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Body composition and nutritional status in neonates and sick children as assessed by dual energy x-ray absorptiometry, bioelectrical impedance analysis and anthropometric methods. Impact of nutrition on postnatal growth

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List of abbreviations

AGA	Appropriate-for-gestational age
BA	Bone area
BIA	Bioelectrical impedance analysis
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BMI-SDS	Body mass index standard deviation scores
BW%	Birthweight percentile
DPA	Dual photon absorptiometry
DONALD	Dortmund Nutritional and Anthropometric Longitudinally Designed Study
DXA	Dual energy X-ray absorptiometry
ECW	Extracellular water
FFM	Fat-free mass
FFM_{DXA}	Fat-free mass measured by dual energy X-ray absorptiometry
FM	Fat mass
HT^2/R	Resistance index
HT^2/Z	Impedance index
Ht^2/I	Impedance index
ICW	Intracellular water
LBM	Lean body mass
LGA	Large-for-gestational age
MM	Mother's milk
NF	Nucleotide supplemented formula
PI	Ponderal index
R	Resistance
RI	Resistance index
ROC	Receiver operating characteristic
RSS	Residual sum of squares
SEE	Standard error of estimate
SF	Standard formula
SGA	Small-for-gestational age
SKF	Skinfold
SPA	Single photon absorptiometry
TBW	Total body water
W/L	Weight-length
Xc	Reactance
Z	Impedance
ZI	Impedance index
%BF	Percentage body fat
$\%BF_{DXA}$	Percentage body fat measured by dual energy X-ray absorptiometry

Chapter I

1. Introduction

1.1. Body composition

1.1.1. Definition

Body composition is a technical term used to describe the different components that, when taken together, make up a person's body weight. Body composition analysis involves subdividing body weight into two or more compartments according to element, chemical, anatomical or fluid components (Heymsfield and Waki 1991; Wang et al. 1993). The classic two-compartment model divides the body mass into fat and fat-free mass (FFM) compartments. The fat consists of all extractable lipids, and the FFM includes water, protein, and mineral components (Siri 1961). **Figure 1** illustrates the body composition models.

