

Combined effect of folate and adiposity on homocysteine in children at three years of age

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BACKGROUND/OBJECTIVES: Cardiovascular diseases is a major cause of death and is responsible for 23.8% of deaths in Korea. Clinical symptoms manifest in adulthood, but susceptibility begins *in utero*. Elevated homocysteine levels and adiposity might be linked to a greater risk in children as well as adults. We hypothesized that those who have simultaneous risk for folate and adiposity would be affected with elevated homocysteine levels at 3 years of age.

SUBJECTS/METHODS: From the ongoing birth cohort at Ewha Womans University Mok-Dong Hospital, we compared adiposity parameters, serum homocysteine, and folate levels in 238 children (118 boys and 120 girls) at three years of age. The relationship between birth outcome, current weight and body mass index (BMI), postnatal growth, and homocysteine level were assessed using correlation and general linear model. Additionally, we assessed the combined effect between blood folate status and adiposity on current homocysteine levels.

RESULTS: Birth characteristics were not correlated with homocysteine. Current weight, BMI, upper-arm circumference, skinfold thickness, waist circumference, and hip circumference were positively correlated with homocysteine at three years of age ($P < 0.05$). Folate level was negatively correlated with homocysteine at three years of age ($P < 0.0001$). A relative high anthropometric measure which is compatible with adiposity and low folate level was associated with high homocysteine levels.

CONCLUSION: We found a combined effect of adiposity and folate levels with homocysteine levels at three years of age. This implicates the beneficial role of folate supplementation in the high-risk population at an early age.

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INTRODUCTION

Cardiovascular disease (CVD) is a major cause of death in developed countries [1] and is responsible for 23.8% of deaths in Korea. Adult CVD has a prolonged latent period and atherosclerosis begins early in childhood; fatty streaks in the aorta are common even in 3 year olds [2]. Similar findings were found in autopsies from participants in the Bogalusa Heart Study in whom adiposity and other previously measured risk factors including blood pressure and blood lipids, correlated with the extent of coronary and aortic atherosclerosis [3]. Furthermore the origin of CVD may be found in fetal life. The fetal origins hypothesis suggests that an adverse early life environment has a lasting effect on health in later life [4].

In addition to emphasizing the significance of birth weight and gestational age, the current concept also focuses on postnatal growth and adiposity [4,5]. To assess the impact of

intrauterine life on later life along with adiposity, it is necessary to follow up a group of children longitudinally from fetal period to adulthood via a birth cohort. While atherosclerosis has been clearly shown to begin in childhood, the process is usually subclinical and the rate of progression is slow, but the appropriate intervention would be preventive of clinical diseases for the high risk population [6].

Homocysteine is a non-proteinogenic, sulphur-containing amino acid derived from the metabolism of methionine [7]. Genetic defects, vitamin deficiencies or renal impairment cause elevated plasma total homocysteine and direct toxic endothelial cell damage generating potent reactive oxygen species that induce oxidative damage and decrease endothelial production of nitric oxide. This impairs the endothelial dependent vascular reactivity and activates platelets to form thrombus [8].

Epidemiological studies have shown that elevated homocysteine level is related to a higher risk of cardiovascular disease,

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stroke and peripheral vascular disease in adults [9]. Meta-analyses of cohort studies show significant positive associations between serum homocysteine and ischemic heart diseases; a 2.5 times higher risk of subsequent coronary events and each 5 mmol/l increment was associated with a 25% higher risk [10]. Throughout life, the level of homocysteine in plasma increases along with aging in both males and females. High plasma total homocysteine in children was related to CVD or death in their parents or close relatives in white and black children and in white children with hypercholesterolemia [11]. Folate level is a well known modifying factor that affects circulating homocysteine level [6]. However, the association between adiposity and homocysteine in children is debated [12-14]. Therefore the present study was undertaken to prospectively study the impact of adiposity including characteristics at birth and postnatal growth on homocysteine levels in children at three years of age in an ongoing birth cohort in South Korea. Additionally, we examined whether those with relatively high adiposity with low folate level are associated with high level of homocysteine.

SUBJECTS AND METHODS

Study subjects

In order to investigate the correlation between fetal environment and adult diseases, an ongoing birth cohort has been established in 2001 at Ewha Womans University Mok-Dong Hospital. Pregnant women are enrolled in the antenatal period during the second trimester. Detailed description for birth cohorts has already been reported elsewhere [15]. The Institutional Research Board on human subjects at Ewha Womans University approved the protocol and informed consents were obtained. Out of 734 children enrolled in the birth cohort, 527 children were contacted from November of 2005 to November of 2007 around their third birthday. Out of the 527 children contacted, 238 children (45.2%) participated in the three year check-up program.

