Early Human Development 95 (2016) 29–33

Contents lists available at ScienceDirect

Early Human Development

journal homepage: www.elsevier.com/locate/earlhumdev

Longitudinal changes in adiponectin multimer levels in preterm infants

Yuya Nakano *, Kazuo Itabashi, Kazushige Dobashi, Katsumi Mizuno

Department of Pediatrics, Showa University School of Medicine, Tokyo, Japan

ARTICLE INFO

Article history:
Received 28 October 2015
Received in revised form 23 January 2016
Accepted 29 January 2016

Keywords:
Adiponectin
Preterm infants
Infancy
Longitudinal changes

ABSTRACT

Background: Preterm infants have altered adiponectin levels at term-equivalent age and have a higher risk of developing components of the metabolic syndrome in later life than term infants.
Aims: To investigate the longitudinal changes in adiponectin levels in preterm infants and to compare the levels between term and preterm infants.
Study design: A cohort study.
Subjects: The study subjects were 43 term infants and 42 preterm infants born at ≤34-week gestation.
Outcome measures: Serum levels of total adiponectin (T-Ad) and high-molecular-weight adiponectin (HMW-Ad) were measured in 42 preterm infants at term-, 6 month-, and 12 month-equivalent ages. Moreover, the levels in 43 term infants investigated previously were reviewed.
Results: In preterm infants, T-Ad and HMW-Ad levels at the 12 month-equivalent age were lower than at the term- and 6 month-equivalent ages (all values $p < 0.001$), which was consistent with previous results in term infants. The difference in ratios of HMW-Ad to T-Ad between term and preterm infants continued at the 6 month-equivalent age but disappeared at the 12 month-equivalent age. Multiple regression analyses revealed that HMW-Ad levels at term-equivalent age were only a significant determinant of the changes in HMW-Ad between the term- and 12 month-equivalent ages in preterm infants ($p < 0.001$).
Conclusions: The HMW-Ad levels decline till the 12 month-equivalent age in both term and preterm infants. The changes in HMW-Ad level during infancy might be determined at least to a certain degree up to term-equivalent age in preterm infants.

1. Introduction

Low birth weight infants are known to be at an increased risk of future coronary heart disease, type 2 diabetes mellitus, and hypertension [1]. Low birth weight infants result from not only intrauterine growth restriction but also preterm birth. Some previous investigations reported that preterm birth was associated with insulin resistance [2] and higher blood pressure [3] in later life, independent of low birth weight; however, such relationships were not found in other studies [4,5]. Although the relationship between preterm birth and such diseases is now controversial, a recent meta-analysis has suggested that there is a higher risk for developing at least some components of the metabolic syndrome [6–8].

Adiponectin is an adipocyte-secreted hormone that exerts positive health effects such as protection against insulin resistance [9] and reduction in proinflammatory cytokine levels [10]. A decrease in adiponectin concentration was reported to be related to the metabolic syndrome, which includes obesity [11], type 2 diabetes mellitus [12], hypertension [13], and dyslipidemia [14]. Adiponectin consists of a carboxyl-terminal globular domain and an amino-terminal collagenous domain. It circulates in serum to form several different molecular weight species including low molecular weight adiponectin (LMW-Ad), middle molecular weight adiponectin (MMW-Ad), and high molecular weight adiponectin (HMW-Ad) [15]. The HMW-Ad level is a better indicator of glucose intolerance than total adiponectin (T-Ad) level [16,17]. In addition, pioglitazone, which improves insulin sensitivity in humans, increases secretion of HMW-Ad from adipocytes, with no significant effects on LMW-Ad or MMW-Ad [18]. Moreover, not only HMW-Ad but also the ratio of HMW-Ad to T-Ad (HMW%) is predictive of insulin resistance and metabolic syndrome [19]. Hence, HMW-Ad is now considered the active form of this protein [20].

Preterm infants have altered adiponectin levels at term-equivalent age, including lower HMW% compared with term infants [21,22], even if they present without extrauterine growth restriction at term-equivalent age [22]. In term infants, we previously showed that HMW-Ad levels at 12 months of age were significantly lower than those at birth and at 6 months. We also showed that the cord serum HMW-Ad levels could significantly determine the levels present at 12 months, implying that postnatal production of HMW-Ad at least during infancy may be regulated before birth [23]. However, there is no information on the longitudinal

* Corresponding author at: Department of Pediatrics, Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8666, Japan. Tel.: +81 3 3784 8677; fax: +81 3 3784 7410.
E-mail address: nakano_yt_tulip@yahoo.co.jp (Y. Nakano).

http://dx.doi.org/10.1016/j.earlhumdev.2016.01.022
0378-3782/

changes in adiponectin multimer levels in preterm infants. Thus, we aimed to investigate the longitudinal changes in adiponectin multimer levels in preterm infants, to compare the levels between term and preterm infants, and to evaluate confounding factors associated with HMW-Ad levels at 12 month-equivalent age in preterm infants.

2. Methods

2.1. Subjects

The Ethics Committee at Showa University School of Medicine and Obihiro Keiai Hospital (primary obstetric center) approved the study protocol, and we obtained written informed consent from the subjects' parents. The subjects in the present study include a portion of a population in which we had previously studied adiponectin levels and compared these levels between 43 term infants and 58 preterm infants [22,23]. Forty-three term infants were recruited from the Obihiro Keiai Hospital between May 1, 2005 and September 30, 2006. All term infants were born as appropriate for gestational age (AGA) infants without any complications such as gestational diabetes mellitus, thyroid disease, pregnancy-induced hypertension, or autoimmune disease noted in the mothers. All preterm infants were recruited from Showa University School of Medicine between August 1, 2010 and July 31, 2012, and were born between 24 and 34 weeks of gestation. Of the 58 preterm infants, sixteen were lost to follow-up assessment because of changes in residence. Hence, the study subjects ultimately consisted of 43 term infants and 42 preterm infants; the preterm infant group including 6 small for gestational age (SGA) infants, defined as those with birth weight standard deviation (SD) scores <−2 SD. None of the preterm infants included had severe complications such as necrotizing enterocolitis, periventricular leukomalacia, hydrocephalus, or severe-grade intraventricular hemorrhage, except one infant who had severe chronic lung disease and required home oxygen therapy.

