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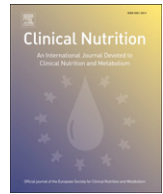
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## Original Article

## The bioelectrical impedance vector migration in healthy infants

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## SUMMARY

**Background & aims:** Detecting young children with high amount of body fat is important to intervene in the development of obesity. The aim of this study is to gain insight in the bioelectrical impedance vector analysis in healthy infants.

**Methods:** Repeated measurements of whole body reactance and resistance were assessed, using a 50 kHz frequency bioelectrical impedance analysis, in 51 boys and 62 girls during infancy. Bivariate vector analysis, which can be used to determine tissue hydration and soft tissue mass, was conducted. The 95% confidence intervals of the mean vectors for different age groups and the 95%, 75% and 50% tolerance intervals were plotted, using resistance and reactance components standardized by the participant's height.

**Results:** During infancy impedance vectors changed significantly: A vector migration of the Xc/H of 8.50 ohm/m and the R/H of −95.68 ohm/m between the age of two months and eight to twelve months ( $p = 0.0001$ ) was observed. Bivariate, reference tolerance intervals of the impedance vectors for healthy infants at the age of two months are presented.

**Conclusion:** Our results show a significant impedance vector migration during the first year of life. New reference tolerance intervals for the second month of life were constructed.

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## 1. Introduction

The prevalence of obesity in children is growing. A relative high amount of total body fat at young age might be the start of the development of obesity.<sup>1</sup> Detecting young children with a high amount of body fat, which points to children with an increased risk for developing obesity, is of great importance, since intervening in the development of obesity is still an option in early infancy.

In children many methods to assess body composition have been described. Dual-energy X-ray absorptiometry and isotope dilution are considered standards to measure total body fat. Their

use is limited because of practicality and costs. The Goran equation, combining skinfold and bioelectrical impedance analysis (BIA) measurements, is most reliable for assessing total body fat in six- and seven-year-old children (L'Abée C et al., Comparisons of methods to assess total body fat in six- and seven-year-old children, submitted). The Horlick equation, based on the BIA measurements alone, can detect six- and seven-year-old children with a high amount of body fat. Other studies confirm that BIA can be considered as a useful tool for assessing body composition.<sup>2–4</sup> The BIA is a user friendly, non invasive, low cost and portable method, which can be used to calculate total body fat in children and adults.

For calculating total body fat BIA equations are often used. These equations assume constant composition of fat/non-fat related to electrolytes.<sup>5,6</sup> Since the density and tissue hydration in infant's changes, the infant body cannot be presumed to be a constant conductor of current.<sup>7,8</sup> Therefore, not a single equation can be developed to calculate total body fat in infants of different ages. Whereas the BIA equations determine body fat percentage, the bioelectrical impedance vector analysis (BIVA) visualizes the impedance measurements whereupon the body composition can be interpreted. In both clinical and research settings, BIVA can be

*Non-standard abbreviations:* BIA, bioelectrical impedance analysis; BIVA, bioelectrical impedance vector analysis; H, height; R, resistance; Xc, reactance.

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used as a stand-alone procedure to assess the hydration of the soft tissue mass (lean and fat) in a person based on patterns of direct impedance measurements. Fig. 1 shows how to interpret the BIVA patterns.<sup>9</sup> In adults, individual impedance measurements can be drawn in age-specific reference tolerance intervals (50%, 75%, and 95% tolerance ellipses) to determine if that person has normal or abnormal hydration of a normal or abnormal soft tissue mass (increased mass as in obese and athletic, or decreased mass as in cachectic and lean subjects) compared to the reference population. In adult's weight loss following energy restriction, BIVA allows the identification of fat versus lean tissue loss.<sup>10</sup> Guida et al. confirmed the usefulness of the BIVA in 8-year-old children.<sup>11</sup> This study showed similar BIVA patterns for obese children compared to obese adults, namely a difference of impedance vector pattern for obese and normal weight children (the obese subjects fell in the lower left region of the reference tolerance ellipses).<sup>10</sup>

Based on one cross-sectional study, reference values of the impedance vector are constructed for infants.<sup>2</sup> The study of Savino found no significant vector migration during the first year of life. Progressive vector shortening from birth, through age 2–15 years, toward the adult's vector position has been described in literature.<sup>7,8,12</sup> Vector migration has never been proven for infants during the first year of life. Therefore, longitudinal data are needed to investigate the change of the vector during the first year of life. If necessary, reference values of the impedance vector for infants should be obtained and can be used for clinical practice to detect young children with a high amount of body fat.