Data

Birth data such as birth date, sex, gestational age, birth weight, and placenta weights were obtained from medical records. As routine definition, < 37 weeks of gestation age and < 2.5 kg of birth weight were defined preterm and low birth weight, respectively. At the three year check-up program, all anthropometric indicators were measured by well-trained examiners. Height and weight were measured to one decimal place while wearing light clothing and without shoes using a stadiometer and calibrated scale (DS-102 model, Dong Sahn Jenix Co. Ltd, Seoul, Korea). Waist circumferences, upper-arm circumferences, skinfold thickness, and hip circumference were also measured as anthropometric indicators for adiposity. Body mass index was calculated as weight in kilograms divided by height in meters squared (kg/m^2). To convert into z score, age was calculated in months. The birth weight and current weight were transferred to an age- and gender- specific z score criteria reference source from the 2007 Korean Children and Adolescents Growth Standards [16]. To explain the postnatal growth, the change of weight z score was calculated by subtracting the birth weight z score from the current weight z score. Catch-up growth was defined

as greater than 0.67 (equal to 75th percentile) at current weight z score if born preterm or of low birth weight and others with a change of weight z score more than 0.67 [16].

Venous samples were collected after 12 hour fasting and centrifuged within 2 hours. Homocysteine levels was measured by the method previously described by Araki *et al.* [17] using the high performance liquid chromatography (HPLC) - fluorescence detection method. A HPLC system was equipped with a column (Waters XTerraTMRP18, 5- μm particle size, 4.6 \times 250 mm column, Waters Co., MA, USA), pumps (Waters 2690, Waters Co., MA, USA), HPLC integrator (Younglin Autochro-win ver 2.0, Younglin, Korea), and Fluorescence Detector (Waters 474, Waters Co., MA, USA), under the condition excitation/emission wave length of 385 nm/515 nm. Folate was measured by radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA, USA). The inter-assay differentiation coefficient was set at 4.0%.

Statistical analysis

Data analysis was performed using the statistical package SAS version 9.3 (SAS Institutes, Cary, NC, USA). As possible independent variables, we considered characteristics at birth (gestational age, birth weight, placenta weight, preterm, and low birth weight), adiposity-related anthropometric measurements at 3 years of age (weights, BMI, waist, upper-arm circumferences, skin-fold thickness, and hip circumferences), postnatal growth (change of weight z scores from birth to current, catch-up growth) and blood folate level. The outcome variable was the homocysteine level. Homocysteine and folate were used as log-transformed value to meet the normality assumption and then results were presented as back transformed values. Pearson correlation was used to investigate the strength of linearity between homocysteine and possible independent variables above mentioned. The impact of adiposity including characteristics at birth and postnatal growth on homocysteine levels were analyzed under controlling for age and sex (model 1), additionally including blood folate level (model 2) using a generalized linear model. Considering the statistical power, we used an operational definition for high risk of obesity and low folate level to assess the combined effect between blood folate level and adiposity on current homocysteine levels. Adiposity-related anthropometric measurements were divided into two groups (< 75th percentile or \geq 75th percentile), folate levels were also divided into two groups (< 25th percentile or \geq 25th percentile, cut point value: 6.9 ng/ml), and defined as low and high, respectively. Indeed, normal concentration of serum folate suggested by WHO ranges from 6 to 20 ng/ml for all aged person [18]. In turn the subjects were grouped into four groups: low adiposity measures with high folate (low risk group), low adiposity measures with low folate, high adiposity measures with high folate, and high adiposity measures with low folate (high risk group). Regarding the characteristics at birth and postnatal growth, preterm, low birth weight, and catch-up growth were considered as risk group on the basis of previous studies [2,4-6,8]. Grouping was followed as mentioned above. After controlling for age and sex, adjusted means differences of homocysteine among groups was tested using a generalized linear model. The multiple comparisons were conducted using Bonferroni method. Statistical significance was noted as $P < 0.05$

based on a two-tail test.

