The Effects of Breastfeeding on Serum Asymmetric Dimethylarginine Levels and Body Composition in Children

Renata Roszkowska, Katarzyna Taranta-Janusz, Edyta Tenderenda-Banasiuk, and Anna Wasilewska

Abstract

Introduction: The purpose of this work was to investigate the association of serum asymmetric dimethylarginine (ADMA) and high-sensitivity C-reactive protein (hs-CRP) levels with duration of breastfeeding and body composition in children.

Patients and Methods: The study group consisted of 88 patients with a median age of 12 months (42 boys, 46 girls), classified as never breastfed or fully breastfed. ADMA and hs-CRP were measured by immunoenzymatic enzyme-linked immunosorbent assay commercial kits. Body composition analysis was performed by bioelectrical impedance.

Results: We found significantly higher serum ADMA levels but not serum hs-CRP levels in never breastfed compared with the fully breastfed group ($p < 0.05$). Serum ADMA was inversely associated with high-density lipoprotein-cholesterol levels and breastfeeding duration ($p < 0.05$). Positive correlation was found between ADMA and body fat mass ($p < 0.05$).

Conclusions: In never breastfed children, increased ADMA is observed; however, further studies are needed to assess whether breastfeeding duration affects body fat and body composition at older ages.

Introduction

A GROWING BODY OF EVIDENCE suggests that the composition of the baby “milk formula” received during early life may have long-term effect on health in later life. Optimal nutrition during infancy is critical not only to support the dramatic growth and development that takes place during the first 12 months following birth, but also to protect against infectious illness throughout childhood and across the life span. Recent World Health Organization recommendations strongly advocate breastfeeding in infancy as not only reducing infant infections but also being associated with a protective effect against cardiovascular disease (CVD) and obesity development later in life,¹ although the evidence has been inconsistent.² Formula contains about 1.6–1.7 times higher protein content than human milk.³

Whether high protein intake in the neonatal period affects the risk of obesity later in life remains debated. Formula-fed infants gain more weight during the first year of life and may be more likely to become obese than breastfed infants.⁴ Another study reported that full-term infants fed a lower protein formula (1.25 g/dL) achieved a slightly lower body mass index (BMI) at 2 years of age, compared with those fed a higher protein formula (2.05 g/dL).⁵ In experimental studies with an animal model, high-protein feeding was associated

with increase in triglyceride and insulin levels at Day 18 of life. However, other studies did not confirm that a 30% increase in protein intake altered body weight, plasma glucose, or insulin levels in normal birth weight pups.⁶

Atherosclerosis begins during childhood. A strong relation between the prevalence and extent of the asymptomatic atherosclerosis lesions and cardiovascular risk factors such as elevation in BMI, blood pressure, and plasma lipid concentrations have been documented even in childhood.⁷ These risk factors depend not only on genetic predisposition, but also on environmental parameters, like diet. Previous studies showed that low-grade inflammation could be implicated in the development of CVD from early stages of life.⁸ Inflammatory markers are independent risk factors for coronary and vascular diseases.

The vascular endothelium has many functions, and, accordingly, endothelial dysfunction is responsible for numerous health problems, including atherosclerosis, hypertension, sepsis, thrombosis, vasculitis, and bleeding, among others. One of the most important function of the endothelium is nitric oxide secretion. Nitric oxide, an important mediator of endothelial cell function, is produced by endothelial cell nitric oxide synthase,⁹ which has been identified in neurons, endothelial cells, macrophages, and hepatocytes in various different isoforms. The nitric oxide synthase is a family of enzymes that

convert L-arginine to L-citrulline and nitric oxide. The activity of nitric oxide synthase is effectively controlled by an endogenous inhibitor. Asymmetric dimethylarginine (ADMA), a recently discussed cardiovascular risk factor, is an endogenous nitric oxide synthase inhibitor.

In the past decade, as the role of inflammation in CVD became appreciated, interest turned to high-sensitivity C-reactive protein (hs-CRP) as a possible risk marker for CVD. Since then, studies have shown that hs-CRP concentration is positively associated with CVD incidence and mortality, even when the concentration was thought to be normal.