The present study was conducted to establish impedance vector migration in healthy children during the first year of life.

## 2. Methods

### 2.1. Subjects

This study was performed within the GECKO (Groningen Expert Center for Kids with Obesity) Drenthe study. The GECKO Drenthe is a population-based birth-cohort study. All children born from April 2006 to April 2007 and living at time of birth in Drenthe, one of the northern provinces of the Netherlands, were asked to participate in the study. Within the Dutch Health Care system, Well Baby Clinics have a central position during the first years of life. More than 95% of

all parents regularly visit these clinics, which also provide free immunizations for the baby. Parents of infants visiting two out of the 61 Well Baby Clinics in Drenthe between the period September 2006 and April 2007, were asked if their child could participate in this study. The participating infants were born at full term, adequate for gestational age, did not show chronic diseases or growth problems, and had a normal body temperature. Informed consent was obtained from parent or guardian. The study was approved by the Ethics Committee of the University Medical Center Groningen and performed in accordance with the Declaration of Helsinki.

### 2.2. Measurements

A 50 kHz frequency bioelectrical impedance analysis (BIA), type BIA 101 (Akern®, Florence, Italy; RJL Systems licensee, Clinton Twp, MI, USA) was used to measure the whole body reactance and resistance. The accuracy of the equipment was checked with a 500 ohm resistor supplied by the manufacturer before the measurements were performed. Electrodes were longitudinally cut into two pieces to fit the infants.<sup>8</sup> These outer electrodes were placed on the dorsal surfaces of the third proximal phalanx of the right hand and the right foot. The inner electrodes were placed on the forearm and pretibial region, leaving 5.5 cm of free skin from the outer electrodes. Participants were laid on a non-conductive surface, with all clothing or any kind of metal jewellery removed. During the measurements the participants laid still and were distracted by toys. No direct contact was made with the infant during the measurements. Three measurements were taken, with arms and legs abducted from the body. The mean of the three measurements was taken for further calculations. Children were measured two times during the first year of life, the first measurement at the age of two months. The second measurement took place between six and twelve months.

At both visits, body weight was measured using an electronic scale with digital reading, which recorded the nearest 0.01 kg. Height was assessed using an infantometer and recorded to the nearest 0.1 cm. BMI was calculated by dividing weight by stature squared ( $\text{kg}/\text{m}^2$ ).

### 2.3. Statistical analyses

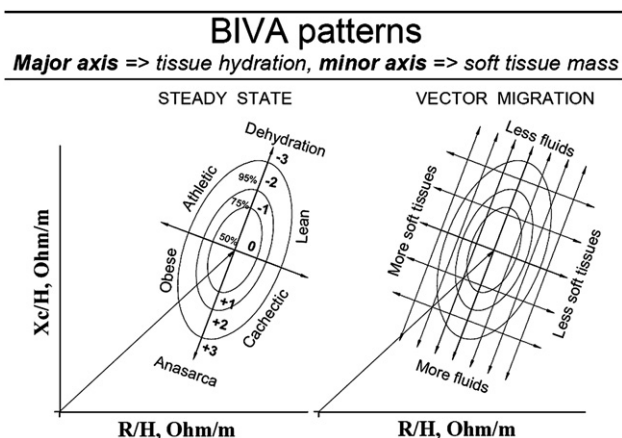
BIVA uses the plot of direct measurements of the vector components resistance (R) and reactance (Xc) from the impedance analyzer (RXc graph).<sup>13</sup> According to the RXc graph method, impedance measurements standardized by the subject's height are plotted as bivariate vectors with their confidence and tolerance intervals, which are ellipses on the R-Xc plane.