RESULTS

Basic characteristics of the study population

The overall mean homocysteine and folate level in the study population were 4.85 $\mu\text{mol/L}$ and 9.90 ng/mL (geometric homocysteine mean: 4.73 $\mu\text{mol/L}$, geometric folate mean: 9.08 ng/mL ; each ranged from 2.72 to 11.10 mmol/L and from 1.8 to 38.5 ng/mL) respectively. The levels were similar among boys and girls. The characteristics of study participants are shown in Table 1. About a quarter of subjects was low birth weight and preterm. All anthropometric data were within the normal range for 3 year olds in Korea. We subdivided the group into boys and girls but no difference was found between the genders except for heights and weights. Current weight, BMI, upper arm circumferences, skinfold thickness, and hip circumference were positively correlated with homocysteine level with a statistical significance ($P < 0.05$). On the other hand, current folate level was negatively correlated with homocysteine level ($P < 0.001$). Gestational age, birth weight, placental weight, and z scores of weight change from birth to three years of age were not correlated with homocysteine.

Analysis of factors related to homocysteine by a generalized linear model

Using a generalized linear model adjusted for sex, age in months, and blood folate level, the adjusted homocysteine levels at 3 years of age by quartile of adiposity measures and

placenta weight including birth outcomes and catch-up growth are presented in Table 2. Placental weight was associated with homocysteine levels at 3 years with a U shape. Those who belong to the highest quartile in weight, BMI, waist circumferences, skinfold thickness, and hip circumferences at 3 years of age showed the highest mean of homocysteine, but it was only a significant for group differences in placenta weight under controlling with sex and age. When adding folate level into consideration (model 2), it showed borderline significant in BMI, upper arm circumferences, and hip circumferences. On the contrary, the birth outcomes and postnatal growth in early life were not associated with homocysteine.

Combined effect of folate and adiposity on homocysteine

Table 3 summarizes the combined effect of blood folate and adiposity measure including birth outcomes and postnatal growth on homocysteine. Compared with those in the low risk group with low adiposity and high blood folate, children with high risk corresponding with weight, BMI, waist circumferences, skinfold thickness, and hip circumferences showed significantly high homocysteine levels. In addition, those who belong to low adiposity and low blood folate also showed higher homocysteine levels compared with in low risk group. In case of high adiposity and high blood folate, children with high upper arm circumferences and high folate level have higher homocysteine level compared with in low risk group. For birth features, those who belong to high placenta weight with low blood folate had high homocysteine levels compared with low placenta weight with high folate level. Low birth weight, and preterm, with low

Table 1. Basic characteristics at birth and at 3 years of age of study subjects (n = 238)

	Total (n = 238)	Boys (n = 118)	Girls (n = 120)	Correlation coefficient with homocysteine
At Birth				
Gestational age (weeks)	37.6 (2.4)	38.0 (2.2)	37.4 (2.6)	-0.04
Preterm (< 37 weeks)	61 (25.63%)	25 (21.19%)	36 (30.00%)	
Birth weight (kg)	2.90 (0.65)	2.96 (0.65)	2.84 (0.65)	0.01
Low birth weight (< 2.5 kg)	60 (25.21%)	28 (23.73%)	32 (26.67%)	
Placenta weight (g)	0.60 (0.08)	0.61 (0.07)	0.59 (0.08)	0.09
At 3 years of age				
Anthropometric measures				
Heights (cm)	98.27 (4.78)	99.43 (4.79)	97.13 (4.50) ^a	0.03
Weights (kg)	15.12 (2.01)	15.51 (2.21)	14.75 (1.71) ^a	0.14**
Body mass index (kg/m^2)	15.63 (1.46)	15.65 (1.62)	15.61 (1.29)	0.15**
Waist circumferences (cm)	49.14 (3.39)	49.29 (3.56)	48.98 (3.21)	0.13***
Upper-arm circumferences (cm)	16.25 (1.60)	16.25 (1.60)	16.26 (1.61)	0.15**
Skinfold thickness (cm)	11.15 (2.82)	10.89 (2.96)	11.40 (2.65)	0.14**
Hip circumferences (cm)	52.81 (3.42)	52.89 (3.60)	52.73 (3.25)	0.15**
Z scores of weight change from birth to 3yars	1.30 (2.04)	1.20 (1.75)	1.39 (2.29)	0.10
Catch up growth	83 (34.87%)	46 (38.98%)	37 (30.83%)	
Serological measures				
Homocysteine ($\mu\text{mol/L}$)	4.73 (1.01)	4.78 (1.02)	4.68 (1.02)	
Folate (ng/mL)	9.08 (1.03)	8.68 (1.05)	9.50 (1.04)	-0.32*

* $P < 0.0001$, ** $P < 0.05$, *** $P < 0.10$

^a $P < 0.05$ in sex difference

Continuous values are expressed as mean and standard deviation and discontinuous values are presented as n with percent (%). Catch-up growth was defined as greater than 0.67 (equal to 75th percentile) at current weight z score if born preterm or of low birth weight and others with a change of weight z score more than 0.67, it calculated by subtracting the birth weight z score from the current weight (3 years old) z score.

