adjustment for Tanner stage, insulin resistance, body mass index, sleep duration, and physical activity (odds ratio: 17.3; 95% CI: 1.25, 239.76; highest compared with lowest tertile), whereas the significant association between adiponectin and the metabolic syndrome observed by using bivariate analysis reflects, in part, an underlying association with obesity.

Conclusion: A-FABP predicts the development of the metabolic syndrome independently of pubertal status, adiposity, and insulin resistance in Korean boys. *Am J Clin Nutr* 2011;93:19–26.

INTRODUCTION

Obesity is associated with an increase in cardiovascular disease risk factors, which persist from childhood to young adulthood, as shown by several cross-sectional and longitudinal studies worldwide (1–3). This constellation of cardiovascular disease risk factors, recognized as the metabolic syndrome in adults (4, 5), is also being observed with increasing frequency in children and adolescents (6, 7). Identification of children at early stages of development of the metabolic syndrome is crucial, because its prevention or treatment should start early in life.

Adipocyte fatty acid–binding protein (A-FABP) is one of the most abundant proteins of those expressed predominantly in adipose tissue and macrophages. Previous studies have suggested that A-FABP plays a key role in linking obesity with various features of the metabolic syndrome (8, 9). Mice with an A-FABP deficiency are protected from the development of dyslipidemia, hyperglycemia, insulin resistance, and atherosclerosis in both genetic and diet-induced obesity (10, 11). Previous prospective studies indicate that serum A-FABP predicts the development of type 2 diabetes (12) and the metabolic syndrome (13) in adults. We have reported that exercise training for 3 mo induced a significant reduction in circulating A-FABP concentrations in

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obese Korean women (14). In a recent cross-sectional study, we found that A-FABP concentrations were significantly correlated with body mass index (BMI) and waist circumference in children (15).

Retinol-binding protein 4 (RBP4) has been identified as an adipokine that may link obesity, insulin resistance, and type 2 diabetes (16). Several previous studies in adults (17, 18) have reported significant associations between RBP4, obesity, and insulin resistance. Therefore, it has been speculated that RBP4 might be a useful predictor of the metabolic syndrome (19). In a cross-sectional study, Aeberli et al (20) reported that serum RBP4 concentrations are correlated with components of the metabolic syndrome in children as well.

Plasma concentrations of adiponectin have been negatively associated with indexes of obesity, insulin resistance, and cardiovascular disease (21). Adiponectin has also been shown to be a predictor of the metabolic syndrome in both adults (13, 22) and children (23) in prospective studies.

Although there is limited evidence of a relation between A-FABP, RBP4, and adiponectin and the metabolic syndrome, to the best of our knowledge, no prospective study has jointly evaluated the relation between these adipokines and future risk of the metabolic syndrome in children. The aim of the present study was to investigate associations between the adipokines A-FABP, RBP4, and adiponectin and obesity and obesity-related metabolic abnormalities in boys. Furthermore, we examined whether baseline A-FABP, RBP4, and adiponectin concentrations predict the development of the metabolic syndrome in these boys prospectively, ie, after 3 y of follow up.

SUBJECTS AND METHODS

Study subjects

Participants in this study were selected from the Korean Metabolic disorders & Obesity Study in Elementary School children (KMOSES), the main aim of which was to comprehensively assess obesity-related metabolic risk factors and to evaluate clinical outcomes in Korean children prospectively. We recruited 216 Korean boys, aged 9 y at baseline, who participated in a school-based health examination in 2006. In 2009, 159 boys were reexamined for the 3-y follow-up assessment, and these boys were included in the present analysis. Reasons for participants not being available for the 3-y follow-up assessment included participant transfer to another school (73.7%), refusal to provide a blood sample (17.5%), and missing data on metabolic risk factors (8.8%). No significant difference in baseline characteristics [height, weight, BMI, waist circumference, systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting plasma glucose, total cholesterol, triglycerides, and HDL cholesterol] were observed between subjects who returned for the follow-up visit and those who did not (*see* Supplemental Table 1 under "Supplemental data" in the online issue). None of the children had a history of cardiovascular disease, diabetes, hypertension, or endocrine disorders, and they were nonsmokers. Written informed consent was obtained from their parents, and the Korea University Institutional Review Board approved the study protocol, in accordance with the Declaration of Helsinki of the World Medical Association.

Anthropometric and laboratory measurements

During both visits, anthropometric measurements were obtained from all children while wearing light clothing and no shoes. Height and weight were measured with an automated height-weight scale to the nearest 0.5 cm and 0.5 kg, respectively. BMI was calculated as weight (kg)/height squared (m). Waist circumference was measured at the midpoint of the lower border of the rib cage and the top of the lateral border of the iliac crest. Blood pressure was measured by a standard brachial cuff technique. Self-reported information on pubertal development was collected by using drawings of the 5 Tanner stages of pubertal development, which involves the development of genitalia and pubic hair in 2009 (24).