To search for gender differences in the different age groups, the bivariate 95% confidence interval for the mean impedance vector of boys and girls was calculated and plotted, using the bivariate normal distribution of R divided by height (R/H) and Xc divided by height (Xc/H). Two-sample Hotelling's  $T^2$  test for vector analysis was performed. Separate 95% confidence ellipses indicate a statistically significant difference between mean vector positions on the R-Xc plane (equivalent to a significant two-sample Hotelling's  $T^2$  test,  $p < 0.05$ ).

Paired one-sample Hotelling's  $T^2$  test was performed to determine if the changes in mean vector of the groups, measured at the first and second time point, were significantly different from zero (null vector). A 95% confidence ellipse excluding the null vector indicates a statistically significant vector displacement with age.

Moreover, we calculated and plotted the bivariate 95%, 75% and 50% tolerance intervals for two-month-old infants.

P-values  $< 0.05$  were considered to indicate statistical significance. All statistic analyses were performed using Microsoft Excel



**Fig. 1.** Interpretation of BIVA patterns;<sup>9</sup> Mean impedance vector with the reference 50%, 75% and 95% tolerance ellipses. The vector of an individual subject is expected to fall within these intervals with probabilities of 50%, 75% and 95%, respectively. Normal hydration is represented by vectors falling within 75% tolerance ellipses. Dehydration is represented by lengthening and steepening of the vector. Obese subjects fall in the lower left region.<sup>10</sup> Abbreviations are: R, resistance; Xc, reactance; H, height.

2003 (using the BIVA software)<sup>14</sup> and SPSS version 14.0 (SPSS, Chicago IL, USA).

### 3. Results

In total 62 girls and 51 boys participated in the study. The baseline characteristics and results for bioelectrical impedance parameters are listed in Table 1.

All 113 children were measured at the age of two months, which was determined as group 1. Group 2a included 53 infants, who had their second measurement at the age of six to seven months; group 2b consisted of 46 infants who were measured the second time between eight to twelve months of age.

No differences, concerning gestational age, birth weight, gender, anthropometry and impedance measured at the age of two months, were found between the group of children measured twice ( $n = 99$ ) and the group of children ( $n = 14$ ) measured once.

Small differences for impedance measurements between boys and girls in the different age groups were found. The sex-specific 95% confidence ellipses of two mean vectors were overlapping, which means that the position between the vector of a boy and a girl of the same age was not statistically different in the R-Xc plane. Hence one group, including both boys and girls, was used to compare the different age groups.

The paired one-sample Hotelling's  $T^2$  test determined a difference in mean vectors between the first measurement and matching second measurement (Fig. 2). The 95% confidence ellipses of two mean vectors were not overlapping, which means that the position between the vector at two months and at 6–12 months was statistically different ( $p < 0.0001$ ) in the R-Xc plane. The  $p$ -value of the paired one-sample Hotelling's  $T$ -test of 0.0089 was found comparing group 2a with the same children of group 1, and the  $p$ -value of 0.0001 comparing group 2b with the matching children of group 1.

The reference intervals (95%, 75% and 50% confidence ellipses) for an individual vector measured at the second months of life are shown in Fig. 3.

### 4. Discussion

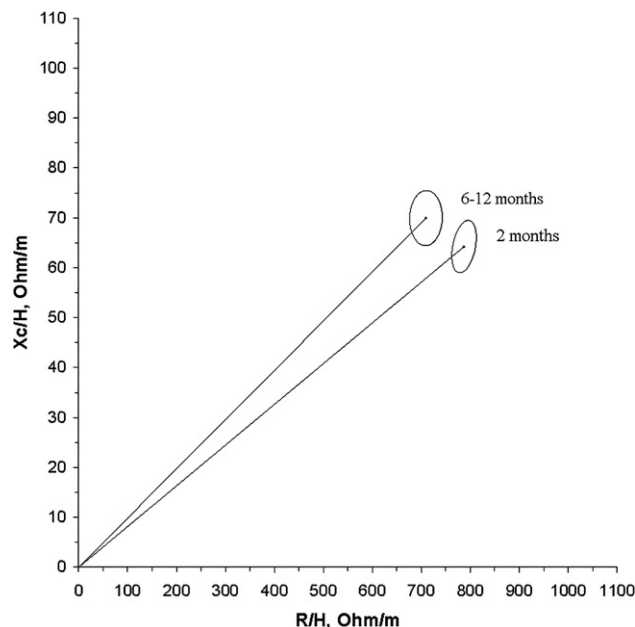
Our results of the repeated measurements show a significant vector displacement during the first year of life. Moreover,

**Table 1**

Clinical characteristics of the infants divided in group 1 (measured at 2 months), group 2a (second measurement at 6–7 months) and group 2b (second measurement at 8–12 months).

