Exclusive breast-feeding can improve cardiovascular risks and anti-inflammatory state in obese adolescents

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Objective: To assess the inflammatory state and carotid artery intima-media thickness in obese adolescent study participants and according to the duration of time the mothers of the participants breast-fed them exclusively [breast-milk only] during infancy.

Methods: This was a cross-sectional and retrospective study with 118 obese adolescent participants. Data regarding the duration of exclusive breast-feeding (EBF) were obtained through interviews with the participants’ parents. Anthropometric profile (measurements of body size, structure, and composition) and blood pressure data were obtained. Participants’ serum glucose, insulin, total and LDL-cholesterol, adiponectin, and leptin concentrations were measured. Carotid intima-media thickness (cIMT) was estimated by ultrasonography, and insulin resistance was assessed by HOMA-IR.

Results: Adiponectin level was higher in participants who had received EBF for 6 months than that in participants who had never received EBF. Conversely, cIMT was significantly lower in participants who had received EBF for 6 months. Body mass, BMI, fat mass (kg), waist circumference (WC), insulin levels, and HOMA-IR were significantly lower in participants who had received EBF than that in those who never received EBF. Insulin and HOMA-IR levels were associated with body mass, BMI, WC, and fat mass. Leptin concentration was inversely associated with adiponectin concentration and positively associated with fat mass and BMI.

Conclusion: Obese adolescents who had been exclusively breastfed for 6 months as infants were found to show improvements in anthropometric parameters, metabolic profile, and anti-inflammatory state, which may contribute in avoiding development of early atherosclerosis.

Key words: Breast-feeding, Adiponectin, Leptin, Carotid Artery Disease, Adolescents, and Obesity
Introduction

Obesity is increasing worldwide, and the proportion of overweight Brazilian people increased from 23.6% to 49.05% in the last decades (1). The proportion of Brazilian children categorized as ‘overweight’ reached 34.8% in boys and 32.0% in girls aged 5 to 9 years. In both adolescent boys and girls, the prevalence was around 20% (2). The increase in the prevalence of overweight and obesity in childhood is alarming, because it is closely associated with increased risk of chronic diseases, such as insulin resistance, dyslipidemia, and hypertension, as well as cardiovascular risks (3-4).

Adipose tissue secretes several adipokines, including leptin, which have pro-inflammatory and pro-atherosclerotic properties when their concentrations in human plasma are elevated (5).

On the other hand, obese individuals have reduced serum levels of adiponectin, which has anti-inflammatory and anti-atherosclerotic properties and is involved in the insulin sensitivity signaling pathway. This imbalance in anti/pro-inflammatory adipokine levels characterizes obesity as an inflammatory and chronic disease related to several comorbidities and cardiovascular risks (5).

Cardiovascular diseases are considered the primary cause of death, and it is estimated that, in 2025, around 80 and 90% of these deaths will occur in low- and middle-income countries, respectively (6). An increase in the carotid intima-media thickness (cIMT) is considered the first subclinical sign of atherosclerosis, and studies have demonstrated an increase in the cIMT as a result of deregulation in metabolic profile and pro/anti-inflammatory adipokines in obese adolescents (7-8).

In early life, the influence of nutrition on presence of chronic disease later in life is termed as metabolic programming. Metabolic programming is a biological phenomenon in which early nutrition can alter the human metabolism and physiological development permanently: sub-optimal nutrition at the start of life can lead to chronic disease development throughout life. This theory was first proposed by Barker et al (9), and it has been confirmed by reviews and meta-analyses, which also demonstrate the protective influence of breastfeeding on risk factors associated with obesity (10-12).

Breast-feeding also has many short-term health benefits for children, such as reduction in precarious mortality, respiratory and gastrointestinal tract infections, otitis media, necrotizing enterocolitis, allergies, and celiac disease (11). Breast-feeding may also have long-term health benefits by reducing weight gain and metabolic syndrome parameters, such as high blood pressure, blood cholesterol, and diabetes (10). Children who receive exclusive breast-feeding (EBF) have lower rate of weight gain in the first month of age than those fed exclusively with infant milk formula (13). A systematic review demonstrated that solid food introduction before 4 months of age can increase the risk of overweight in infancy (14).