During both visits, serum was obtained from each subject after they had fasted overnight for $\geq$8 h. Fasting plasma glucose, triglycerides, and HDL cholesterol concentrations were analyzed by using an autoanalyzer (LX20; Beckman Coulter, Fullerton, CA). Plasma insulin concentrations were measured by radioimmunoassay (Roche, Indianapolis, IN). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting insulin (in μIU/mL) $\times$ fasting glucose (in mmol/L)/22.5 (25). Serum A-FABP concentrations were measured by enzyme-linked immunosorbent assay (Cayman, Ann Arbor, MI), and the intra- and interassay CVs were 3.3–7.1% and 2.1–5.7%, respectively. Serum RBP4 concentrations were measured by enzyme-linked immunosorbent assay (AdipoGen, Seoul, Korea), and the intra- and interassay CVs were 1.92–3.68% and 6.57–8.59%, respectively. Serum adiponectin concentrations were measured by enzyme-linked immunosorbent assay (Mesdia, Seoul, Korea), and the intra- and interassay CVs were 4.1–5.9% and 3.7–6.3%, respectively.

Definition of obesity and the metabolic syndrome

Children were classified as overweight or obese on the basis of the Korean Pediatric Society 2005 guidelines (26), ie, having a BMI value greater than or equal to the 85th percentile for age and sex (27). Normal weight was defined as a BMI below the 85th percentile for age and sex.

The age-modified standards of the Adult Treatment Panel III (ATP) metabolic syndrome criteria defined by the National Cholesterol Education Program (NCEP) (28) are widely used to identify the metabolic syndrome. In this study, these criteria were modified for children to include elevated blood pressure (BP), defined as systolic or diastolic BP greater than the 90th percentile, sex, height, and age based on the Korean Pediatric Society, 2005 guidelines (26, 29); an HDL-cholesterol concentration $\leq$40 mg/dL; a triglyceride concentration $\geq$110 mg/dL; and glucose intolerance defined as a fasting blood glucose concentration $\geq$110 mg/dL. Children with a waist circumference above the 90th percentile for age and sex based on the Korean Pediatric Society guidelines (26) were defined as having abdominal obesity. Subjects who met $\geq$3 of the above-mentioned 5 criteria were classified as having the metabolic syndrome phenotype.

Statistical analysis

Statistical analysis was performed by using SPSS version 12.0 (SPSS Inc, Chicago, IL), and $P < 0.05$ was considered significant. The results are expressed as means $\pm$ SDs. Clinical and

laboratory characteristics between the normal-weight and overweight children were compared by using Student's t test for normally distributed data, and the Wilcoxon's 2-sample test was used for data not normally distributed. Adiponectin was not normally distributed; it was log transformed for subsequent analyses. Pearson's partial correlations were calculated to characterize the associations between adiponectin (log transformed), RBP4, and A-FABP and components of the metabolic syndrome after adjustment for Tanner stage, sleep duration, physical activity, baseline HOMA-IR, and BMI. Logistic regression analyses were performed by using the metabolic syndrome in 2009 as a dependent variable. Adjusted odds ratios (ORs) for the metabolic syndrome were obtained by using simple and multiple logistic regression analysis after adjustment for Tanner stage, sleep duration, physical activity, baseline HOMA-IR, and BMI. P for trend was conducted across categories by using the Cochran-Armitage test.

RESULTS

Thirty percent (n = 48) of the children were classified as overweight at baseline. Three years later (in 2009) the prevalence of overweight was 34.6% (n = 55); 24.5% of boys were at pubertal Tanner stage I, 44.0% at stage II, 20.1% at stage III, 9.4% at stage IV, and only 1.9% at stage V. Anthropometric and metabolic characteristics of the study participants at baseline and 3 y later are presented in **Table 1**. Differences in the mean values of the following variables between the normal-weight and overweight study participants were significant at baseline: height, weight, BMI, waist circumference, SBP, DBP, serum insulin, HOMA-IR, high-sensitivity C-reactive protein (hs-CRP), adiponectin, RBP4, and A-FABP.

Baseline adiponectin concentrations were negatively correlated with baseline height, weight, BMI, waist circumference, SBP, and DBP. Baseline RBP4 concentrations were positively correlated with height, weight, BMI, waist circumference, triglyceride concentrations, insulin concentrations, and HOMA-IR, whereas A-FABP concentrations were positively correlated with height, weight, BMI, waist circumference, SBP, DBP, insulin concentrations, and RBP4 concentrations (**Table 2**). Importantly, a negative association between A-FABP and adiponectin concentrations ($r = -0.36$, $P < 0.01$) and a positive correlation between A-FABP and RBP4 concentrations ($r = 0.38$, $P < 0.01$) were also found at baseline.

In relation to the changes in anthropometric and metabolic variables, adiponectin was negatively correlated with changes in height, weight, and triglyceride concentrations and positively correlated with SBP and DBP changes (**Table 3**). Furthermore, baseline RBP4 concentrations were negatively correlated with changes in HDL cholesterol and positively correlated with changes in height, weight, and waist circumference. On the other hand, A-FABP concentrations were positively correlated with changes in height, weight, BMI, and waist circumference. After adjustment for Tanner stages, some associations, but not all, became nonsignificant.

None of the study participants had the metabolic syndrome at baseline. Ten boys developed the metabolic syndrome 3 y later; of these 10 boys, only 1 had normal weight; the other 9 were overweight at follow-up. Thus, the prevalences of the metabolic syndrome were 0.94% and 6.7% in normal-weight and overweight boys, respectively.