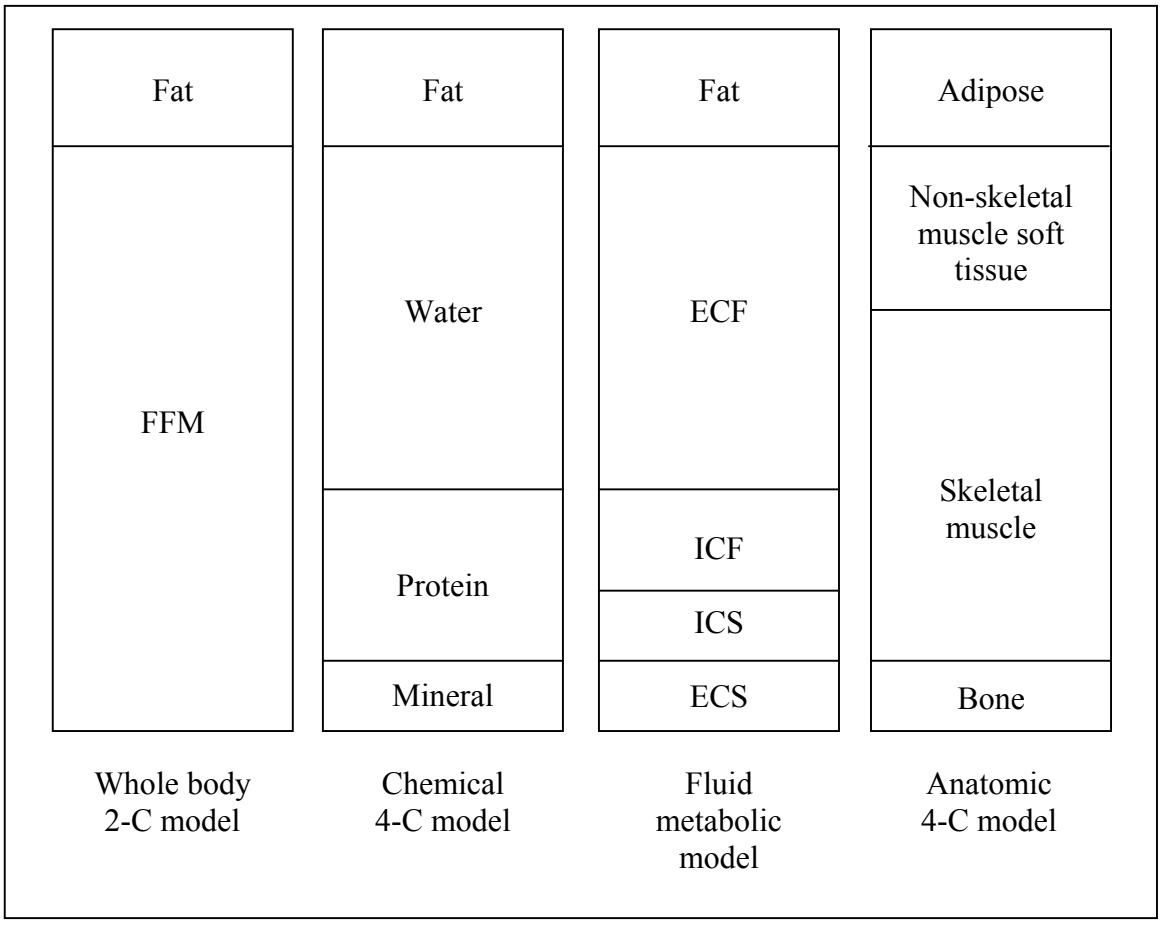

FIGURE 1. Two-compartment and multicompartment body composition models. FFM = fat-free mass; ECF = extracellular fluid; ICF = intracellular fluid; ICS = intracellular solids; ECS = extracellular solids.

1.1.2. Fat mass

Fat mass is defined as all extractable lipids from adipose and other tissues in the body (Heyward and Stolarczyk 1996). The total amount of body fat consists of essential fat and storage fat. Fat in the marrow of bones, in the heart, lungs, liver, spleen, kidneys, intestines, muscles, and lipid-rich tissues throughout the central nervous system is called essential fat, whereas fat that accumulates in adipose tissue is called storage fat. Essential fat is necessary for cell membrane formation, transport and storage of fat-soluble vitamins A, D, E and K, functioning of the nervous system, the menstrual cycle, the reproductive system, as well as growth and maturation during pubescence. Storage fat is located around internal organs (visceral fat) and directly beneath the skin (subcutaneous fat). It provides protection for the body and serves as an insulator to conserve body heat (Heyward and Stolarczyk 1996).

1.1.3. Percentage body fat (%BF)

%BF is the percentage of fat that a body contains. %BF can be measured by several methods but dual energy X-ray absorptiometry (DXA) is the most convenient one (Fusch et al. 1999). DXA provides information on three components of body composition: fat mass (grams), lean mass (grams), bone mineral content (BMC) (grams). Total %BF determined by DXA was calculated as 100 x [fat mass/(fat mass + lean mass + BMC)] (Taylor et al. 1998).

Nutritional status of an individual can be divided into 4 categories: underweight, normal weight, overweight and obesity. Similar to the classification of nutritional status based on Body mass index (BMI)-for age in children and adolescents, the classification of nutritional status based on %BF, in theory, is possible. In reality, an accurate measure of %BF for a large number of subjects is time-consuming and costly. It leads to the lacks in international or national reference data of %BF.

Ideally, body fat would be measured and obesity would be defined as the amount and distribution of body fat, which is associated with increased morbidity and mortality. Unfortunately, the long-term health outcomes for different amounts of adiposity at different ages have not been described, leading to the lack of clear %BF level at which to define obesity and some authors have used arbitrary %BF values. Lazarus et al. (1996) used 85th percentile of %BF measured by DXA as the criteria to define obesity. Williams et al. (1992) proposed cut-off point of 25% fat in boys and 30% fat in girls to define obesity. Dwyers and

Blizzard (1996) suggested the same value for girls, but a cut-off point of 20% body fat for boys.