Although the protective role of breast-feeding in child growth, health, and development is clearly defined (10-11, 15), its mechanisms and influence on pro/anti-inflammatory adipokine levels and the atherosclerotic process needs to be further explored in the literature (16). The present study aimed to assess the inflammatory profile and carotid intima-media thickness in obese adolescents according to the duration of EBF that participants (118 obese adolescents) had received.

Methods

This was a cross-sectional study, with 118 obese adolescent participants (49 girls, 69 boys). The participants for the present study had participated in the Obesity Study Group - GEO from Universidade Federal de São Paulo, São Paulo-SP, Brazil. The data were collected between 2006 and 2010.

This study complied with the principles of the Helsinki Declaration and was formally approved by the Institutional Ethical Committee from Universidade Federal de São Paulo (0045/12HE). All participants and/or their parents signed the informed consent form. All participants met the inclusion criteria of post-pubertal Tanner Stage V and had a body mass index (BMI) > 95th percentile, according to the criteria defined by the Centers for Disease Control.

Anthropometric measurements and body composition

Measurements of body mass (kg), height (m), and waist circumference (WC) (cm) were obtained with participants wearing light clothing and barefoot, according to standard procedures. Body mass index (kg/m2) was calculated. Body composition was determined using air displacement plethysmography (BOD POD® body composition system). This is a quick and easy way to determine the body composition that uses the inverse relation between pressure (p) and volume (v), based on Boyle’s law (P1V1 = P2V2) to determine the body volume. These procedures were already previously validated in the literature (17).

Serum analysis

Blood samples were collected by venipuncture in the forearm after a 12-hour overnight fast. Concentrations of glucose, insulin, total cholesterol, and low-density lipoprotein-cholesterol (LDL-c) were determined by enzymatic colorimetric methods (CELM, Barueri, Brasil). Leptin and adiponectin levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit from R&D Systems (Minneapolis, MN, USA), according to the manufacturer’s instructions. Leptin data were analyzed according to reference values described by Gutin et al (18). Insulin resistance was assessed by homeostasis model assessment of insulin resistance (HOMA-IR = [fasting insulin (µU/mL) x fasting blood glucose (mmol/mL)/22.5] (19).
Blood pressure measurement

Blood pressure was measured using an auscultatory sphygmomanometer by a medical professional who was unaware of any breast-feeding information. The participant remained seated for at least 5 minutes, and the pressure was measured in the right arm. Systolic blood pressure (SBP) was determined on auscultation of the first sound (Korotkoff phase I) and diastolic blood pressure (DBP) was determined by the disappearance of sound (Korotkoff phase V).

Measurements of carotid artery intima-media thickness

Carotid artery IMT was measured using high-resolution ultrasound equipment (logic 5 and logic 7, GE, SP, Brazil) with a 7-14 MHz linear array transducer, by an experienced radiologist who was unaware of the medical and biochemical statuses of the participants. Participants were examined in the supine position with the neck in hyperextension. The protocol involved three repeated measurements of the right and left common carotid far wall at 2 cm proximal to the bulb bifurcation, as previously described (7). The mean of the highest measures of each side represented the cIMT in this study.

Breast-feeding information

Parents of the participants were interviewed using a structured questionnaire. We collected information regarding the past history, whether or not the participant had been breast-fed exclusively, and, if so, duration of EBF. This method has been previously described in the literature (20).

In this study, the participants were divided in three groups according to the duration of EBF: those who never received exclusive breast-feeding, those who were breast-fed exclusively for 1 to 5 months, and those who were breast-fed exclusively for 6 months. According to the World Health Organization (WHO) defines EBF as when the infant receives only breast milk. No other liquids or solids are given – not even water – with the exception when the infant receives only breast milk. No other liquids

Statistical analysis

The Gaussian distribution of variables was verified with a Kolmogorov Smirnov test and homogeneity, by a Levene test. Variables with normal distribution were expressed as mean ± standard deviation (SD), while variables without normal distribution were expressed as median (minimum - maximum) in a descriptive table. For comparing groups, one-way analysis of variance (ANOVA) followed by a Tukey post-hoc test were used for parametric variables. A Kruskal Wallis test followed by Mann-Whitney test was used for non-parametric variables. Pearson’s and Spearman correlation coefficients were calculated to assess possible relationships between normally and non-normally distributed variables, respectively. Simple regression analysis was applied. The significance level was 5%.