Table 2. The relations between the birth outcomes and anthropometric measures and homocysteine levels at 3 years of age using a generalized linear model

	Hcy	
	Model 1	Model 2
Preterm (<37weeks)		
No	4.73 (1.02)	4.72 (1.02)
Yes	4.73 (1.03)	4.77 (1.03)
Low birth weights (< 2.5 kg)		
No	4.69 (1.02)	4.69 (1.02)
Yes	4.85 (1.03)	4.88 (1.03)
Placenta weight (g) ¹⁾		
< 570	4.75 (1.05) ^a	4.82 (1.05) ^a
570 - 600	4.60 (1.07)	4.67 (1.07)
600 - 650	4.40 (1.03)	4.41 (1.03)
≥ 650	5.35 (1.04)	5.24 (1.04)
Weights at 3 yrs		
< 13.8	4.60 (1.03)	4.68 (1.03)
13.8 - 15.0	4.55 (1.03)	4.55 (1.03)
15.0-16.25	4.76 (1.03)	4.70 (1.03)
≥ 16.25	4.98 (1.03)	4.99 (1.03)
Body mass index at 3 yrs		
< 14.56	4.54 (1.03)	4.60 (1.03) ^b
14.56-15.52	4.57 (1.03)	4.51 (1.03)
15.52-16.52	4.90 (1.03)	4.88 (1.03)
≥ 16.52	4.92 (1.03)	4.95 (1.03)
Waist circumferences at 3 yrs		
< 47	4.52 (1.03)	4.51 (1.03)
47-49	4.67 (1.03)	4.73 (1.03)
49-51	4.74 (1.04)	4.73 (1.03)
≥ 51	4.91 (1.03)	4.89 (1.03)
Upper-arm circumferences at 3 yrs		
< 15.5	4.56 (1.03)	4.57 (1.03) ^b
15.5-16.2	4.55 (1.03)	4.53 (1.03)
16.2-17.0	4.88 (1.04)	4.94 (1.04)
≥ 17.0	4.87 (1.02)	4.87 (1.02)
Skinfold thickness at 3 yrs		
< 9.0	4.60 (1.03)	4.65 (1.03)
9.0-11.0	4.58 (1.03)	4.62 (1.03)
11.0-13.0	4.69 (1.03)	4.67 (1.03)
≥ 13.0	4.99 (1.03)	4.94 (1.03)
Hip circumferences at 3 yrs		
< 50.5	4.52 (1.03)	4.67 (1.03) ^b
50.5-52.5	4.55 (1.03)	4.72 (1.03)
52.5-54.8	4.81 (1.03)	4.91 (1.03)
≥ 54.8	4.96 (1.03)	5.15 (1.03)
Catch-up growth		
No	4.67 (1.02)	4.68 (1.02)
Yes	4.85 (1.03)	4.83 (1.02)

^a $P < 0.05$; ^b $P < 0.10$

Results represented as least squares means with standard error and P value for mean differences by groups obtained from generalized linear model.

All anthropometric indicators and placenta weight were classified into the quartile groups.

Model 1: adjusted for sex, life month Model 2: adjusted for sex, life month, and blood folate level

¹⁾ Sample size differ due to missing data (n = 119; Q1 = 30, Q2 = 11, Q3 = 47, Q4 = 31).

Catch-up growth was defined as greater than 0.67 (equal to 75th percentile) at current weight z score if born preterm or of low birth weight and others with a change of weight z score more than 0.67, it calculated by subtracting the birth weight z score from the current weight (3 years old) z score.