The definition of underweight based on %BF has not been examined so intensively. Mei et al. (2002) defined underweight as being below the 15th percentile %BF measured by DXA in non-hispanic white and black children aged 3-19 y. According to this, 15th percentile %BF gave three values cut-off points of %BF to define underweight in children aged 3-5 y, 6-11 y and 12-19 y as followings: 10.2%, 11.3% and 12.5%, respectively.

1.1.4. Fat-free mass (FFM)

FFM (or fat-free body) is defined as all residual, lipid-free chemicals and tissues, including water, muscle, bone connective tissue and internal organs. Lean body mass (LBM) differs from FFM. LBM represents the weight of your muscles, bones, ligaments, tendons, and internal organs. Since there is some essential fat in the marrow of your bones and internal organs, the LBM includes a small percentage of essential fat (Heyward and Stolarczyk 1996). The body's water, glycogen, and protein mass make up the lean mass. FFM measured by DXA is the sum of lean mass plus BMC.

1.1.5. Body composition applications

Too high or too low body fat is a risk factor of many health problems. Obesity is a serious health problem that reduces life expectancy by increasing the risk of developing coronary artery disease, hypertension, Type II diabetes, obstructive pulmonary disease, osteoarthritis, and certain types of cancer. Too little body fatness, as found in individuals with eating disorders (anorexia nervosa), exercise addition, and certain diseases such as cystic fibrosis, can lead to serious physiological dysfunctions (Heyward and Stolarczyk 1996). Therefore, health professionals need to understand the principles underlying the assessment of total body composition and regional fat distribution. Medical, health, and fitness professionals measure body composition in order to:

1. Identify one's health risk associated with excessively low or high levels of total body fat.
2. Identify one's health risk associated with excessive accumulation of intra-abdominal fat.
3. Promote the one's understanding of health risks associated with too little or too much body fat.
4. Monitor changes in body composition that are associated with certain diseases.

5. Assess the effectiveness of nutrition and exercise interventions in altering body composition.
6. Estimate ideal body weight of clients and athletes.
7. Formulate dietary recommendations and exercise prescriptions.
8. Monitor growth, development, maturation, and age-related changes in body composition.

1.2. Body composition models

1.2.1. Two-compartment model (2-C model)

Two-compartment model divides the body into fat and FFM. The assumption of this model, is that the constituents of the fat mass and FFM compartments have constant densities (0.900 and 1.100 kg/l), respectively (Visser et al. 1997). With this assumption, this model ignores interindividual variability in the composition of FFM. The direct measurement of body fat mass has never been easy and remains a significant challenge for most body composition techniques. By determining the amount of a given constituent, such as water, potassium, or nitrogen present in the body, the magnitude of the FFM can easily be calculated. Total body water is determined by isotope dilution (D_2O, tritium, or ^{18}O), total body potassium by assay of ^{40}K, a natural isotope, and total body nitrogen by neutron activation. Body fat can be defined indirectly as the difference between body weight and FFM.

The 2-C model, which has been used in body composition research for more than 50 years, continues to serve a vital role, especially in the evaluation of newer technologies focusing on body fat assessment (Ellis 2000). Practical methods of assessing body composition such as skinfolds, bioelectrical impedance analysis (BIA), and hydrostatic weighing are based on the 2-C model of body composition. Another methods based on the 2-C model are total body water, total body potassium, total body nitrogen, nitrogen balance, urinary creatinin excretion and anthropometry (Forbes 1999).

1.2.2. Three-compartment model (3-C model)

The 3-C model divides the body into fat, water, and the remaining solids (predominately protein and minerals), which is assumed to have a constant ratio of protein to mineral (Wells et al. 1999). The advantage of this model over the two-compartment model is that it avoids the assumption that the water content of FFM is constant between individuals of a given age and sex, and it can also provide an estimate of the hydration and density of FFM.