Compared with those who did not develop the metabolic syndrome after 3 y, those who did develop the metabolic syndrome had significantly higher A-FABP (25.9 ± 10.5 compared with 15.6 ± 7.4 μg/L, $P < 0.001$) and significantly lower adiponectin (2.20 ± 0.53 compared with 2.67 ± 0.53 μg/mL, $P < 0.001$) concentrations at baseline. However, baseline RBP4 concentrations were not significantly different between the groups (66.0 ± 12.0 compared with 62.4 ± 16.7 μg/mL, P = 0.393).

TABLE 1
Anthropometric and metabolic characteristics of study participants at baseline and after 3 y[1]

	Baseline		3 y later	
	Normal weight (n = 111)	Overweight (n = 48)	Normal weight (n = 104)	Overweight (n = 55)
Height (cm)	132.2 ± 4.9	136.9 ± 4.9[2]	150.5 ± 7.9	154.6 ± 6.5[3]
Weight (kg)	28.5 ± 3.5	40.6 ± 4.7[2]	40.9 ± 6.7	59.1 ± 6.7[3]
BMI (kg/m^2)	16.2 ± 1.4	21.6 ± 1.7[2]	17.96 ± 1.84	24.64 ± 7.3[3]
WC (cm)	56.3 ± 3.8	69.4 ± 5.7[2]	61.6 ± 5.5	78.8 ± 2.01[3]
SBP (mm Hg)	108.7 ± 14.5	120.6 ± 18.0[2]	108.8 ± 10.6	117.7 ± 6.3[3]
DBP (mm Hg)	69.0 ± 11.7	77.5 ± 14.0[2]	69.6 ± 8.8	72.5 ± 9.3[3]
FBS (mmol/L)	4.38 ± 0.43	4.41 ± 0.38	4.62 ± 0.53	4.52 ± 0.50
TC (mmol/L)	4.27 ± 0.63	4.63 ± 0.84	4.20 ± 0.74	4.50 ± 1.08
TG (mmol/L)	1.00 ± 0.59	1.15 ± 0.61	0.86 ± 0.44	1.35 ± 0.64[3]
HDL-C (mmol/L)	1.63 ± 0.32	1.50 ± 0.36[2]	1.30 ± 0.27	1.14 ± 0.30[3]
Insulin (μIU/mL)	3.58 ± 3.3	5.71 ± 4.66[2]	10.06 ± 7.32	17.96 ± 13.85[3]
HOMA-IR	0.71 ± 0.70	1.10 ± 1.05[2]	2.10 ± 1.59	3.68 ± 2.99[3]
hs-CRP (mg/L)	0.60 ± 0.91	1.08 ± 1.21[2]	—	—
RBP4 (μg/mL)	59.68 ± 15.27	69.34 ± 17.10[2]	—	—
A-FABP (μg/L)	12.84 ± 5.05	23.63 ± 8.15[2]	—	—

[1] All values are means ± SDs. Overweight was defined as a BMI >85th percentile. WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBS, fasting blood sugar; TC, total cholesterol; TG, triglyceride; HDL-C, HDL cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; RBP4, retinol-binding protein 4; A-FABP, adipocyte fatty acid–binding protein.
[2] Significantly different from normal weight at baseline, $P < 0.05$ (Student's t test).
[3] Significantly different from normal weight 3 y later, $P < 0.05$ (Student's t test).

TABLE 2
Pearson's correlations between adipokines and anthropometric and metabolic variables at baseline[1]

	Height (cm)	Weight (kg)	BMI (kg/m^2)	Waist (cm)	SBP (mm Hg)	DBP (mm Hg)	FBS (mmol/L)	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	Insulin (μIU/mL)	HOMA-IR	Log adiponectin (μg/mL)	RBP4 (μg/mL)	A-FABP (μg/L)
Height (cm)	1.0														
Weight (kg)	0.70[2]	1.0													
BMI (kg/m^2)	0.42[2]	0.94[2]	1.0												
Waist (cm)	0.54[2]	0.91[2]	0.91[2]	1.0											
SBP (mm Hg)	0.27[2]	0.38[2]	0.35[2]	0.28[2]	1.0										
DBP (mm Hg)	0.21[3]	0.30[2]	0.29[2]	0.22[3]	0.82[2]	1.0									
FBS (mmol/L)	0.14	0.07	0.01	0.02	0.05	0.05	1.0								
TC (mmol/L)	0.03	0.15	0.18[3]	0.17[3]	0.13	0.04	−0.01	1.0							
TG (mmol/L)	0.08	0.14	0.13	0.16[3]	0.07	0.01	0.07	0.14	1.0						
HDL-C (mmol/L)	0.07	−0.16[3]	−0.17[3]	−0.22[3]	0.05	0.02	0.05	0.40[2]	-0.38[2]	1.0					
Insulin (μIU/mL)	0.18[3]	0.30[2]	0.28[2]	0.31[2]	0.20[3]	0.19[3]	0.27[2]	0.04	0.28[2]	−0.12	1.0				
HOMA-IR	0.17[3]	0.26[2]	0.25[3]	0.28[2]	0.16[3]	0.19[3]	0.38[2]	0.04	0.21[3]	−0.11	0.93[2]	1.0			
Log adiponectin (μg/mL)	−0.26[2]	−0.33[2]	−0.29[2]	−0.30[2]	−0.29[3]	−0.25[3]	−0.05	−0.11	0.08	0.15	−0.03	−0.05	1.0		
RBP4 (μg/mL)	0.28[2]	0.39[2]	0.37[2]	0.31[2]	0.15	0.07	0.04	0.31[2]	0.22[2]	0.09	0.24[3]	0.24[3]	−0.04	1.0	
A-FABP (μg/L)	0.35[2]	0.67[2]	0.69[2]	0.61[2]	0.39[2]	0.32[2]	−0.11	0.23[2]	0.11	−0.09	0.19[3]	0.17	−0.37[2]	0.38[2]	1.0