	Group 1 2 months	Group 2a 6–7 months	Group 2b 8–12 months
Population (n)	113	53	46
Gender: male (n)	51	23	23
male (%)	45.1	43.4	50.0
Mean weight (g)	5402	8328	9097
SD	741	809	996
Mean height (cm)	58.7	70.3	73.1
SD	2.2	2.0	3.2
Mean BMI (kg/m <sup>2</sup> )	15.6	16.8	17.0
SD	1.7	1.3	1.3
Mean R/H (ohm/m)	789	723	692
SD	97	117	145
Mean Xc/H (ohm/m)	63	68	73
SD	21	23	21
r (R/H, Xc/H)	0.29	0.09	0.05

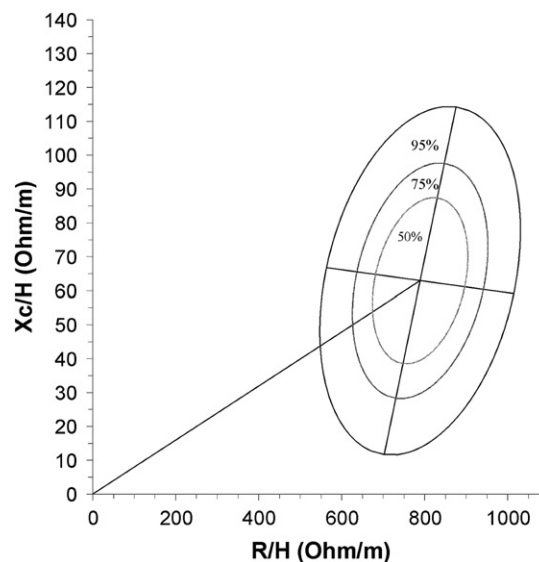
R, resistance; H, height; Xc, reactance; r(R/H, Xc/H), linear correlation coefficient between R/H and Xc/H.



**Fig. 2.** Mean impedance vector with the 95% confidence ellipse from subjects measured at the age of two months and six to twelve months. Abbreviations are: R, resistance; Xc, reactance; H, height.

reference, bivariate, 95%, 75% and 50% tolerance intervals of the impedance vectors for two-month-old healthy infants are presented. With the use of these intervals, two-month-old infants with abnormal soft tissue mass can be detected. More studies are necessary to obtain reference values for the period between two months and two years.

Few studies on BIVA have been done in infants. The strength of this study is the repeated measurements in this group of infants. In the first year of life the children were measured twice, at the age of two months and between the age of six and twelve months. To our knowledge, longitudinal data of bioelectrical impedance vectors in infants have not been shown in the literature before.



**Fig. 3.** Bivariate reference intervals for the age of two months with 50%, 75% and 95% tolerance ellipse for an individual impedance vector. The arrow represents the mean impedance vector. Abbreviations are: R, resistance; Xc, reactance; H, height.

However, progressive vector shortening through ages is expected during infancy based on cross-sectional studies.<sup>7,8</sup> Our longitudinal data confirm progressive vector migration during the first year of life.