Table 3. The combined effect between the birth outcomes and anthropometric measures and blood folate level on homocysteine levels at 3 years of age using generalized linear model

	Folate		P combined
	< 25 th percentile	≥ 25 th percentile	
Low birth weight			
No	5.12 (1.03) ^b	4.57 (1.02)	< 0.001
Yes	5.69 (1.06) ^a	4.65 (1.03)	
Preterm			
No	5.22 (1.03) ^a	4.59 (1.02)	< 0.01
Yes	5.31 (1.07)	4.59 (1.03)	
Placenta weight (g) ¹⁾			
< 75 th percentile	5.16 (1.05) ^b	4.40 (1.03)	< 0.0001
≥ 75 th percentile	5.95 (1.07) ^a	5.06 (1.05)	
Weights at 3 years			
< 75 th percentile	5.11 (1.03) ^b	4.52 (1.02)	< 0.001
≥ 75 th percentile	5.67 (1.06) ^a	4.80 (1.03)	
Body mass index at 3 years			
< 75 th percentile	5.18 (1.03) ^a	4.52 (1.02)	< 0.001
≥ 75 th percentile	5.43 (1.06) ^b	4.79 (1.03)	
Waist circumferences at 3 years			
< 75 th percentile	5.11 (1.04) ^b	4.51 (1.02)	0.001
≥ 75 th percentile	5.40 (1.05) ^a	4.78 (1.03)	
Upper-arm circumferences at 3 years			
< 75 th percentile	5.33 (1.04) ^a	4.45 (1.02)	0.0001
≥ 75 th percentile	4.98 (1.05)	4.85 (1.03) ^b	
Skinfold thickness at 3 years			
< 75 th percentile	5.07 (1.04)	4.52 (1.02)	< 0.001
≥ 75 th percentile	5.40 (1.05) ^a	4.83 (1.03)	
Hip circumferences at 3 years			
< 75 th percentile	5.11 (1.03) ^a	4.50 (1.02)	< 0.001
≥ 75 th percentile	5.53 (1.06) ^b	4.83 (1.03)	
Catch-up			
No	5.28 (1.04) ^a	4.52 (1.02)	< 0.001
Yes	5.18 (1.05)	4.74 (1.03)	

Results represented as least squares means and standard error after adjusting for sex and life months. For multiple comparison test, Bonferroni method was used.

¹⁾ Sample size differ due to missing data (n = 119; < 75th percentile n = 88 and ≥ 75th percentile n = 31).

^a P value < 0.01 when compared with low risk group (folate ≥ 25th with anthropometric measure < 75th)

^b P value < 0.05 when compared with low risk group

Catch-up growth was defined as greater than 0.67 (equal to 75th percentile) at current weight z score if born preterm or of low birth weight and others with a change of weight z score more than 0.67, it calculated by subtracting the birth weight z score from the current weight (3 years old) z score.

folate level also showed higher level of homocysteine than normal birth outcomes with high folate level, although it was only significant in low birth weight children.

DISCUSSION

As part of the ongoing birth cohort study, this study found that homocysteine correlated with several adiposity indicators and those who have simultaneous risk of adiposity and poor folate level demonstrated elevated homocysteine level at 3 years of age.

These results are obtained from younger children than

previous studies except for one study conducted in Spain for subjects aged 2 months to 18 years old [16]. Although the association between homocysteine in the general population as a childhood antecedent of CVD is unclear, several studies reported that homocysteine in children was associated with parental CVD risk [19]. In addition, the Homocysteine Studies Collaboration analyzed results of 12 prospective and 18 retrospective studies covering 16,786 healthy adults and demonstrated a 25% increase in the serum homocysteine is associated with a 12% higher risk of ischemic heart in the retrospective studies and a 20% increase in the prospective studies [20]. In another meta-analysis on homocysteine and cardiovascular risk by Humphrey *et al.* [21] concluded that high homocysteine levels may be independently and moderately increased by about 20% for the risks for developing cardiovascular diseases.

Barker *et al.* [22] published that intrauterine malnutrition might program cardiovascular health several decades after the original insult. This has been supported by epidemiological studies demonstrating that low birth weight infants had impaired glucose tolerance, type 2 diabetes, and high blood pressure. These subjects had endothelial dysfunction and increased arterial stiffness even by the end of the first decade of life [23]. In this study, the homocysteine levels were higher in low birth weight than in normal birth weight. Although those who in the highest quartile of placenta weight showed the highest values of the homocysteine levels, those in the lowest quartile of placenta weight also showed an increasing tendency. In addition, when simultaneously considered with poor folate level, mean difference of homocysteine has become clear. To our knowledge, there was no information for association between placenta weight and offspring's homocysteine level. Alternatively, study from population based Netherlands cohort study showed that higher homocysteine level in early pregnancy was related with low placenta weight [24]. Several other studies reported that maternal obesity, higher gestational weight gain, and gestational diabetes were associated with larger placenta weight [25]. Although it was suggested that influences of the placenta may be linked with development of cardiovascular diseases in offspring, mechanisms underlying the placenta origin and disease development remain unclear [26].