[1] SBP, systolic blood pressure; DBP, diastolic blood pressure; FBS, fasting blood sugar; TC, total cholesterol; TG, triglyceride; HDL-C, HDL cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; RBP4, retinol-binding protein 4; A-FABP, adipocyte fatty acid–binding protein.

[2] $P < 0.001$.

[3] $P < 0.05$.

TABLE 3
Pearson's correlations for the association between baseline adipokines and changes (Δ) in metabolic variables during the 3-y follow-up[1]

	Unadjusted coefficients			Coefficients adjusted for Tanner stage		
	Log adiponectin (μg/mL)	RBP4 (μg/mL)	A-FABP (μg/L)	Log adiponectin (μg/mL)	RBP4 (μg/mL)	A-FABP (μg/L)
ΔHeight (cm)	−0.16[2]	0.24[2]	0.17[2]	−0.11	0.30[3]	0.16
ΔWeight (kg)	−0.29[2]	0.28[2]	0.43[3]	−0.23[2]	0.31[3]	0.45[3]
ΔBMI (kg/m^2)	−0.12	0.07	0.17[2]	−0.09	0.13	0.18[2]
ΔWaist (cm)	−0.13	0.18[2]	0.25[2]	−0.12	0.22[2]	0.27[2]
ΔSBP (mm Hg)	0.22[2]	−0.02	−0.11	0.13	0.02	−0.10
ΔDBP (mm Hg)	0.18[2]	−0.04	−0.15	0.01	−0.06	−0.18[2]
ΔFBS (mmol/L)	0.06	0.04	0.02	−0.07	0.08	0.05
ΔTC (mmol/L)	0.04	−0.03	0.02	−0.04	−0.02	0.02
ΔTG (mmol/L)	−0.22[2]	0.05	0.16	−0.27[2]	0.08	0.13
ΔHDL-C (mmol/L)	0.10	−0.20[2]	−0.15	0.18	−0.27[3]	−0.08
ΔInsulin (μIU/mL)	−0.06	0.04	0.09	−0.17	0.02	0.06
ΔHOMA-IR	−0.06	0.03	0.07	−0.07	0.02	0.13

[1] SBP, systolic blood pressure; DBP, diastolic blood pressure; FBS, fasting blood sugar; TC, total cholesterol; TG, triglyceride; HDL-C, HDL cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; RBP4, retinol-binding protein 4; A-FABP, adipocyte fatty acid–binding protein. The interaction models were not significant.
[2] $P < 0.05$.
[3] $P < 0.001$.

In multiple logistic regression analysis, the results of which are presented in **Table 4**, children in the highest tertile of baseline A-FABP concentrations had an increased risk of developing the metabolic syndrome at year 3 than did those with A-FABP concentrations in the lowest tertile (OR: 17.33; 95% CI: 1.25, 239.76; $P < 0.001$, P for trend = 0.005) after adjustment for Tanner stage, sleep duration, physical activity, HOMA-IR at baseline, and BMI at baseline. On the contrary, children in the highest tertile of baseline adiponectin concentrations had a lower risk of developing the metabolic syndrome at year 3 than did subjects in the lowest tertile (OR: 0.08; 95% CI: 0.01, 0.79; $P < 0.001$, P for trend =0.017) after adjustment for Tanner stage and HOMA-IR at baseline. However, after further adjustment for sleep duration, physical activity, and BMI at baseline, the association between adiponectin and development of the metabolic syndrome became nonsignificant (Table 4).

To study potential interactions, we then divided children into 4 groups, ie, those with high adiponectin concentrations and low A-FABP concentrations at baseline, low adiponectin concentrations and low A-FABP concentrations at baseline, high adiponectin concentrations and low A-FABP concentrations at baseline, and low adiponectin concentrations and high A-FABP concentrations at baseline. These 4 groups were associated with several risk factors for the metabolic syndrome (**Table 5**), but interaction terms were not significant in terms of predicting the development of metabolic syndrome.