Although previous studies suggested that ADMA as well as hs-CRP levels might correlate with cardiovascular risk or chronic kidney disease in adults, the relationship between ADMA and hs-CRP and early nutrition has not been characterized.

The goal of this study was to investigate the association of serum ADMA and hs-CRP levels with duration of breastfeeding in children and with body composition.

Patients and Methods

The protocol was approved by the Bioethics Committee of the Medical University of Białystok, Białystok, Poland in accordance with the Declaration of Helsinki.

Eighty-eight patients (42 boys, 46 girls) with a median age of 12 months (interquartile range, 7–21 months) with complete data on breastfeeding were included into the study. Patients were recruited from referrals to the Department of Pediatrics and Nephrology of the Medical University of Białystok from 2012 until 2013. The reason for hospitalization was suspicion of urinary tract defect, which was ruled out during diagnostic procedures. Information about breastfeeding initiation and continuation was obtained from questionnaires used by researchers for data collection. Questionnaire construction on breastfeeding practices included questions on whether the child was ever breastfed, at what age the child completely stopped being breastfed, when infant formula was started, the age at which the child was introduced to solid foods, and the health status of the breastfeeding mothers. Children were classified as never breastfed or fully breastfed (breastfeeding, breastmilk with water and water-based drinks). Children in the breastfeeding group were exclusively breastfed until at least 6 months of age in accordance with the World Health Organization recommendations, or shorter, if the examined child was younger than 6 months of age. Children were also divided according to age at the time of examination as ≤ 12 months and > 12 months. Infants who were partially breastfed (breastfed and formula-fed) were not included in the study. Health status of the breastfeeding mothers was unaffected during the period of breastfeeding.

Participants were required to meet the following inclusion criteria: (1) age 4–36 months, (2) no clinical and laboratory signs of infection, (3) not treated with antibiotics within the last 4 weeks, and (4) the parents signed the informed consent. The following exclusion criteria were used: chronic disease, history of CVD, metabolic syndrome, diabetes mellitus, gouty arthritis, renal or hepatic dysfunction, systemic inflammatory conditions, or autoimmune diseases, and taking any medication.

Demographic and clinical data were assessed. In all children, full clinical history, including underlying comorbidities,

and physical examination were done. Body weight and height were measured using a balance beam scale and pediatric wall-mounted stadiometer. BMI was calculated as weight (kg) divided by the square of height (m^2). BMI Z-scores, which reflect the SD score for age- and gender-appropriate BMI distribution, were calculated using the following formula: $Z = (X - \mu) / \sigma$, where X is the BMI measured for the patient, whereas μ and σ represent the mean and the standard deviation, respectively, for age- and gender-matched healthy children.¹⁰ Based on the international norms from the World Health Organization with age- (to the nearest 1 month) and gender-specific BMI, BMI cutoffs were the following: overweight, BMI $> +2$ SD; obesity, BMI $> +3$ SD.¹¹ Age- and height-specific reference values for BMI and height were generated by the least mean squares method.¹²

Body composition analysis was performed by bioelectrical impedance (BIA) using a BioScan 916 (Maltron, Rayleigh, United Kingdom) analyzer and a current with a frequency of 50 kHz. Bioimpedance analyses were performed to estimate body fat mass, fat-free mass, total body water, body cell mass, protein mass, mineral content, and muscle mass.

Collected and centrifuged blood samples were investigated by routine laboratory methods on the same day, or isolated serum aliquots were stored at -80°C until assay for determination of estimated parameters.

The routine biochemical work-up included serum creatinine (measured by updated Jaffe reaction), urea, fasting plasma glucose, lipid profile, and serum uric acid concentration.

ADMA was measured in serum using the enzyme-linked immunosorbent assay method (Immundiagnostik AG, Bensheim, Germany) according to the manufacturer's guidelines. Serum ADMA levels were expressed in $\mu\text{mol/L}$. The intra- and interassay coefficients of variance were 6.5–7.0% and 6–7%, respectively.

Serum hs-CRP was quantified by enzyme-linked immunosorbent assay using enzyme-linked immunoassay kits (LDN, Nordhorn, Germany). The hs-CRP serum values were expressed in ng/mL . The intraassay and interassay coefficients of variation for hs-CRP were 5.0–15.2% and 7.8–9.9%, respectively.