Results

Comparison between groups

Participants who had received EBF for 6 months had significantly lower body mass, BMI, fat mass (kg), and WC than participants who had never received EBF. In addition, participants who had received EBF for 1-5 months had significantly lower BMI and systolic blood pressure than those who had never received EBF (Table 1).

The participants who had never received EBF had significantly lower serum values of adiponectin than all those who did. In addition, the participants who received EBF for 6 months had the lowest cIMT. Leptin, glucose, and total and LDL-cholesterol levels did not differ between groups (Table 1, Figure 1).

Correlation between variables

Body mass was positively correlated with insulin (r=0.324, p<0.001), HOMA-IR (r=0.341, p<0.001), and systolic (r=0.359, p<0.001) and diastolic blood pressure (r=0.275, p=0.003). BMI was also positively correlated with leptin levels (r=0.271, p=0.011), insulin levels (r=0.315, p=0.001), HOMA-IR (r=0.328, p=0.000), and systolic (r=0.372, p=0.000) and diastolic blood pressure (r=0.233, p=0.011). Fat mass (kg) also correlated with insulin levels (r=0.306, p=0.001), HOMA-IR (r=0.335, p=0.000), and leptin levels (r=0.380, p=0.000). Waist circumference correlated with insulin levels (r=0.395, p<0.001), HOMA-IR (r=0.400, p<0.001), and systolic (r=0.266, p<0.004) and diastolic blood pressure (r=0.314, p<0.001). Leptin levels were negatively correlated with adiponectin levels (r=-0.235, p=0.038).

In order to confirm these results, simple linear regression analyses were performed. We verified that insulin and HOMA-IR were predicted by body mass, BMI, fat mass, and waist circumference. On the other hand, systolic and diastolic blood pressures were predicted by body mass, fat mass, and waist circumference. In addition, leptin was predicted by fat mass, BMI, and adiponectin (Table 2).

Discussion

Several mechanisms have been proposed to explain the association between breast-feeding and its protective effect against obesity, including differences in the composition of human milk versus formula, self-regulation, and feeding practices by the infant (10). Additionally, human milk contains essential fatty acids, hormones, growth factors, immune-related components, enzymes, and other bioactive factors, which could modulate growth factors, inhibiting the differentiation of adipocytes (22).

Human milk contains adipokines, such as adiponectin, which play an anti-inflammatory role, influence infants feeding behavior, and regulate growth and appetite later in life (26). The concentration of adiponectin in breast milk decreases during lactation by approximately 5-6% each month. This suggests that breast-feeding in the first months of life is important so that adiponectin in the breast milk is beneficial (27). Therefore, one of the most important findings in the present investigation is that the group of participants who never received EBF had significantly lower serum concentrations of adiponectin than those who did (Figure 1). A low concentration of adiponectin is related to cardiometabolic risk factors in childhood (28), and can be involved in an increase in leptin concentrations (29).

Leptin is an adipokine expressed by adipose tissue and it is involved in neuroendocrine control of energy balance. However, hyperleptinemia occurs in obese individuals, which can deregulate neuropeptides levels and make weight loss difficult (30). We also observed significant and positive associations between leptin concentration and body mass and BMI (Table 2). Although leptin concentration did not differ between groups, it is important to highlight that participants who had never received EBF were found to have a more intense hyperleptinemic state (Figure 1). This state may impair the attenuation of inflammation, particularly by impeding the increase in adiponectin concentration that is directly involved in vascular protection (29). The negative association between leptin and adiponectin was confirmed in the current study by a simple regression analysis (Table 2).