TABLE 4
Odds ratios (and 95% CIs) from a logistic regression analysis of baseline adipokines as predictors of the development of the metabolic syndrome at the 3-y follow-up by tertile (T)[1]

	T1	T2	T3	P for trend
Adiponectin (μg/mL)	2.42–11.04	11.19–18.47	18.66–41.08	
Model 1	1.0	0.10 (0.01, 1.04)	0.07 (0.01, 0.73)	0.017
Model 2	1.0	0.10 (0.01, 0.91)	0.08 (0.01, 0.79)	
Model 3	1.0	0.27 (0.03, 2.65)	0.15 (0.01, 1.76)	
Model 4	1.0	0.16 (0.02, 1.20)	0.10 (0.01, 1.02)	
RBP4 (μg/mL)	24.1–55.2	55.5–67.8	67.8–108.0	
Model 1	1.0	5.42 (0.61, 48.04)	4.25 (0.46, 39.31)	0.252
Model 2	1.0	5.31 (0.59, 47.42)	3.12 (0.32, 30.86)	
Model 3	1.0	6.38 (0.58, 69.68)	1.99 (0.17, 23.12)	
Model 4	1.0	9.93 (0.77–127.6)	2.56 (0.29, 22.84)	
A-FABP (μg/L)	5.0–11.0	12.0–17.0	18.0–43.0	
Model 1	1.0	1.09 (0.06, 18.81)	10.82 (1.15, 101.52)	0.005
Model 2	1.0	1.02 (0.06, 16.79)	9.56 (1.15, 79.64)	
Model 3	1.0	1.29 (0.08, 21.47)	12.00 (1.34, 107.36)	
Model 4	1.0	1.90 (0.08, 44.66)	17.33 (1.25, 239.76)	

[1] RBP4, retinol-binding protein 4; A-FABP, adipocyte fatty acid–binding protein. Model 1 was adjusted for Tanner stage; model 2 was adjusted for Tanner stage and homeostasis model assessment of insulin resistance (HOMA-IR) at baseline; model 3 was adjusted for Tanner stage, HOMA-IR at baseline, and BMI at baseline; and model 4 was adjusted for Tanner stage, sleep duration, physical activity, HOMA-IR at baseline, and BMI at baseline. The interaction models were not significant.

TABLE 5
Comparisons of risks of metabolic syndrome 3 y later by baseline adipocyte fatty acid–binding protein (A-FABP) and adiponectin interactions[1]

	High adiponectin + low A-FABP	Low adiponectin + low A-FABP	High adiponectin + low A-FABP	Low adiponectin + high A-FABP	*P* value
Height (cm)	150.5 ± 8.1	149.5 ± 6.9	151.4 ± 7.6	155.5 ± 6.8	<0.01
Weight (kg)	42.1 ± 8.1	41.9 ± 9.0	48.9 ± 9.0	56.7 ± 9.7	<0.01
BMI (kg/m^2)	18.5 ± 2.5	18.6 ± 3.2	21.2 ± 2.9	23.4 ± 3.3	<0.01
WC (cm)	62.7 ± 7.5	63.1 ± 8.0	69.8 ± 8.6	75.6 ± 9.5	<0.01
SBP (mm Hg)	109.7 ± 10.2	108.9 ± 10.5	115.2 ± 12.1	115.3 ± 10.1	<0.01
DBP (mm Hg)	70.2 ± 8.4	68.1 ± 7.5	71.6 ± 9.6	70.8 ± 8.8	0.14
FBS (mmol/L)	4.80 ± 0.52	4.66 ± 0.54	4.50 ± 0.41	4.53 ± 0.54	0.05
TC (mmol/L)	4.25 ± 0.78	4.02 ± 0.54	4.60 ± 0.96	4.55 ± 0.87	0.02
TG (mmol/L)	0.93 ± 0.51	0.89 ± 0.45	0.98 ± 0.55	1.27 ± 0.64	<0.01
HDL-C (mmol/L)	1.30 ± 0.30	1.24 ± 0.26	1.29 ± 0.30	1.18 ± 0.31	0.24
Insulin (μIU/mL)	11.8 ± 7.4	11.4 ± 9.5	11.0 ± 6.7	16.9 ± 15.4	0.04
HOMA-IR	2.49 ± 1.63	2.39 ± 2.03	2.20 ± 1.38	3.44 ± 3.32	0.08

[1] All values are means ± SDs. WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBS, fasting blood sugar; TC, total cholesterol; TG, triglyceride; HDL-C, HDL cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance. The interaction models were not significant. The groups were compared by ANOVA.

DISCUSSION

A-FABP, which is expressed in adipocytes and macrophages, has been suggested to be a central regulator of insulin sensitivity, lipid metabolism, and inflammation associated with atherosclerosis (11, 30). In animal models, FABP-deficient mice are protected from insulin resistance, type 2 diabetes, and atherosclerosis (11, 31, 32). In humans, several studies have also shown positive associations between A-FABP concentrations and components of the metabolic syndrome and insulin resistance (8, 9); therefore, A-FABP might be a useful biomarker of obesity-related metabolic and cardiovascular disorders. We performed the first prospective study in a pediatric population and, consistent with previous findings (15, 33, 34), we observed that obese children had significantly greater A-FABP concentrations than did the normal-weight children. Furthermore, we found that baseline A-FABP concentrations at age 9 y were significantly associated with changes in both weight and waist circumference over 3 y of follow-up and that baseline A-FABP concentrations in children who developed the metabolic syndrome were significantly higher than those in children who did not develop the metabolic syndrome. These findings suggest that A-FABP might contribute to the development of the metabolic syndrome in children, because it was previously shown in adults (13). In children, evidence thus far has indicated positive associations between circulating A-FABP concentrations, central and total adiposity, and insulin resistance (35), whereas substantial weight loss due to an obesity-intervention program may lead to a significant decrease in A-FABP concentrations (33). Furthermore, a recent publication by Khalyfa et al (34) reported that obese children exhibit higher circulating concentrations of A-FABP, and the frequency of the rs1054135 allelic variant was associated with increased A-FABP concentrations, which suggested that both genetic and environmental conditions alter A-FABP concentrations. It was also reported that A-FABP may contribute to the risk of reduced insulin sensitivity and increased systemic inflammation, and that the presence of selective single nucleotide polymorphisms in the *FABP4* gene may account for an increased risk of insulin resistance or systemic inflammation in children (34). These data agree with our data by supporting that A-FABP plays a central role in obesity and in the metabolic syndrome in children.