Statistical analysis

Data were analyzed with the Statistica program (version 10.0; StatSoft, Tulsa, OK), and the Kolmogorov–Smirnov test was used to determine normality of variables. Discrete variables were expressed as counts (percentage), and continuous variables were expressed as median and quartiles, unless stated otherwise. The comparison between the two groups was done using chi-squared and Fisher's exact tests for categorical variables and t test for continuous variables for normally distributed data, with Mann–Whitney or analysis of variance tests for non-normally distributed data. Correlations between estimated parameters and other variables were evaluated by Pearson's or Spearman's test, as appropriate, in both groups. A value of $p < 0.05$ was considered statistically significant.

Results

Eighty-eight children were enrolled into the study. Of these, 55% of participants (median age, 14 months) (24 boys,

TABLE 1. SOCIODEMOGRAPHIC, ANTHROPOMETRIC, AND CLINICAL CHARACTERISTICS OF THE STUDIED GROUP

	Median (interquartile range)		p
	Breastfed	Not breastfed	
Number	49	39	—
Males:females	24:25	18:21	—
Age (months)	14 (8–22)	11 (7–20)	0.47
Maternal age at child's birth (years)	29 (26–33)	28 (24–32)	0.18
Gestational age at delivery (weeks)	40 (39–40)	40 (38–40)	0.92
Birth weight (kg)	3.50 (3.10–3.87)	3.25 (2.99–3.45)	0.02
Body weight Z-score	0.58 (–0.29 to 1.11)	0.32 (–0.57 to 1.57)	0.06
Height/length Z-score	1.17 (–0.50 to 2.39)	0.86 (0.13–2.65)	0.49
Waist/hip ratio (cm)	0.95 (0.92–0.99)	0.97 (0.94–1.00)	0.07
BMI (kg/m ²)	16.22 (15.31–17.33)	15.69 (14.22–17.59)	0.36
BMI Z-score	–0.22 (–0.93 to 1.30)	–0.63 (–1.99 to 1.04)	0.23

BMI, body mass index.

25 girls) were reported by mothers to have been breastfed. The median age of those individuals who were not breastfed was 11 months (interquartile range, 7–20 months). The age and sex of breastfed children did not differ from those of never breastfed children ($p > 0.05$). Sociodemographic variables and study characteristics grouped by breastfeeding status are shown in Table 1. In breastfed children, the median duration of breastfeeding was 8 months (range, 4–24 months). Table 1 shows that all parameters were comparable between the groups, except for a lower birth weight in those who were not breastfed ($p < 0.05$).

Biochemical characteristics of participants are presented in Table 2. Children who had been exclusively breastfed had lower serum uric acid and urea than those who had never been breastfed ($p < 0.05$). Additionally, the analyses were also performed depending on participant's age (47 children ≤ 12 months age and 41 children > 12 months of age). We found significantly higher serum hemoglobin and urea levels in children ≤ 12 months old in the never breastfed group compared with the breastfed group ($p < 0.05$). Never breastfed

children > 12 months showed also higher uric acid and urea serum concentrations than in the breastfed group ($p < 0.05$). In breastfed patients > 12 months hemoglobin levels were higher in comparison with never breastfed subjects ($p < 0.05$).

According to BMI data, 18 of the 88 children (20.45%) were classified as overweight (breastfed, $n = 8$; never breastfed, $n = 10$). In the first 6 months of life more overweight and obese children were found in the breastfed than in the never breastfed group (15% vs. 10%). However, starting from the age of 12 months more overweight/obese children were found in never breastfed children compared with breastfed children (13.5% vs. 12% at 12 months and 33.3% vs. 18% at 24 months of age).

Serum concentration of ADMA and hs-CRP revealed higher serum ADMA ($p < 0.05$) but not serum hs-CRP levels in not breastfed children in comparison with breastfed participants. (Fig. 1). Similarly, when the group was stratified according to age, we found that serum ADMA but not hs-CRP levels differ between the groups ($p < 0.05$).