Table 1. Anthropometric and metabolic profile of obese adolescents according to the duration of exclusive breastfeeding.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Never EBF n=18</th>
<th>1 to 5 months n=60</th>
<th>6 months n=40</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Age (years)</td>
<td>15.50±1.25</td>
<td>16.15±1.57</td>
<td>16.20±1.49</td>
<td>0.219</td>
</tr>
<tr>
<td>*Body mass (kg)</td>
<td>111.82±20.99a</td>
<td>103.41±14.87ab</td>
<td>98.95±16.67b</td>
<td>0.025</td>
</tr>
<tr>
<td>*BMI (kg/m²)</td>
<td>39.33±5.86a</td>
<td>36.35±6.44b</td>
<td>35.73±6.09b</td>
<td>0.023</td>
</tr>
<tr>
<td>*Body fat (kg)</td>
<td>52.48±12.60a</td>
<td>46.62±10.77ab</td>
<td>44.27±10.30b</td>
<td>0.043</td>
</tr>
<tr>
<td>*Fat - free mass (kg)</td>
<td>59.81±9.21</td>
<td>57.07±9.19</td>
<td>53.83±10.28</td>
<td>0.069</td>
</tr>
<tr>
<td>*Wrist circumference (cm)</td>
<td>108.57±9.47a</td>
<td>103.81±10.43ab</td>
<td>101.87±8.72b</td>
<td>0.046</td>
</tr>
<tr>
<td>*Glucose (mg/dL)</td>
<td>92.5±7.16</td>
<td>90.03±6.22</td>
<td>90.50±7.76</td>
<td>0.416</td>
</tr>
<tr>
<td>*Insulin (μU/mL)</td>
<td>20.80±6.85a</td>
<td>18.47±8.98ab</td>
<td>15.69±4.19b</td>
<td>0.047</td>
</tr>
<tr>
<td>#HOMA-IR</td>
<td>4.74 (2.66-8.86)a</td>
<td>3.82 (1.64-13.10)ab</td>
<td>3.57 (1.51-5.71)b</td>
<td>0.032</td>
</tr>
<tr>
<td>#Median (minimum-maximum)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#Total cholesterol (mg/dL)</td>
<td>168.78±38.27</td>
<td>165.88±36.02</td>
<td>161.10±31.03</td>
<td>0.269</td>
</tr>
<tr>
<td>#LDL-cholesterol (mg/dL)</td>
<td>101.66±28.94</td>
<td>99.29±30.65</td>
<td>95.55±28.14</td>
<td>0.725</td>
</tr>
<tr>
<td>#SBP (mmHg)</td>
<td>125 (110-150)a</td>
<td>120 (100-160)b</td>
<td>120 (100-150)ab</td>
<td>0.017</td>
</tr>
<tr>
<td>#DBP (mmHg)</td>
<td>80 (60-100)</td>
<td>80 (60-95)</td>
<td>80 (60-90)</td>
<td>0.355</td>
</tr>
</tbody>
</table>

EBF: Exclusive breastfeeding;
#Median (minimum-maximum) – Kruskal Wallis Test and Mann-Whitney Test;
*Mean±standard deviation – ANOVA and Tukey post hoc test.
a,b Means and median followed by the same letter do not differ at 5% probability

Table 2. Simple regression analysis.

<table>
<thead>
<tr>
<th></th>
<th>βUnadjusted</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>0.400</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.328</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>0.330</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.395</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>0.377</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.290</td>
<td>0.002</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>0.287</td>
<td>0.003</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.351</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic Blood Pressure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>0.365</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.352</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.277</td>
<td>0.003</td>
</tr>
<tr>
<td>Diastolic Blood Pressure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>0.229</td>
<td>0.013</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.230</td>
<td>0.012</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.299</td>
<td>0.001</td>
</tr>
<tr>
<td>Leptin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>0.390</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.290</td>
<td>0.006</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>-0.233</td>
<td>0.040</td>
</tr>
</tbody>
</table>

Participants in our study who received EBF had a lower body mass, BMI, fat mass (kg) and waist circumference than participants who had never received EBF in infancy (Table 1). Furthermore, body mass, BMI, fat mass, and waist circumference were positively associated with insulin levels and insulin resistance (Table 2), which have been reported to be positively correlated with cardiovascular risk factors in obese adolescents (7, 23-24). Flint et al (2010) demonstrated, in a large cohort of adults, that BMI and WC strongly predicted future risk of coronary heart disease (25). These results suggest a possible relation between EBF and anthropometric profile control later in life.

Human milk contains adipokines, such as adiponectin, which play an anti-inflammatory role, influence infants feeding behavior, and regulate growth and appetite later in life (26). The concentration of adiponectin in breast milk decreases during lactation by approximately 5-6% each month. This suggests that breast-feeding in the first months of life is important so that adiponectin in the breast milk is beneficial (27). Therefore, one of the most important findings in the present investigation is that the group of participants who never received EBF had significantly lower serum concentrations of adiponectin than those who did (Figure 1). A low concentration of adiponectin is related to cardiometabolic risk factors in childhood (28), and can be involved in an increase in leptin concentrations (29).
Furthermore, the prevalence of overweight/obesity and type 2 diabetes was lower among breastfed subjects. Altogether, these alterations can contribute to endothelial dysfunction, increasing the cIMT (10, 31).