One of the limitations of this study is that BIA measurements were not conducted among strictly standardized conditions like being fasting or supine for ten minutes. All researchers who investigate young children with the BIA face this limitation.<sup>2,7,8</sup> Because of the children's age, we decided not to fast the children, with a difference in amount of food intake and a difference in "fasting" time as a result. However, consumption of food and beverage may decrease impedance by 4–15 ohm over two to four hours after meals, representing an error smaller than 3%.<sup>3,15</sup> Further, the measurement was not taken after lying supine for 10 min, what is thought to be ideal, because of the hydrostatic fluid shift when changing position.<sup>16</sup> Nevertheless, Kushner et al. described a difference of 1.2% between lying for 0 min compared to lying for 10 min.<sup>3</sup> Our data were obtained according to the normal clinical condition. Another limitation is the fixed interelectrode distance during the measurements. In our study, the interelectrode distance of 5.5 cm was used to prevent interaction between the pairs of electrodes. However, Gartner suggested standard (anatomical) electrode placement when different age groups are being compared, since fixed signal electrode does not represent the same part of the limb to be measured as conductor.<sup>17</sup> However, the fixed distance between the signal electrodes used to measure the infants is in line with previous studies.<sup>2,7,8</sup>

Literature on bioelectrical impedance vectors in young children are limited and based on cross-sectional studies.<sup>2,7,8,17</sup> Piccoli and colleagues described a group of neonates in the first week after birth.<sup>8</sup> De Palo and colleagues made reference values of bioelectrical impedance vector in healthy Italian children of two to fifteen years.<sup>7</sup> One cross-sectional study assessed the bioelectrical impedance vector distribution in 153 healthy Italian infants between birth and one year.<sup>2</sup> This study found no significant vector migration during the first year of life. Based on the progressive vector shortening through ages, vector migration during the first year of life is expected.<sup>7,8</sup> Longitudinal data are needed to investigate vector migration during the first year of life. If necessary, reference values of the impedance vector for infants should be obtained and can be used for clinical practice to assess children's body composition to detect young children with a high amount of body fat. Detecting children with a high amount of total body fat is of great importance, since these children are at risk for developing overweight in later life.

Our results show a significant vector displacement during the first year of life in healthy children. Possible explanation for the conflicting results with the study of Savino is the use of cross-sectional data of Savino.<sup>2</sup> Moreover, the study population of Savino of 153 children was classified by gender and age (intervals of four months). The possibility exists that the intervals were too large and the groups too small to detect the migration.

Our results confirm the progressive vector migration through ages.<sup>7,8</sup> The position of tolerance ellipses at the age of two months is intermediate between neonates and two-year-old children. Body fluid is known to decrease during the first year of life.<sup>18</sup> The impedance decrease during childhood indicates that soft tissue mass increases. The R/H decrease and Xc/H increase (shift to a more athletic body composition) after two months, maybe indicating a greater increase in physical activity in the second half of the first year.

This study presents reference, bivariate, 95%, 75% and 50% tolerance intervals of the impedance vectors in healthy infants at the age of two months. This reference tolerance interval fills the gap between the already existent reference intervals for neonates (age 1–7 days) and children age 2–15 years.<sup>7,8</sup> This reference tolerance

interval can be used for epidemiological studies and clinical practice to provide a bedside assessment of body composition. Reference intervals of impedance vectors allow classification of subjects with normal or abnormal body impedance (i.e. body composition) and monitoring of repeated measurements. Notable, reference data are dependent on the different BIA machines produced by different manufacturers. The 50 kHz frequencies BIA, type BIA 101 (Akern®, Florence, Italy; R/L Systems licensee, Clinton Twp, MI, USA) should be used when applying the reference values. Namely, all available reference values are based on the same BIA machines produced by the same manufacturer.<sup>2,7,8</sup> Additionally, validation study is necessary to establish whether in children BIVA patterns are as valid as in adult subjects.

In conclusion, our longitudinal study showed a significant impedance vector migration during the first year of life. Moreover, reliable reference intervals (95%, 75%, and 50% tolerance ellipses) for two-month-old healthy infants are presented.

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### Statement of authorship

CA was responsible for design of the study and coordination, carried out the data analyses, and drafted the manuscript; PP collected the data, helped with interpretation of the data and with revising the manuscript; EG assisted in the data collection, and helped with interpretation of the data and with the revising the manuscript; AP gave significant advices concerning the BIVA software, data interpretation and revision of the manuscript; RS participated in the design of the study, helped with interpretation of the data and with revising the manuscript; PS participated in the design of the study, and gave advices concerning the data interpretation and revision of the manuscript. All authors read and approved the final manuscript.

### Conflict of interest

This study is free of any conflicts of interest.

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