TABLE 2. BIOCHEMICAL CHARACTERISTICS OF THE CHILDREN EXAMINED

	Median (interquartile range)		p
	Breastfed (n = 49)	Not breastfed (n = 39)	
WBCs ($10^3/\mu\text{L}$)	8.86 (6.69–10.15)	9.20 (6.44–11.39)	0.73
RBCs ($10^6/\mu\text{L}$)	4.60 (4.29–4.78)	4.53 (4.07–4.74)	0.20
HGB (g/dL)	12.0 (11.1–12.7)	11.9 (11.2–12.5)	0.70
Platelet count ($10^3/\mu\text{L}$)	336 (284–438)	370 (287–446)	0.47
Fibrinogen (mg/dL)	242 (202–305)	287 (208.5–353)	0.43
Fasting plasma glucose (mg/dL)	85.5 (80.5–92.5)	88 (80–91)	0.92
Serum creatinine (mg/dL)	0.22 (0.20–0.27)	0.24 (0.20–0.28)	0.48
Serum uric acid (mg/dL)	3.38 (3.13–3.87)	3.66 (3.14–4.63)	0.03
Serum urea (mg/dL)	17 (12–21)	20 (15–24)	0.01
Serum cholesterol (mg/dL)	148.5 (134.5–171.5)	137 (118–168)	0.12
HDL-cholesterol (mg/dL)	41 (31–50)	37.5 (29–48)	0.56
Triglycerides (mg/dL)	88.0 (60.5–127.5)	108.5 (70–128)	0.21
hs-CRP (ng/mL)	420.90 (100.60–920.08)	197.06 (102.55–1796.99)	0.80
ADMA ($\mu\text{mol/L}$)	0.749 (0.658–0.805)	0.825 (0.722–0.958)	0.01

ADMA, asymmetric dimethylarginine; HDL, high-density lipoprotein; HGB, hemoglobin; hs-CRP, high-sensitivity C-reactive protein; RBC, red blood cell; WBC, white blood cell.

FIG. 1. Comparison of (A) asymmetric dimethylarginine (ADMA) and (B) high-sensitivity C-reactive protein (hs-CRP) levels depending on age and feeding status of infants. ANOVA, analysis of variance; BF, breastfed; NBD, never breastfed.

BIA data (Table 3) showed a significant difference in fat-free mass (%) and body fat (%) among the two categories of assessed children ($p < 0.05$). Comparison of evaluated BIA parameters in children divided according to age presented with significantly increased body fat (%) in never breastfed children > 12 months old ($p < 0.05$).

Serum ADMA (Table 4) was inversely associated with high-density lipoprotein-cholesterol levels and breastfeeding duration in studied children ($p < 0.05$). Positive correlation was found between ADMA and body fat mass ($p < 0.05$). No correlation between serum ADMA and hs-CRP was found. The factors that were found to have a significant correlation with serum ADMA in the single regression analyses were used as explanatory variables to create the multiple regression models. In the model (Table 5), two parameters (body fat mass and breastfeeding duration) accounted for more than 23.1% of the variation in the serum ADMA level ($p < 0.05$).

Discussion

The current study showed that baseline characteristics, such as age, BMI, and sociodemographic status, were similar in both groups examined. Infants in the formula-fed group had significantly lower birth weight than those breastfed. The results of this cross-sectional study have also demonstrated elevated ADMA levels in never breastfed children. Another important finding is that we have not found a significant increase in serum hs-CRP between breastfed participants and those never breastfed. We also confirmed negative correlation of serum ADMA with high-density lipoprotein-cholesterol and breastfeeding duration in the examined groups. It is interesting to note that we created a multivariable linear regression model and found that body fat and breastfeeding duration accounted for more than 23% of the variation in serum ADMA.

To our best knowledge this is the first clinical study on the association of serum ADMA and hs-CRP levels with duration of breastfeeding in children and with body composition.

Since the early studies in the 1960s¹³ it has been suggested that breastfed infants had a lower risk of CVD, obesity, high cholesterol concentration, type 2 diabetes, and hypertension. The concept that nutrition in infancy may have a long-term effect on risk factors for CVD first emerged in the pioneer work of McCance.¹⁴ He showed that rats raised in small litters, and therefore overfed early in postnatal life, were programmed for greater body size as adults.