1.2.3. Four-compartment model (4-C model)

The 4-C model divides the body into fat, water, protein, and mineral; thereby further avoiding the assumption that the ratio between mineral and protein in FFM is constant. However, the ratio of bone mineral to total body mineral is still assumed to be constant. The ability of the 4-C model to adjust for body mineral mass may result in improved accuracy in the estimation of the hydration and density of FFM, compared with the 3-C model. To obtain a measure of the mass of each of these body compartments, two additional measurements (neutron activation analysis for body protein and DXA for BMC) would be needed.

1.3. Body composition methods

There are many methods for measuring body composition. In this thesis, only bioelectrical impedance analysis, dual energy X-ray absorptiometry, skinfold and dilution methods are mentioned.

1.3.1. Bioelectrical impedance analysis (BIA)

BIA is a rapid, non-invasive, painless, and relatively inexpensive method for evaluating body composition in field and clinical settings. It requires little subject cooperation, and does not require a high level of technical skill and can be used to estimate body composition in obese individuals (Schaefer et al. 1994). Thomasset's pioneering work in the early 1960s established basic BIA principles (Thomasset 1962). With this method, low-level electrical current is passed through the body, and the impedance (Z), or opposition to the flow of current, is measured with a BIA analyzer. The total body water (TBW) can be estimated from the impedance measurement because the electrolytes in the body's water are excellent conductors of electrical current. When the volume of TBW is large, the current flows more easily through the body with less resistance (R). The resistance to current flow will be greater in individuals with large amounts of body fat, because adipose tissue contains relatively small water, it is a poor conductor of electrical current. The water content of FFM is relatively large (73% water), FFM can be predicted from TBW estimates.

1.3.1.1. Assumption

BIA is based on the two following assumptions (Heyward and Stolarczyk 1996):

1. The human body is shaped like a cylinder with a uniform length and cross-sectional area.

2. Assuming the body is a perfect cylinder, at a fixed signal frequency (e.g., 50 kHz), the impedance (Z) to current flow through the body is directly related to the length (L) of the conductor (height) and inversely related to its cross-sectional area (A). It means $Z = p(L/A)$, where p is the specific resistivity of the body's tissues and is a constant. Multiplying both sides of the equation by L/L gives: $Z = pL^2/AL$, where AL is equal to volume (V). Substituting gives $Z = pL^2/V$ or $V = pL^2/Z$. Thus, the volume of the FFM or TBW of the body is directly related to L^2, or height squared (HT^2), and indirectly related to Z.

1.3.1.2. Principles

There are two principles that BIA method is based on:

1. Biological tissues act as conductors or insulators, and the flow of current through the body will follow the path of least resistance. To measure total body impedance, a low-level excitation current (500 μA to 800 μA) at 50 kHz is used. At low frequencies (approximately 1 kHz), the current passes through the extracellular fluids only; at higher frequencies (500 kHz to 800 kHz), it penetrates cell membranes and passes through the intracellular fluids, as the well as the extracellular fluid (Lukaski 1987).

2. Impedance is a function of resistance and reactance, where $Z = \sqrt{R^2 + X_c^2}$. Resistance (R) is a measure of pure opposition to current flow through the body; reactance (Xc) is the opposition to current flow caused by capacitance produced by the cell membrane (Kushner et al. 1992).

1.3.1.3. BIA prediction equations

BIA prediction equations are based on either population-specific or generalized models. These equations estimate FFM and TBW because of theoretical and empirical relationships established among FFM, TBW, and bio-impedance measures.

Many **population-specific** BIA equations have been developed for homogenous subgroups to account for differences due to age, ethnicity, gender, and physical activity level, and level of body fatness. These equations are valid for and can only be applied to individuals whose physical characteristics are similar to those in the specific population subgroup.