RBP4 was recently identified as an adipocyte-derived factor influencing systemic insulin sensitivity in an experimental mouse model (16). Overexpression of human RBP4 or injection of recombinant RBP4 induces insulin resistance in mice, whereas RBP4 knockout mice showed enhanced insulin sensitivity (16). Early reports in humans have shown strong associations between RBP4 and insulin resistance as well as features of the metabolic syndrome (17). However, several subsequent studies have failed to confirm these associations (36, 37). A recent cohort study including overweight black adolescents has shown that baseline RBP4 concentrations were prospectively associated with insulin resistance and hypertriglyceridemia (38). We performed the first prospective study in children and found no independent association between RBP4 concentrations and development of the metabolic syndrome, despite the fact that RBP4 concentrations were significantly higher in obese than in normal-weight children at baseline.

Adiponectin is an insulin-sensitizing hormone, the concentration of which decreases with intraabdominal obesity (39). Adiponectin concentration is a predictor of the development of type 2 diabetes and cardiovascular disease in adults (21, 40). Several cross-sectional studies have evaluated adiponectin in relation to the metabolic syndrome or cardiovascular disease risk factors in adults (41) and children (42, 43). We found that children with higher adiponectin concentrations at baseline, compared with children who had lower adiponectin concentrations, had lower risk of developing the metabolic syndrome after adjustment for pubertal status and insulin resistance as confounding factors. Further adjustment for obesity (BMI), however, resulted in a suggestive, but nonsignificant, association between adiponectin and development of the metabolic syndrome at the conventional $P = 0.05$ level, which indicated that adiponectin mediates, in part, the effects of obesity on the risk of developing the metabolic syndrome.

Evidence from animal studies suggests the A-FABP may influence systemic inflammation (11, 31). A-FABP$^{-/-}$ macrophages had reduced nuclear transcription factor κB (NF-κB) activity resulting in suppression of inflammatory function,

including the production of cytokines such as tumor necrosis factor-α and proinflammatory enzymes (44). Adiponectin upregulates AMP-activated protein kinase (AMPK), which results in decreased NF-κB activity (45) and subsequent synthesis and secretion of proinflammatory cytokines. Although both A-FABP and adiponectin are expressed in adipocytes, unfavorable effects of A-FABP appear to contrast with the beneficial effects of adiponectin, including antiatherogenic, antidiabetogenic, and antiinflammatory actions (21) The present study found that A-FABP concentrations were significantly and negatively correlated with adiponectin concentrations ($r = -0.37$, $P < 0.01$), even after adjustment for BMI ($r = -0.19$, $P = 0.02$). Furthermore, we showed no significant interactions between adiponectin and A-FABP in predicting metabolic syndrome risk in children. The common underlying factor responsible for the association between adiponectin and A-FABP remains to be elucidated by future larger studies. Similarly, larger studies are needed to confirm our data on lack of significant interaction between these 2 molecules.

The present study had several strengths, including its prospective study design and the state-of-the-art method used to measure study variables. However, the study had some limitations. First, error in the assessment of the hormones of interest and a certain degree of misclassification in the assessment of other variables is unavoidable, but such error is random and could have only suppressed effect estimates toward the null and P values toward nonsignificance. Second, the most important limitation was the small number of subjects who developed the metabolic syndrome during the 3-y follow-up. Although statistically significant for prediction of the development of metabolic syndrome, the 95% CIs for adiponectin, RBP4, and A-FABP were extremely wide. Third, there was a lack of age-, sex-, and ethnicity-specific values for metabolic syndrome in children. Fourth, the observational nature of this study did not allow direct proof of causality, but the prospective design allowed for the incorporation of the time sequence criterion for causality. Fifth, although physician assessment of the Tanner stage is the gold standard, we used self-reported Tanner staging because of the nature of the school-based examination. Finally, we could not assess diet or dietary patterns in this study. Several studies have shown that A-FABP concentrations are associated with dietary patterns, especially fat intake (32, 35). Obesity is an important risk factor for sleep apnea, and sleep apnea is a major risk factor for the metabolic syndrome, particularly in obese children. Recent studies showed that sleep apnea is also associated with increased A-FABP concentrations (46, 47), but we did not assess the prevalence of sleep apnea in this study because we estimated that it might be present in only $\approx$10 subjects, given the prevalence estimates reported in other obese populations. Because we controlled for obesity, however, which is upstream of sleep apnea in the causal pathway, we feel that our results are accurate and reliable and are generalizable to populations similar to the Korean population. Whether similar results will be obtained in Western populations remains to be shown.