Gestational age was assessed on the basis of the mother's menstrual history and ultrasound assessment according to the first trimester crown-rump length scan. The preterm deliveries were induced because of threatened premature delivery (19 infants), non-reassuring fetal status (7 infants), premature rupture of membrane (11 infants), and hypertension in the mother (5 infants). Among the 42 mothers whose infants were born preterm, seven had pregnancy-induced hypertension, and glucocorticoids were administrated to 29 mothers to prepare for their preterm delivery.

All of the preterm infants were fed breast milk and infant formula between birth and term-equivalent age. Breast milk was fortified for all the preterm infants having a birth weight of <1500 g. Parenteral amino acids, which amount to 1.5–2.5 $g \cdot kg^{-1} \cdot day^{-1}$, were administered to all preterm infants whose birth weight was <1700 g as soon as possible after birth. All term and preterm infants were supplemented with infant formula from term-equivalent age to 12 month-equivalent age when mothers judged that intake of breast milk was not sufficient for their infants; the introduction of solid food and its content were left to the mother's discretion. We interviewed the subject's parents to assess how the infants were being fed (breast-fed or formula), when and whether they were weaned, and whether they were given any solid foods at 6 month- and 12 month-equivalent ages. Of the 42 preterm subjects, 16 infants were exclusively fed breast milk, five infants were fed a combination of breast milk and formula milk, and 21 infants were fed formula milk at 12 month-equivalent age.

2.2. Anthropometric measurements

General physical measurements such as body weight and length were determined by experienced nurses in preterm infants at birth, term-, 6 month-, and 12 month-equivalent ages. We reviewed the anthropometric measurements obtained at birth, 6 months, and 12 months in 43 term AGA infants investigated previously. Body weight was measured using a standard electronic scale. Body weight standard deviation (SD) scores for gestational age, 6 months, and 12 months were determined according to Japanese reference data. Body mass index (BMI) was calculated as body weight / length^2 (kg/m^2).

2.3. Measurements of total-, high-, middle-, and low-molecular weight adiponectin levels

To determine serum T-Ad, HMW-Ad, MMW-Ad, and LMW-Ad levels, blood samples were collected from veins at least 2 or 3 h after feeding in preterm infants at term-, 6 month-, and 12 month-equivalent ages. 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 concentrations of T-Ad, HMW-Ad, MMW-Ad, and LMW-Ad were determined by sandwich ELISA using a commercial kit (Daiichi Pure Chemicals, Tokyo, Japan). Intra-assay variation (CV) values for T-Ad, HMW-Ad, and MMW-Ad + HMW-Ad were 5.3%, 3.3%, and 4.1%, as described previously [24]. We reviewed serum concentrations of T-Ad, HMW-Ad, MMW-Ad, and LMW-Ad measured at birth, 6 months, and 12 months in 43 term AGA infants investigated previously. All samples in term and preterm infants were analyzed with the same assay in order to reduce interassay variance. HMW% was calculated as (HMW-Ad/T-Ad).

2.4. 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 compared T-Ad, HMW-Ad, MMW-Ad, and LMW-Ad levels between genders, using Mann–Whitney test. In addition, we evaluated the longitudinal changes in T-Ad, HMW-Ad, MMW-Ad, and LMW-Ad levels, as well as HMW%, in term and preterm infants using the Friedman test and Wilcoxon test. In addition, we also used the Mann–Whitney test to compare serum levels of T-Ad, HMW-Ad, and HMW% between in term and preterm infants at the term-, 6 month-, and 12 month-equivalent age groups.

We used a simple regression analysis to evaluate the association between HMW% at term-equivalent age and changes in body weight SD score between the term- and 12 month-equivalent ages. In addition, simple regression analyses were also performed to assess the influences of multiple variables on changes in T-Ad, HMW-Ad, and HMW% between the term- and 12 month-equivalent ages. Multiple regression analyses were performed to determine significant predictors of changes in T-Ad, HMW-Ad, and HMW% between term- and 12 month-equivalent ages, considering serum levels of T-Ad, HMW-Ad, and HMW% at term-equivalent age, gestational age, birth weight SD score, breastfeeding duration, and changes in body weight SD score during this period. Differences were considered statistically significant when 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 of the preterm infants were 1581 g, −0.6 SD, and 31.9 weeks, respectively. Serum concentrations of T-Ad, HMW-Ad, MMW-Ad, LMW-Ad, and HMW% in preterm infants at term-, 6 month-, and 12-month-equivalent ages did not differ between male and female infants. In preterm infants, a simple regression analysis revealed that T-Ad levels had a strong positive correlation with HMW-Ad levels at term- ($r = 0.921$, $p < 0.001$), 6 month- ($r = 0.960$, $p < 0.001$), and 12 month-equivalent ages ($r = 0.929$, $p < 0.001$). The HMW% was not significantly related to T-Ad at the term-equivalent age in preterm infants ($r = 0.240$, $p = 0.126$), which is inconsistent with the results obtained previously in term infants, but was positively correlated to T-Ad at the 6 month- ($r = 0.394$, $p = 0.010$) and 12 month-equivalent ages ($r = 0.597$, $p < 0.001$).