Generalized BIA equations have been developed for heterogeneous populations varying in age, gender, and body fatness. This approach accounts for the biological variability among

population subgroups by including factors such as age and gender as predictor variables in BIA equations estimating FFM or TBW.

The human body is not a perfect cylinder with a uniform cross-sectional area, and the specific resistivity of tissues is not constant. Thus, including body weight in the equation may be one way of accounting for the complex geometric shape of the body, as well as individual differences in trunk size. The predictive accuracy of BIA equations typically is improved by including body weight, along with HT^2 and R, in the BIA regression model (Kushner 1992).

The size of resistance (R) is much large than Xc (at a 50 kHz frequency) when measuring whole body impedance; therefore, R is a better predictor of FFM and TBW than impedance (Z) (Lohman 1989). For this reasons, the resistance index (HT^2/R), instead of impedance index (HT^2/Z), is often used in many BIA models to predict FFM and TBW. However, several models used HT^2/Z as the predictor variable to predict FFM (Deurenberg et al. 1990; Schaefer et al. 1994; de Lorenzo et al. 1998; Pietrobelli et al. 2003).

BIA prediction equations should be selected based on the age, gender, ethnicity, physical activity level, and level of body fatness. Use of inappropriate equations can lead to systematic prediction errors in estimating FFM.

1.3.1.4. Using the BIA method

This technique uses four electrodes applied to the hand, wrist, foot, and ankle at defined positions on the right side of the body. An excitation current of (500 μA to 800 μA) at 50 kHz is applied at the source (distal) electrodes on the hand and foot, and the voltage drop due to impedance is detected by the sensor (proximal) electrodes on the wrist and ankle. Traditionally, there are two approaches to measure impedance: two electrode and four-electrode techniques. In the two-electrode technique, the electrodes that sense the voltage drop are the same as those that introduce the current. This technique gives highly accurate measurements with a very low amplitude current. However there are two major disadvantages to this method: first, the impedance measured reflects both the impedance of the body as well as that due to electrode polarization, which may be high at low frequencies (Ackmann and Seitz 1984); second, needle electrodes must be used to avoid the high impedance of the skin. These needle electrodes must be inserted subcutaneously in a standardized fashion and may result in minor pain and local tissue trauma that reduce both the acceptability and the accuracy of the impedance measurement. The four surface-electrode technique overcomes the main

disadvantages of the two-electrode approach. In this technique, because the electrodes that inject the current are separate from those that detect the potential, impedance due to electrode polarization can be eliminated. The use of spot or band electrodes that are attached to the surface of the skin, rather than penetrating it, avoids problems associated with pain and tissue trauma. Therefore, the four-electrode approach is used more frequently.

The accuracy and precision of the BIA method are affected by instrumentation, subject factors, technician skill, environmental factors, and the prediction equation used to estimate FFM (Kushner 1992). The instrumentation is a substantial source of error and one limitation of the BIA method. To control for this error, the same instrument should be used when monitoring changes in body composition. Subject factors like eating, drinking, dehydrating, and exercising alter the individual's hydration state, thereby affecting total body resistance and the estimate of FFM (Heyward and Stolarczyk 1996). To decrease the errors in estimating FFM or TBW, the technician should require the subjects to lie in a supine position on a nonconductive surface in a room with normal ambient temperature of 22^{o} C with arms and legs comfortably apart, at about a 45^{o} angle to each other. In order to ensure accuracy when using the BIA method, subjects should follow the guidelines as described below:

- No eating or drinking within 4 hours of the test.
- No exercise within 12 hours of the test.
- Urinate within 30 minutes of the test.
- No alcohol consumption within 48 hours of the test.
- No diuretic medications within 7 days of the test.

1.3.2. Dual energy X-ray absorptiometry (DXA)

DXA uses two different energy levels of X-rays to pass through tissue and the attenuation is measured. The DXA procedure is based on an analysis of gamma rays emitted from the body in response to a low intensity neutron beam. Lean tissue, bone and fat tissues emit different gamma ray responses from which an estimation of body composition can be made (Ellis et al. 1994).