Moreover, HOMA-IR is considered an independent predictor of cIMT in obese adolescents (23), and BMI and WC are also positively associated with elevated values of cIMT (32). In the current study, BMI, WC, and HOMA-IR were significantly higher in participants who never received EBF than in those who received EBF for 6 months (Table 1); this can favor inflammation and deregulation in the metabolic profile. In addition, previous findings demonstrated that lower concentration of adiponectin/leptin ratio can contribute to higher values of cIMT in obese adolescents (33).

Another possible mechanism underlying the association between EBF and cIMT is the composition of breast milk, which contains polyunsaturated fatty acids. Long-chain polyunsaturated fatty acids are present in breast milk, but not in most brands of formula, and these substances are important structural components of tissue membrane systems, including the vascular endothelium (10, 16, 31).

In the present study, insulin concentration was lower in participants who had received EBF. However, the difference was statistically significant only when participants who had never received EBF were compared with participants who had received EBF for 6 months (Table 1). This suggests that formula-fed infants have higher basal and postprandial concentrations of insulin. This hormone has an important anabolic role and is involved in the mechanism of fat deposition. The higher insulin concentration may lead to higher fat mass, and earlier development of insulin resistance and type 2 diabetes. Moreover, differences in protein intake and energy metabolism during infancy may be one of the biological mechanisms linking early eating habits to later obesity and its comorbidities (10).

Furthermore, body mass, BMI, and WC were related to higher values of systolic and diastolic blood pressure (Table 2), leading to cardiovascular risk factors associated with metabolic syndrome. Additionally, Kudo et al (2013) demonstrated a positive correlation between systolic and diastolic blood pressure and body fat amounts in a sample of 147 teens (34).

In the current analysis, we observed significantly better health profiles in participants who had received EBF for 6 months. However, those participants who had received EBF for 1-5 months also had significant positive differences, like lower BMI and blood pressure and higher adiponectin levels. It is important to highlight that EBF is recommended for the first 6 months, followed by continued breastfeeding with complementary food up to 24 months of life, according to the WHO and the Ministry of Health and Brazilian Society of Pediatrics (21, 35-36). Recently Davis et al. (2014) demonstrated that breastfeeding for ≥12 months was associated with a 47% reduction in obesity.

Figure 1. Serum concentration of adiponectin and leptin, and carotid intima-media thickness in obese adolescents according to duration of exclusive breastfeeding.
prevalence in children aged 2-4 years (37). Moreover, it is estimated that there is 15% to 30% reduction in adolescent and adult obesity rates if any breastfeeding occurred in infancy compared with no breastfeeding. The duration of breastfeeding is also inversely related to the risk of overweight, since each month of breastfeeding has being associated with a 4% reduction in risk (11).

Whether breast milk protects against many chronic diseases remains debatable; however, the current findings demonstrate for the first time that adiponectin levels are higher and cIMT is lower in obese adolescents who had received EBF for 6 months. The results observed cannot establish a causal effect. Therefore, prospective and large cohort studies are necessary in order to confirm these results. A major limitation of the study is that mothers of participants could have misclassified the duration of breastfeeding in the breastfeeding questionnaire. However, this method has been used by other studies in order to investigate the effects of breastfeeding later in life (16, 20). Moreover, the introduction of complementary food, even after EBF for 6 months, can influence the child’s development and metabolism. This was not assessed in the present analysis and can be considered another bias. Despite this, the investigation of the inflammatory profile of obese individuals according to exclusive breastfeeding could be extremely important, since its influence on inflammatory profile in obese subjects is not well defined. The notion that nutrition during early phases of human development can alter organ function and thereby predispose individuals to a later onset of adult disease is an area of considerable interest to researchers and of great concern to public health (10).

Conclusion

In summary, these findings suggest that obese adolescents who had been breastfed exclusively for 6 months have a better anti-inflammatory profile and improvement in cardiovascular risks, which contributes to the prevention of early atherosclerosis development during adult stage. These results reinforce the importance of recommending breastfeeding exclusively for the first 6 months after birth in order to control obesity and comorbidities.

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