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Accumulation of subcutaneous fat, but not visceral fat, is a predictor of adiponectin levels in preterm infants at term-equivalent age

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ABSTRACT

Background: Preterm infants have altered fat tissue development, including a higher percentage of fat mass and increased volume of visceral fat. They also have altered adiponectin levels, including a lower ratio of high-molecular-weight adiponectin (HMW-Ad) to total adiponectin (T-Ad) at term-equivalent age, compared with term infants.
Aims: The objective of this study was to investigate the association between adiponectin levels and fat tissue accumulation or distribution in preterm infants at term-equivalent age.
Study design: Cross-sectional clinical study.
Subjects: Study subjects were 53 preterm infants born at ≤34 weeks gestation with a mean birth weight of 1592 g.
Outcome measures: Serum levels of T-Ad and HMW-Ad were measured and a computed tomography (CT) scan was performed at the level of the umbilicus at term-equivalent age to analyze how fat tissue accumulation or distribution was correlated with adiponectin levels.
Results: T-Ad ($r = 0.315, p = 0.022$) and HMW-Ad levels ($r = 0.338, p = 0.013$) were positively associated with subcutaneous fat area evaluated by performing CT scan at term-equivalent age, but were not associated with visceral fat area in simple regression analyses. In addition, T-Ad ($\beta = 0.487, p = 0.003$) and HMW-Ad levels ($\beta = 0.602, p < 0.001$) were positively associated with subcutaneous fat tissue area, but they were not associated with visceral fat area also in multiple regression analyses.
Conclusion: Subcutaneous fat accumulation contributes to increased levels of T-Ad and HMW-Ad, while visceral fat accumulation does not influence adiponectin levels in preterm infants at term-equivalent age.

1. Introduction

The hormone adiponectin is secreted exclusively by adipocytes and has a beneficial role in insulin sensitivity. Decreased production of adiponectin is associated with type 2 diabetes [1] and obesity [2], and especially with visceral fat accumulation in adults [3] and children [4]. Since high-molecular-weight adiponectin (HMW-Ad) is one of the active adiponectin multimers [5], HMW-Ad is reported to be a better marker of obesity-related complications than total adiponectin (T-Ad) [6]. In addition, the ratio of HMW-Ad to T-Ad (HMW%) is also significantly associated with insulin resistance [7].

Low birth weight infants have an increased risk of adult-onset diseases, including type 2 diabetes mellitus, cardiovascular disease, and obesity [8]. Not only small for gestational age (SGA) infants, but also low birth weight infants (caused by preterm birth) have a higher risk of insulin resistance in later life than term-appropriate for gestational age (AGA) infants [9], although the mechanisms have yet to be fully elucidated [10]. A recent investigation has shown that preterm infants have altered adiponectin levels, including decreased HMW% at term-equivalent age compared with term infants at birth [11]; this effect is present even if the infant does not present with extra-uterine growth restriction (EUGR) [12]. In addition, preterm infants have a higher percentage of fat mass [13] and increased volume of visceral fat [14], which may influence adiponectin production at term-equivalent age.

Some previous investigations have suggested that postnatal growth, as indicated by rate of weight gain [15] or body weight standard deviation (SD) score for example, increases from birth to term-equivalent age [12] and is one of the significant predictors of the increases in T-Ad and HMW-Ad levels in preterm infants during this period. These results indicate that fat tissue accumulation during this period may increase adiponectin production (the opposite effect of that in children and adults with obesity). However, growth during the postnatal period results from fat tissue accumulation; in addition, 'true' growth also occurs, such as increases in muscle mass, body length, and head circumference. Hence,

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it remains unclear whether fat tissue accumulation really depends on an increase in the levels of T-Ad and HMW-Ad in preterm infants during this period because no information regarding the direct association between fat tissue accumulation and adiponectin levels in preterm infants at term-equivalent age is available. Moreover, it also remains unknown how the fat distribution influences T-Ad and HMW-Ad levels in preterm infants at term-equivalent age.

Hence, in this study, we measured serum T-Ad and HMW-Ad levels and investigated visceral fat area (VFA) and subcutaneous fat area (SFA), using commercially available software; we were able to calculate the values from a fat density evaluation by computed tomography (CT) scan at the levels of the umbilicus. Our aim was to clarify whether the amount of fat tissue is correlated with T-Ad and HMW-Ad levels in preterm infants at term-equivalent age and whether visceral fat accumulation influences the levels of T-Ad and HMW-Ad in preterm infants at term-equivalent age.

2. Methods

2.1. Subjects

The Ethics Committee at Showa University School of Medicine approved the study protocol, and we obtained written informed consent from the subjects' parents. The study subjects were 53 preterm infants (23 male and 30 female), born at 24–34 weeks of gestation. All the infants were recruited from Showa University School of Medicine between August 1, 2010 and May 3, 2012. The subjects in the present study were part of a population in which we had already studied adiponectin levels in term and preterm infants. We have reported the clinical profile of the preterm subjects and their mothers in detail [12]. We obtained written informed consent for the CT scan in this study from 53 subjects among 58 preterm subjects in the previous study (that is, 5 parents of other subjects declined the CT scan). The subjects include 7 SGA infants, defined as birth weight <− 2 SD and also include 5 EUGR at term-equivalent age, defined as body weight <− 2 SD at term-equivalent age. All subjects were fed breast milk and infant formula. Breast milk was fortified for all subjects with a birth weight of less than 1500 g. Parenteral amino acids, which amounted to 1.5–2.5 $g \cdot kg^{-1} \cdot day^{-1}$, were administered as soon as possible after birth to all subjects whose birth weight was <1700 g. No subject whose birth weight was >1700 g received support with parenteral amino acids.