DXA is a technology that is gaining recognition as a reference method for body composition research. DXA is also an attractive alternative to hydrodensitometry as a reference method because it is safe, rapid, requires minimal subject cooperation, and most importantly, accounts for individual variability in bone mineral (Heyward and Stolarczyk 1996).

1.3.2.1. History and development of DXA

The widespread use of single and dual photon absorptiometry from 1963 to 1984 preceded the recent development of DXA. Prior to the development of DXA, single (SPA) and dual photon (DPA) absorptiometry were used to estimate regional BMC (g/cm) and bone mineral density (BMD) (g/cm^2). The SPA technique was developed first and used iodine-125 as the photon source (Cameron and Sorenson 1963; Mazess and Cameron 1972). Next, the DPA technique was developed and validated, iodine-125 was replaced with gadolinium-153, which has gamma emissions at both 44 and 100 keV (Gotfredsen et al. 1984). Due to the decay of the radioactive source over time from DPA technique leading to a lack of precision in estimating BMD changes in the same subject, the DXA was developed in which the radioactive source is replaced by an x-ray tube with a filter to convert the polychromatic x-ray beam into low and high energy peaks (Lohman 1992).

1.3.2.2. Radiation exposure from DXA

The general applicability of DXA in human population of all ages results from the low radiation exposure. The exposure for a whole-body scan ranges from 0.05 mrem to 1.5 mrem (1 mrem = 10 μSv) depending on the instrument and the scan speed. This exposure is less than that during one transcontinental flight across the U.S (4 to 6 mrem), much less than the typical radiation exposure with conventional x-rays of 25 to 270 mrem (chest x-ray, CT scan), thus, DXA is used widely for subjects of all ages. Since some radiation is involved, DXA is not recommended for use with pregnant women (Roche et al. 1996).

1.3.2.3. Application of DXA

The primary application of DXA has been to obtain site-specific measurements of areal BMD (gram/cm^2) at the lumbar spine, femur, and forearm. The BMD is defined as the ratio of BMC to bone area (BA), where BA is the total area in the planar scan image for all pixels classified as containing bone (Lunt et al. 1997). DXA provides information on three components of body composition: fat mass (gram), lean mass (in grams), BMC (grams), FFM is calculated as the sum of lean tissue plus BMC (grams), and fat mass as percent of body mass (%) in different body regions (i.e., trunk, head, arms and legs) and in whole body. DXA has clearly attained a dominant role in the measurement of bone loss for clinical diagnosis of osteopenia and osteoporosis (Laskey 1996). In clinical practice, DXA helps to monitor the disease processes, the treatment outcome and to evaluate nutrition and exercise interventions.

1.3.3. Skinfold method

The thickness of subcutaneous adipose tissue was measured by taking skinfold (SKF) measurement (Brozek and Keys 1951). Although SKF thickness varied at different sites, there were moderate to high relationships among SKF measurements (Brozek and Keys 1951). Over the years, the SKF method has been widely used to estimate total body fatness in field and clinical settings. Because the SKF test is cheap, easy to measure, it is suitable for large-scale epidemiological surveys (Kuczmarski et al. 1994) and clinical nutritional assessment. A SKF indirectly measures the thickness of subcutaneous adipose tissue. Therefore, some basic relationships are assumed when using the SKF method to estimate total body density to derive %BF (Heyward and Stolarczyk 1996).

1.3.3.1. Assumptions

1. The SKF is a good measure of subcutaneous fat. It has been shown that the subcutaneous fat, assessed by SKF measurements at 12 sites, is similar to the value obtained from magnetic resonance imaging (Hayes et al. 1988).
2. The distribution of fat subcutaneously and internally is similar for all individuals within each gender.
3. Because there is relationship between subcutaneous fat and total body fat, the sum of several skinfolds can be used to estimate total body fat.