Of above mentioned factors influencing placental weight, maternal obesity and gestational weight gain, gestational diabetes were associated with development of offspring obesity. In the present study, weight, BMI, waist, upper-arm circumference, skinfold thickness and hip circumference at 3 year of age were positively correlated with the homocysteine levels. In addition, the highest quartile in BMI and hip circumferences showed high level homocysteine even adjusted for blood folate levels, although the high level of homocysteine was of borderline significant.

When simultaneously controlling with preterm or low birth weight, the trend of the association was similar to the presented results of model 2 in Table 2 (data not shown). An effects of adiposity quartile groups on current homocysteine levels in BMI, upper arm circumferences and hip circumference still showed a borderline significance level, while birth outcomes were not significant. These findings suggest that those who were vulnerable to development of obesity have the risk of increased

homocysteine levels. Children who have a greater risk for obesity as defined by various anthropometric measurements along with poor folate level showed significantly high homocysteine levels. Some studies [13,27] reported the positive association between homocysteine and adiposity but not others [12,16].

Generally, homocysteine level is determined by genetic, dietary [28] or secondary diseases. Although C677T mutation for MTHFR enzyme is well known that it greatly contributes to current homocysteine level [28], variation by genetic polymorphisms is random due to the law of independent assortment of gene. Thus, it didn't seem to have great influence on these results.

Homocysteine is a modifiable factor [14] and it is controlled by folate level [29]. However, folate is involved in homocysteine metabolism and the potential role in lowering homocysteine is still much debated. Homocysteine in children is inversely related to serum folate levels [11]. If dietary changes could decrease the elevated homocysteine, an independent risk factor for cardiovascular disease, and decrease the rate of CVD as well, it would be of great interest as a preventive measure [8]. One study by Wald *et al.* suggested that a 3 $\mu\text{mol/l}$ decrease in serum homocysteine achievable with 0.8mg/day folic acid lowers the risk of myocardial infarction by 15% and stroke by 24% after adjustment for other confounding risk factors [30]. However, some reports indicate that folate does not lower the risk of recurrent CVD or reduce the risk of major cardiovascular events in preexisting vascular diseases [31]. Nonetheless, children at young age are relatively free from preexisting vascular diseases and thus may be the potential candidate for such intervention. Taken together the evidence supports a modest protective effect of folic acid supplementation to lower the homocysteine levels [30] especially in the high risk subjects starting at an early age.

This study carries a few limitations. The sample size is small, a possibility of selection bias exists due to follow up loss, and it was not allowed for generalization of results since the birth cohort is based in the tertiary hospital. Being a follow up study always carries a risk of selection bias and a further study will address these shortcomings. Our study subjects are composed higher proportion of preterm (25.6%) and low birth weight (25.2%) than national statistics. Thus, characteristics of study subjects need to be considered when interpreting results from our study. However, as this was a prospective cohort study, the data are subject to less recall bias of old measurements, and may thus elucidate a true cause-and-effect association. Despite the paucity of information about possibility of correlation of homocysteine levels between childhood and adulthood, it is necessary to track homocysteine levels from childhood to prevent further CVD risks [32]. Therefore, further study of tracking homocysteine and the change of homocysteine resulting from the change of folate level is needed to suggest the strategy of intervention. Although this study did not cover dietary factors, blood folate level was considered as a biomarker. Some studies reported that the correlation between homocysteine and folate was stronger than vitamin B12 [27,33]. In addition, because study subjects are still young, the variation by diet was relatively lower than older children or adults. Finally, this study

was conducted in general children and study subjects are young and therefore we alternatively defined as low and high level based on value of 75th percentile as adiposity status and 25th percentile as poor folate status. This approach followed a previous study [34]. In addition, it was similar with the 2007 Korean Children and Adolescents Growth Standards and growth chart recommended by WHO [18], respectively.

In summary, our data showed that homocysteine is positively associated with adiposity indicators and negatively associated with folate. In addition, combined effect of adiposity and poor folate level influences the vulnerability to CVD as demonstrated by a significant association with increased homocysteine at three years of age early in life. Therefore folate supplementation with weight control to the children with greater body mass in early childhood is speculated to decrease the homocysteine levels and modify the risk status of CVD.

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