2.2. Measurement of VFA and SFA in CT scan

Visceral and subcutaneous fat volumes were evaluated by measuring VFA and SFA determined in a CT scan image, by using commercially available software (Virtual Place Advance; AZE, Ltd, Tokyo, Japan). All subjects were examined in the supine position. A single slice of cross-sectional CT scan (LightSpeed; GE Healthcare Japan Co., Tokyo, Japan) was performed at the level of the umbilicus (from L4 to L5). The software automatically defined a region of interest by tracking its contour on each scan, and the attenuation range of CT values (in Hounsfield units) for fat tissue was calculated. A histogram for fat tissue was automatically computed by the software on the basis of mean attenuation ± 1 SD. Tissue with attenuation values within the mean ± 1 SD was considered to be fat tissue on the basis of a previously reported concept [16] within that region of interest. Trained radiologists made manual adjustments if needed, choosing a midway point between adipose and non-adipose tissue peaks when the peaks had considerable overlap and misclassification could occur [17]. The software automatically divided total fat area into SFA and VFA, and radiologists also made manual adjustments if needed. To test the variability of SFA and VFA, the inter-observer and intra-observer coefficients for all 53 subjects were calculated. The inter-observer intraclass correlation coefficient for SFA and VFA was 0.980 and 0.956 (coefficient variation (CV), 3.8% and 9.1%), respectively. To assess intra-observer variability, the same observer repeated the adjustment of measurements of SFA and VFA on two different occasions. The intra-observer intraclass correlation coefficient for SFA and VFA was 0.992 and 0.982 (CV, 2.4% and 5.6%). The ratio of VFA to SFA was designated as the V/S ratio.

2.3. Anthropometric measurements

Physical measurements such as body weight and length were determined immediately by experienced nurses after birth and at term-equivalent age. The medical records of the subjects were reviewed retrospectively. Body weight was measured by using a standard electronic scale. Body weight SD scores for gestational age were determined according to Japanese reference data [18], which were differentiated by sex, number of deliveries, and gestational days. Their mother's body weight and height before pregnancy were self-reported. Body mass index (BMI) was calculated as body weight/length^2 (kg/m^2).

2.4. Measurements of T-Ad, HMW-Ad, and leptin

To determine serum T-Ad, HMW-Ad, and leptin, blood samples were collected from the dorsum manus vein 2 or 3 h after feeding in preterm infants at term-equivalent age. Sera for the assays were obtained by centrifugation of the blood samples and were immediately frozen. The specimens were stored at −40 °C before analysis. Serum T-Ad and HMW-Ad concentrations were determined by ELISA using a commercial kit (Daiichi Pure Chemicals, Tokyo, Japan), and serum leptin levels were measured using a commercial RIA kit (Linco Research, St Louis, MO, USA). The intra-assay variation (CV) for the T-Ad and HMW-Ad assays was 5.3% and 3.3%, as described previously [19], and that of the leptin assay was <8%. HMW% was calculated as (HMW-Ad / T-Ad) × 100.

2.5. Statistical analyses

All analyses were performed with the Statistical Package for the Social Sciences (SPSS) Statistics Desktop for Japan Version 19.0 (IBM Company, Tokyo, Japan). We used the Mann–Whitney test to compare adiponectin levels between male and female infants or between 19 very preterm infants born before 32 weeks and 34 other preterm infants born at 32 weeks gestation or more. We evaluated the correlation of SFA, VFA, and V/S ratio to other variables by using bivariate Pearson's correlation in a simple linear regression and a model of multiple regression analysis. In addition, we also evaluated the influence of an amount and distribution of fat tissue on T-Ad, HMW-Ad, HMW%, and leptin, in the same way. Multiple regression analyses were performed to assess the influence of multiple variables such as SFA, VFA, sex, gestational age, and body weight SD score at term-equivalent age on serum T-Ad, HMW-Ad, HMW%, and leptin at term-equivalent age; we excluded birth weight and body weight at term-equivalent age from the dependent variables because they are strongly correlated with other factors such as gestational age. The associations were considered statistically significant when the p values were <0.05.

3. Results

The clinical characteristics of the subjects are shown in Table 1. The mean birth weight, birth weight SD score, and gestational age were 1592 g, −0.7 SD, and 32.1 weeks, respectively. The mean body weight, body weight SD score, and age of the subjects at term-equivalent age were 2737 g, −0.8 SD, and 39.3 weeks. The subjects included 5 infants that presented with EUGR, defined as body weight SD score <− 2 SD at term-equivalent age. The mean SFA, VFA, and V/S ratio evaluated at term-equivalent age were 9.4 cm^2, 3.4 cm^2, and 0.39, respectively. The mean levels of T-Ad, HMW-Ad, and HMW% measured at term-equivalent age were 22.8 μg/mL, 13.7 μg/mL, and 59.3%, respectively. T-Ad and HMW-Ad levels were significantly higher in