TABLE 3. BIOELECTRICAL IMPEDANCE ANALYSES IN THE STUDY POPULATION

	Median (interquartile range)		p
	Breastfed	Not breastfed	
Fat-free mass (%)	94.18 (90.60–96.97)	87.70 (81.64–92.92)	0.03
Fat-free mass (kg)	10.71 (9.19–11.93)	9.31 (8.09–11.33)	0.26
Body fat mass (%)	5.93 (3.03–9.40)	12.74 (7.08–20.19)	0.007
Body fat (kg)	0.56 (0.36–1.23)	1.23 (0.66–2.31)	0.05
Total body water (L)	7.90 (6.87–9.91)	8.01 (6.48–9.06)	0.68
Body cell mass (kg)	4.34 (3.67–5.25)	4.28 (3.54–5.08)	0.60
Protein mass (kg)	1.18 (0.93–1.45)	1.23 (1.02–1.79)	0.21
Mineral content (kg)	0.44 (0.35–0.50)	0.47 (0.41–0.67)	0.11
Muscle mass (kg)	3.11 (2.59–3.86)	3.09 (2.62–3.52)	0.75

TABLE 4. STATISTICALLY SIGNIFICANT CORRELATIONS (BY SPEARMAN CORRELATION ANALYSIS) WITH ASYMMETRIC DIMETHYLARGININE IN ALL CHILDREN EXAMINED

	r	P
HDL-cholesterol	−0.24	0.02
Body fat (kg)	0.29	0.04
Body fat (%)	0.33	0.02
Breastfeeding duration	−0.28	0.008

HDL, high-density lipoprotein.

Breastfeeding, in particular, appears to protect against the development of later obesity. The mechanisms involved, are poorly understood. Singhal et al.¹⁵ suggested that the previously reported association between birth weight and later CVD might be related to rapid growth in the first weeks after birth and have coined this association as the “growth acceleration hypothesis.” This hypothesis was supported by data from animal studies, which suggested the first few postnatal weeks as a critical window for programming long-term health in both humans and animals.¹⁶ Animal studies have shed light on the mechanisms that link early growth and nutrition with long-term obesity risk. Of particular interest is the programming of appetite, which could contribute to obesity.

Central to the hypothesis that relative undernutrition associated with breastfeeding protects against later obesity is evidence that breastfed infants grow more slowly than those that are formula-fed.¹⁷ In our study we did not find any differences in early weight gain and BMI between both groups. Formula-fed children had lower birth weights than breastfed children. Lower birth weight is connected with reduced number of nephrons. This phenomenon can lead to increased ADMA levels because ADMA is metabolized to citrulline by the specific enzyme dimethylarginine–dimethylaminohydrolase, present mainly in the liver and kidneys.¹⁸ However, there was no relationship between estimated markers and birth weight, which is not surprising given that all our participants were born at term and were of normal birth weight.

It is noteworthy that in our study higher rates of overweight and obesity have been found among never breastfed children > 12 months of age. In our study formula-fed infants gained more weight during the first 2 years of life and may be more likely to become obese than breastfed infants. These findings are in parallel to the findings of the study by Butte et al.,¹⁹ which was performed in normal birth weight babies. The authors reported higher growth rates in early infancy among formula-fed compared with breastfed infants.

TABLE 5. MULTIPLE LINEAR REGRESSION ANALYSIS OF THE SERUM ASYMMETRIC DIMETHYLARGININE LEVELS

Variable	B	SE B	P
HDL	−0.06	0.15	0.67
Body fat (%)	0.32	0.15	0.04
Breastfeeding duration (months)	−0.24	0.16	0.014

HDL, high-density lipoprotein; SE, standard error.

Although several studies have reported a protective effect of breastfeeding on blood pressure,²⁰ others have shown no significant association between breastfeeding and adult blood pressure.²¹ We did not estimate blood pressure in our study as we have limited power to detect reliable values of blood pressure in such a young children.