1.3.3.2. Principles

1. There is a relationship between the sum of SKFs and body density.
2. Age is an independent predictor of body density for both men and women.

1.3.3.3. Skinfold prediction models

SKF prediction equations are developed using either linear (population-specific) or quadratic (generalized) regression models. There are many **population-specific** equations to predict body density from various combinations of SKFs, circumferences, and bony diameters. These equations were developed for relatively homogeneous populations and are assumed to be valid only for those having similar characteristics, such as age, gender, ethnicity, or level of physical activity (Heyward and Stolarczyk 1996). **Generalized** equations are applicable to individuals with age range from 18-60 years, and body fat up to 45% (Jackson and Pollock 1978; Lohman 1981). These equations take into account the effect of age on the distribution

of subcutaneous and internal fat. The advantage of the generalized equations is that one equation, instead of several, can give accurate estimates of %BF (Heyward and Stolarczyk 1996).

Most equations use two or three SKFs to predict body density. Then, body density is converted to %BF using the appropriate population-specific conversion formula. Some authors developed SKF equations to predict directly %BF rather than body density in children (Slaughter et al. 1988).

1.3.3.4. Using the skinfold method

The validity and reliability of SKF measurements and SKF method are affected by the technician's skill, type of SKF caliper, subject factors, and the prediction equation used to estimate body fatness (Lohman et al. 1984). To increase the accuracy and precision of the SKF measurements, experts recommend practicing SKF technique of the technician on 50 to 100 subjects to develop a high level of skill and proficiency (Katch and Katch 1980). The accuracy of the caliper should be checked periodically and the same caliper should be used when monitoring changes in the SKF thickness. Variability in SKF measurements among individuals may be attributed to hydration levels of the subjects, SKF measurement should not be done immediately after exercise. SKF prediction equations should be selected based on the age, gender, ethnicity, and physical activity level (Heyward and Stolarczyk 1996).

1.3.4. Dilution method

1.3.4.1. Basic principle

The basic principle of the dilution techniques for body composition analysis is that the volume of a compartment can be defined as the ratio of the dose of a tracer, administered orally or intravenously, to its concentration in that body compartment within a short time after the dose is administered (Ellis 2000). Two fluid samples (either blood, saliva, or urine) are collected: the first sample just before administration of the dose, to determine the natural background levels; the other sample after waiting a sufficient amount of time for penetration of the tracer within the compartment of interest.

1.3.4.2. Basic assumptions

Any tracer dilution technique is based on the four basic assumptions as follows:

1. The tracer is distributed only in the exchangeable pool.
2. The tracer is equally distributed within this pool.
3. The tracer is not metabolized during the equilibration time.
4. The tracer equilibration is achieved relatively rapidly.

1.3.4.3. Application of dilution method

The dilution method is used to measure total body water (**TBW**)**,** extracellular (**ECW**) and intracellular water (**ICW**). The earliest and the most direct, in vivo measurement technique for **TBW** uses a tracer dose of labeled water: tritium-a radioactive isotope of hydrogen (T or ^{3}H); deuterium-isotope of hydrogen with an atomic weight of 2.014 or also called as heavy water (deuterium oxide, D_2O); or stable isotopes of oxygen (oxygen-18, ^{18}O) and collection of two body fluid samples (blood, urine, or salvia), one predose and the second after an equilibration time of 2-3 hours. The method of analysis is dependent on the choice of tracer: radioactive β-counting for tritium, mass spectroscopy for ^{18}O, and infrared absorption, gas chromatography, or mass spectroscopy for deuterium (Ellis 2000). **TBW** has been measured using ^{18}O and D_2O for more than 40 years. It is a very useful and highly popular technique to estimate lean body mass. The principle is based on the theory that water is distributed in all parts of the body except body fat. It is said that in healthy adults, water constitutes approximately 73% of the FFM or 60% of body weight for nonobese subjects (Kotler et al. 1999); in full-term birth, health infants **TBW** constitutes typically 80-83% of the FFM (Ellis 2000). From the ratio between **TBW** and FFM, it is possible to estimate FFM, and fat mass is calculated as body weight minus FFM. Most researchers use D_2O as the tracer because of its low cost.