In conclusion, both baseline A-FABP and adiponectin serum concentrations are associated prospectively with the development of the metabolic syndrome in children after a 3-y follow-up, in contrast with RBP4, which is not. Moreover, A-FABP was found to be an independent predictor of the metabolic syndrome in Korean boys after adjustment of pubertal status, baseline insulin resistance, and obesity, whereas the predictive role of adiponectin is, in part, mediated by an underlying association with obesity at baseline. We found no significant interaction between these variables. Future studies are needed to confirm our conclusions, which need to be replicated and confirmed in larger population studies and/or in girls, in other age-groups, and in other ethnic groups.

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REFERENCES

1. Katzmarzyk PT, Perusse L, Malina RM, Bergeron J, Despres JP, Bouchard C. Stability of indicators of the metabolic syndrome from childhood and adolescence to young adulthood: the Quebec Family Study. J Clin Epidemiol 2001;54:190–5.
2. Botton J, Heude B, Kettaneh A, et al. Cardiovascular risk factor levels and their relationships with overweight and fat distribution in children: the Fleurbaix Laventie Ville Sante II study. Metabolism 2007;56: 614–22.
3. Geiss HC, Parhofer KG, Schwandt P. Parameters of childhood obesity and their relationship to cardiovascular risk factors in healthy prepubescent children. Int J Obes Relat Metab Disord 2001;25:830–7.
4. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 1988;37:1595–607.
5. Alberti KG, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 2009;120:1640–5.
6. Tailor AM, Peeters PH, Norat T, Vineis P, Romaguera D. An update on the prevalence of the metabolic syndrome in children and adolescents. Int J Pediatr Obes 2010;5:202–13.
7. Weiss R, Dziura J, Burgert TS, et al. Obesity and the metabolic syndrome in children and adolescents. N Engl J Med 2004;350:2362–74.
8. Xu A, Wang Y, Xu JY, et al. Adipocyte fatty acid-binding protein is a plasma biomarker closely associated with obesity and metabolic syndrome. Clin Chem 2006;52:405–13.
9. Stejskal D, Karpisek M. Adipocyte fatty acid binding protein in a Caucasian population: a new marker of metabolic syndrome? Eur J Clin Invest 2006;36:621–5.
10. Uyeda K, Repa JJ. Carbohydrate response element binding protein, ChREBP, a transcription factor coupling hepatic glucose utilization and lipid synthesis. Cell Metab 2006;4:107–10.
11. Maeda K, Cao H, Kono K, et al. Adipocyte/macrophage fatty acid binding proteins control integrated metabolic responses in obesity and diabetes. Cell Metab 2005;1:107–19.
12. Tso AW, Xu A, Sham PC, et al. Serum adipocyte fatty acid binding protein as a new biomarker predicting the development of type 2 diabetes: a 10-year prospective study in a Chinese cohort. Diabetes Care 2007;30:2667–72.
13. Xu A, Tso AW, Cheung BM, et al. Circulating adipocyte-fatty acid binding protein levels predict the development of the metabolic syndrome: a 5-year prospective study. Circulation 2007;115:1537–43.
14. Choi KM, Kim TN, Yoo HJ, et al. Effect of exercise training on A-FABP, lipocalin-2 and RBP4 levels in obese women. Clin Endocrinol (Oxf) 2009;70:569–74.
15. Yun KE, Kim SM, Choi KM, Park HS. Association between adipocyte fatty acid-binding protein levels and childhood obesity in Korean children. Metabolism 2009;58:798–802.
16. Yang Q, Graham TE, Mody N, et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature 2005;436:356–62.
17. Graham TE, Yang Q, Bluher M, et al. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. N Engl J Med 2006;354:2552–63.