Some studies suggested that longer duration of breastfeeding is associated with a lower BMI. BMI provides only information about body weight, whereas it does not distinguish between fat and lean mass. Because an unfavorable fat distribution may be related more strongly to CVD and metabolic diseases, it is important to explore the associations of breastfeeding with measures of fat distribution. Only a few studies have examined the relationships between breastfeeding in infancy and direct measures of adiposity in childhood.²² A study among adult males from Brazil did not show any association between breastfeeding and adult body fat, whereas a large British study reported a protective effect of breastfeeding duration on mean body fat measured with X-ray absorptiometry in children 9–10 years of age.²³ In contrast, two studies assessing the link between breastfeeding and direct measures of body composition at ages of 2–5 years did not show any association.²²

We used BIA as a measure of body composition. Our data showed higher body fat at an age of > 12 months in never breastfed children and are in line with previous studies showing in children who were not exclusively breastfed higher central fat mass at the age of 24 months.²⁴ However, our current report revealed no association between breastfeeding duration and body composition during the first 2 years of life. Our findings are partially consistent with Durmus et al.,²⁴ who reported that shorter breastfeeding was associated with higher fat mass at the age of 6 months, but not at the age of 24 months.

Nutrition in childhood has direct effects on vascular biology associated with the early atherosclerotic process. Atherosclerosis is generally accepted as an inflammatory disease. Several markers of inflammation and atherosclerosis have been shown to be increased, such as interleukin-6, tumor necrosis factor- α , monocyte chemotactic protein-1, hs-CRP, and ADMA.

ADMA is an endogenous modulator of endothelial function and oxidative stress, and increased levels of this molecule have been reported in metabolic disorders and CVD. Very little was found in the literature on role of ADMA in patients with metabolic syndrome. Palomo et al.²⁵ found that ADMA levels were significantly increased in metabolic syndrome; however, the levels of ADMA were modestly correlated only with waist circumference but not with the other components of metabolic syndrome. We found higher ADMA levels in never breastfed individuals. There was also a negative correlation between serum ADMA concentrations and breastfeeding duration ($p < 0.05$). This may suggest a clear relationship between ADMA levels and endothelial dysfunction. However, a raised level of ADMA alone is insufficient, as some participants are particularly susceptible to the effects of raised ADMA, and they are at most risk of developing complications. Findings of our study suggest that the infant feeding method affects an important marker of atherosclerosis and CVD later in life.

Data from the current study revealed higher serum uric acid and urea concentrations in never breastfed children. This

result may be explained by the fact that formula-fed infants get more protein than breastfed infants.

In our study serum ADMA was inversely associated with high-density lipoprotein-cholesterol levels ($p < 0.05$). Previous studies have reported that high-density lipoprotein-cholesterol can exert biological effects on endothelial cells via stimulation of endothelial nitric oxide synthase, which mediates nitric oxide production and vasodilation.²⁶ Positive correlation was found between ADMA and body fat. A possible explanation for this might be the fact that obesity is connected with increased inflammatory activity in adipocytes, especially increased synthesis of tumor necrosis factor- α , which has been shown to stimulate the expression of ADMA in endothelial cells.²⁷ Similarly, Kanazawa et al.²⁸ found a significant positive association between ADMA and BMI, blood pressure, low-density lipoprotein, high-density lipoprotein, and total cholesterol independently of age. In obese women studied by Krzyzanowska et al.,²⁹ ADMA correlated with hs-CRP at baseline and after weight loss, but no association with blood pressure or plasma lipids was observed. Our research did not show any correlation between serum ADMA and hs-CRP.

Associations among inflammation, metabolic syndrome, and CVD have been reported. A clinical marker of inflammation is hs-CRP. In the present study hs-CRP levels in infants who had been breastfed did not differ from those who had never been breastfed. Similarly, Martin et al.¹ did not find any relationship between breastfeeding and inflammation. Our results contrast with two previous reports suggesting an influence of breastfeeding on inflammatory status. Williams et al.³⁰ found a significant inverse linear correlation between the duration of breastfeeding in infancy and the C-reactive protein levels in adulthood. Rudnicka et al.³¹ found that any breastfeeding for at least 1 month was associated with lower C-reactive protein. As both studies were performed in adult women, we can hypothesize that infancy and childhood are too early to detect benefit of breastfeeding. There are several possible explanations for the absence of an association between breastfeeding and inflammatory markers. Breastfeeding might influence low-grade inflammation only in people who are genetically predisposed to CVD. Also, breastfeeding might affect inflammatory markers only in subgroups of the population who have more pronounced low-grade inflammatory status.³² Another hypothesis is that infancy is too early to detect a significant benefit of breastfeeding on cardiovascular risk.³³

Further research with a prospective longitudinal design, in a larger sample, may be needed to draw definite conclusions. Our new analysis, together with published literature, does not provide strong evidence that breastfeeding is related to risk of CVD.

This study has certain limitations, including being a single-center, cross-sectional study with a relatively small cohort. We could not assess all possible confounding factors, such as parental weight, food preference, and smoking habits, and their potential effects cannot be excluded completely. Another limitation was that our study participants are of white European ancestry; therefore, these findings may not be generalizable to other ethnic populations.

In conclusion, increased circulating ADMA in never breastfed children might be linked to some risk of CVD in later life; however, our study provides no support for a cardioprotective effect of breastfeeding into later life.

Our results suggest that a shorter duration of breastfeeding, as well as never breastfeeding, affects early body composition during the first 2 years of life. Follow-up studies are needed to assess whether breastfeeding duration affects body fat and other measures of body composition at older ages.

The idea that the infant feeding method influences CVD risk seem to be very attractive, possibly because of the potential to identify novel preventive interventions that could be started early in life. This observation is in accordance with other studies examining the association of breastfeeding duration with CVD risk factors.

Acknowledgments

This work was supported by a grant (134-41723L) from the Medical University of Białystok, Poland.

Disclosure Statement

No competing financial interests exist.

References

1. Martin RM, Ben-Shlomo Y, Gunnell D, et al. Breast feeding and cardiovascular disease risk factors, incidence, and mortality: The Caerphilly study. *J Epidemiol Community Health* 2005;59:121–129.
2. Fall CH, Borja JB, Osmond C, et al. Infant-feeding patterns and cardiovascular risk factors in young adulthood: Data from five cohorts in low- and middle-income countries. *Int J Epidemiol* 2011;40:47–62.
3. Socha J, Socha P, Weker H, et al. Nutrition of children and health: Yesterday, today and tomorrow. *Pediatr Współcz Gastroenterol Hepatol Żywnienie Dziecka* 2010;12:34–37.
4. von Kries R, Koletzko B, Sauerwald T, et al. Breast feeding and obesity: Cross sectional study. *BMJ* 1999;17:147–150.
5. Koletzko B, von Kries R, Closa R, et al. Lower protein in infant formula is associated with lower weight up to age 2 y: A randomized clinical trial. *Am J Clin Nutr* 2009;89:1836–1845.
6. des Robert C, Li N, Caicedo R, et al. Metabolic effects of different protein intakes after short term undernutrition in artificially reared infant rats. *Early Hum Dev* 2009;85:41–49.
7. Girardet JP, Rieu D, Bocquet A, et al. Childhood diet and cardiovascular risk factors. *Arch Pediatr* 2010;17:51–59.
8. Sahin G, Akay OM, Bal C, et al. The effect of calcineurin inhibitors on endothelial and platelet function in renal transplant patients. *Clin Nephrol* 2011;76:218–225.
9. Aldámiz-Echevarría L, Andrade F. Asymmetric dimethylarginine, endothelial dysfunction and renal disease. *Int J Mol Sci* 2012;13:11288–11311.
10. Kulaga Z, Litwin M, Tkaczyk M, et al. The height-, weight-, and BMI-for-age of Polish school-aged children and adolescents relative to international and local growth references. *BMC Public Health* 2010;10:109.
11. De Onis M, Lobstein T. Defining obesity risk status in the general childhood population: Which cut-offs should we use? *Int J Pediatr Obes* 2010;5:458–460.
12. WHO Multicentre Growth Reference Study Group. *WHO Child Growth Standards: Length/Height-for-Age, Weight-for-Age, Weight-for-Length, Weight-for-Height and Body Mass Index-for-Age: Methods and Development*. World Health Organization, Geneva, 2006.
13. Osborne J. Health in adolescence. *Practitioner* 1963;191:292–298.