18. von Eynatten M, Lepper PM, Liu D, et al. Retinol-binding protein 4 is associated with components of the metabolic syndrome, but not with insulin resistance, in men with type 2 diabetes or coronary artery disease. Diabetologia 2007;50:1930–7.
19. von Eynatten M, Humpert PM. Retinol-binding protein 4 & atherosclerosis: risk factor or innocent bystander? Atherosclerosis 2009;206: 38–9.
20. Aeberli I, Biebinger R, Lehmann R, L'Allemand D, Spinas GA, Zimmermann MB. Serum retinol-binding protein 4 concentration and its ratio to serum retinol are associated with obesity and metabolic syndrome components in children. J Clin Endocrinol Metab 2007;92: 4359–65.
21. Kadowaki T, Yamauchi T. Adiponectin and adiponectin receptors. Endocr Rev 2005;26:439–51.
22. Yamamoto Y, Hirose H, Saito I, Nishikai K, Saruta T. Adiponectin, an adipocyte-derived protein, predicts future insulin resistance: two-year follow-up study in Japanese population. J Clin Endocrinol Metab 2004; 89:87–90.
23. Kynde I, Heitmann BL, Bygbjerg IC, Andersen LB, Helge JW. Hypoadiponectinemia in overweight children contributes to a negative metabolic risk profile 6 years later. Metabolism 2009;58:1817–24.
24. Taylor SJ, Whincup PH, Hindmarsh PC, Lampe F, Odoki K, Cook DG. Performance of a new pubertal self-assessment questionnaire: a preliminary study. Paediatr Perinat Epidemiol 2001;15:88–94.
25. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
26. Committe on Health and Statistics. Korean physical growth standards for children and adolescents. Seoul, Korea: Korean Pediatrics Society 2007.
27. Lambert M, Paradis G, O'Loughlin J, Delvin EE, Hanley JA, Levy E. Insulin resistance syndrome in a representative sample of children and adolescents from Quebec, Canada. Int J Obes Relat Metab Disord 2004; 28:833–41.
28. Cook S, Weitzman M, Auinger P, Nguyen M, Dietz WH. Prevalence of a metabolic syndrome phenotype in adolescents: findings from the third National Health and Nutrition Examination Survey, 1988-1994. Arch Pediatr Adolesc Med 2003;157:821–7.
29. National High Blood Pressure Education Program Working Group on Hypertension Control in Children and Adolescents. Update on the 1987 Task Force Report on High Blood Pressure in Children and Adolescents: a working group report from the National High Blood Pressure Education Program. Pediatrics 1996;98:649–58.
30. Makowski L, Hotamisligil GS. The role of fatty acid binding proteins in metabolic syndrome and atherosclerosis. Curr Opin Lipidol 2005;16: 543–8.
31. Maeda K, Uysal KT, Makowski L, et al. Role of the fatty acid binding protein mal1 in obesity and insulin resistance. Diabetes 2003;52:300–7.
32. Hotamisligil GS, Johnson RS, Distel RJ, Ellis R, Papaioannou VE, Spiegelman BM. Uncoupling of obesity from insulin resistance through a targeted mutation in aP2, the adipocyte fatty acid binding protein. Science 1996;274:1377–9.
33. Reinehr T, Stoffel-Wagner B, Roth CL. Adipocyte fatty acid-binding protein in obese children before and after weight loss. Metabolism 2007; 56:1735–41.
34. Khalyfa A, Bhushan B, Hegazi M, et al. Fatty-acid binding protein 4 gene variants and childhood obesity: potential implications for insulin sensitivity and CRP levels. Lipids Health Dis 2010;9:18.
35. Aeberli I, Beljean N, Lehmann R, I'Allemand D, Spinas GA, Zimmermann MB. The increase of fatty acid-binding protein aP2 in overweight and obese children: interactions with dietary fat and impact on measures of subclinical inflammation. Int J Obes (Lond) 2008;32: 1513–20.
36. Takashima N, Tomoike H, Iwai N. Retinol-binding protein 4 and insulin resistance. N Engl J Med 2006;355:1392, author reply 1394–5.
37. Janke J, Engeli S, Boschmann M, et al. Retinol-binding protein 4 in human obesity. Diabetes 2006;55:2805–10.
38. Goodman E, Graham TE, Dolan LM, Daniels SR, Goodman ER, Kahn BB. The relationship of retinol binding protein 4 to changes in insulin resistance and cardiometabolic risk in overweight black adolescents. J Pediatr 2009;154:67–73e1.
39. Ziemke F, Mantzoros CS. Adiponectin in insulin resistance: lessons from translational research. Am J Clin Nutr 2010;91(suppl):258S–61S.
40. Heidemann C, Sun Q, van Dam RM, et al. Total and high-molecular-weight adiponectin and resistin in relation to the risk for type 2 diabetes in women. Ann Intern Med 2008;149:307–16.
41. Mantzoros CS, Li T, Manson JE, Meigs JB, Hu FB. Circulating adiponectin levels are associated with better glycemic control, more favorable lipid profile, and reduced inflammation in women with type 2 diabetes. J Clin Endocrinol Metab 2005;90:4542–8.
42. Cambuli VM, Musiu MC, Incani M, et al. Assessment of adiponectin and leptin as biomarkers of positive metabolic outcomes after lifestyle intervention in overweight and obese children. J Clin Endocrinol Metab 2008;93:3051–7.
43. Yoshinaga M, Sameshima K, Tanaka Y, et al. Adipokines and the prediction of the accumulation of cardiovascular risk factors or the presence of metabolic syndrome in elementary school children. Circ J 2008;72: 1874–8.
44. Makowski L, Brittingham KC, Reynolds JM, Suttles J, Hotamisligil GS. The fatty acid-binding protein, aP2, coordinates macrophage cholesterol trafficking and inflammatory activity. Macrophage expression of aP2 impacts peroxisome proliferator-activated receptor gamma and IkappaB kinase activities. J Biol Chem 2005;280:12888–95.
45. Devaraj S, Torok N, Dasu MR, Samols D, Jialal I. Adiponectin decreases C-reactive protein synthesis and secretion from endothelial cells: evidence for an adipose tissue-vascular loop. Arterioscler Thromb Vasc Biol 2008;28:1368–74.
46. Gozal D, Capdevila OS, Kheirandish-Gozal L. Metabolic alterations and systemic inflammation in obstructive sleep apnea among nonobese and obese prepubertal children. Am J Respir Crit Care Med 2008;177: 1142–9.
47. Verhulst SL, Rooman R, Van Gaal L, De Backer W, Desager K. Is sleep-disordered breathing an additional risk factor for the metabolic syndrome in obese children and adolescents? Int J Obes (Lond) 2009;33: 8–13.