14. McCance RA. Food, growth and time. *Lancet* 1962;2:671–676.
15. Singhal A, Cole TJ, Fewtrell M, et al. Is slower early growth beneficial for long-term cardiovascular health? *Circulation* 2004;109:1108–1113.
16. Ozanne SE, Hales CN. Lifespan: Catch-up growth and obesity in male mice. *Nature* 2004;427:411–412.
17. Kramer MS, Guo T, Platt RW, et al. Feeding effects on growth during infancy. *J Pediatr* 2004;145:600–605.
18. Bassareo PP, Fanos V, Puddu M, et al. Advanced intra-uterine growth restriction is associated with reduced excretion of asymmetric dimethylarginine. *Early Hum Dev* 2014;90:173–176.
19. Butte NF, Wong WW, Hopkinson JM, et al. Infant feeding mode affects early growth and body composition. *Pediatrics* 2000;106:1355–1366.
20. Singhal A. The early origins of atherosclerosis. In: Kozletko B, Decsi T, Molnar D, et al., eds. *Early nutrition programming and health outcomes in later life: Obesity and beyond*. Springer, New York, 2009, pp. 51–58.
21. Wells JC, Fewtrell MS. Measuring body composition. *Arch Dis Child* 2006;91:612–617.
22. Burdette HL, Whitaker RC, Hall WC et al. Breastfeeding, introduction of complementary foods, and adiposity at 5 y of age. *Am J Clin Nutr* 2006;83:550–558.
23. Toschke AM, Martin RM, von Kries R, et al. Infant feeding method and obesity: Body mass index and dual-energy X-ray absorptiometry measurements at 9–10 y of age from the Avon Longitudinal Study of Parents and Children (ALSPAC). *Am J Clin Nutr* 2007;85:1578–1585.
24. Durmuş B, Ay L, Duijts L, et al. Infant diet and subcutaneous fat mass in early childhood: The Generation R Study. *Eur J Clin Nutr* 2012;66:253–260.
25. Palomo I, Contreras A, Alarcón LM, et al. Elevated concentration of asymmetric dimethylarginine (ADMA) in individuals with metabolic syndrome. *Nitric Oxide* 2011;24:224–228.
26. Lorin J, Guillard JC, Korandji C, et al. High levels of asymmetric dimethylarginine are strongly associated with low HDL in patients with acute myocardial infarction. *PLoS One* 2013;8:e64796.
27. Eid HMA, Arnesen H, Hjerkin EM, et al. Relationship between obesity, smoking, and the endogenous nitric oxide synthase inhibitor, asymmetric dimethylarginine. *Metabolism* 2004;53:1574–1579.
28. Kanazawa I, Yano S, Notsu Y, et al. Asymmetric dimethylarginine as a risk factor for cardiovascular disease in Japanese patients with type 2 diabetes mellitus. *Clin Endocrinol (Oxf)* 2011;74:467–472.
29. Krzyzanowska K, Mittermayer F, Kopp HP, et al. Weight loss reduces circulating asymmetrical dimethylarginine concentrations in morbidly obese women. *J Clin Endocrinol Metab* 2004;89:6277–6281.
30. Williams MJ, Williams SM, Poulton R. Breast feeding is related to C reactive protein concentration in adult women. *J Epidemiol Community Health* 2006;60:146–148.
31. Rudnicka AR, Owen CG, Strachan DP. The effect of breastfeeding on cardiorespiratory risk factors in adult life. *Pediatrics* 2007;119:e1107–e1115.
32. Najjar RA, Ghaderian SMH, Panah AST. C-reactive protein (CRP) gene polymorphisms: Implication in CRP plasma levels and susceptibility to acute myocardial infarction. *Mol Biol Rep* 2012;39:3705–3712.
33. Wener MH, Daum PR, McQuillan GM. The influence of age, sex, and race on the upper reference limit of serum C-reactive protein concentration. *J Rheumatol* 2000;27:2351–2359.

Address correspondence to:
Katarzyna Taranta-Janusz, MD
Department of Pediatrics and Nephrology
Medical University of Białystok
Waszyngtona 17
15-274 Białystok, Poland

E-mail: katarzyna.taranta@wp.pl