To measure the volume of **ECW**, body fluid as the plasma is used (Wong et al. 1989). The tracer is nonradioactive Br administered orally, with a second plasma sample usually collected 3-4 hours later (Van Marken Lichtenbelt et al. 1996). The analytical Br assay in most common use is high-pressure liquid chromatography (Miller et al. 1989), a few investigators have used X-ray fluorescence, spectrophotometric, or mass spectrometry techniques (Price et al. 1975; Janghorbani et al. 1988; Trapp and Bell 1989).

To estimate **ICW**, the most common practice in use today is an oral administration of a combined D_2O plus Br dose, from which **TBW** and **ECW** are determined. Then, **ICW** is defined as their difference (**ICW** = **TBW** - **ECW**) (Ellis 2000).

1.4. Anthropometric indices

1.4.1. Body mass index (BMI)

BMI was developed by the Belgian mathematician Adolphe Quetelet in the period between 1830 and 1850. Therefore it was also known as the Quetelet Index. BMI is a measurement of the relative percentages of fat and muscle mass in the human body (Garn et al. 1986). It is equal to the body weight (W, in kg), divided by the square of the body height (H, in meters), the formula is as follows: $BMI = W/H^2$ (Quetelet 1869).

BMI is the one most commonly recommended and widely used for classifying overweight and obesity in adults (World Health Organization 1995) and has also been recommended for screening overweight and obesity in children and adolescents (Himes and Dietz 1994; Goulding et al. 1996; Taylor et al. 1997; Dietz and Robinson 1998; Pietrobelli et al. 1998).

In adults the BMI offers a reliable and valid measure of adiposity (Garrow and Webster 1985). The association of BMI with increased morbidity and mortality rates provides a reasonable justification for the use of graded increases in BMI to assess the severity of obesity (Dietz and Robinson 1998). A BMI value of 25 to 29.99 constitutes grade 1 overweight, a BMI value of 30.0 to 39.99 constitutes grade 2 overweight, and a BMI value of 40.0 or greater constitutes grade 3 obesity (World Health Organization 1995).

In children and adolescents, body fatness changes over the years as they mature and differs, between boys and girls during growth. Therefore, BMI for children is gender and age specific (Hammer et al. 1991; Pietrobelli et al. 1998). **Figure 2** shows the BMI percentile chart for German boys and girls aged from 0-18 years (Kromeyer et al. 2001). BMI is often used to determine underweight, overweight and obesity, usually by comparison of individuals to age and sex-specific percentiles from a reference population (Maynard et al. 2001). An expert committee on Clinical Guidelines for Overweight in Adolescent Preventive Services suggested that adolescents with BMI values ≥ 95th percentile for age and sex should be characterized as overweight, while those at or above the 85th percentile but < 95th percentile should be considered at risk for overweight, those with BMI < 5th percentile should be considered underweight (Himes and Dietz 1994). In paediatric samples there is no internationally accepted definition of overweight and obesity (Pietrobelli et al. 1998). Up to now, BMI reference data have been separately published for North American, French, British, Swedish, Italia and German children (Must et al. 1991; Rolland-Cachera et al. 1991; Cole et

al. 1995; Lindgren et al. 1995; Luciano et al. 1997; Kromeyer et al. 2001).

According to the BMI reference data in German children, in the guidelines of the "Arbeitsgruppe Adipositas im Kindes- und Jugendalter" the 90th and 97th BMI percentiles as calculated in this reference population are proposed as cut-off points for the definition of overweight and obesity in German children and adolescents. Similarly, the 3rd and 10th BMI percentiles are proposed as cut-off points for the definition of severe malnutrition and malnutrition (Kromeyer et al. 2001).

BMI standards have limitations when applied to paediatric populations. First, BMI standards are population-based measures and are not directly related to any biological disturbances in children. Second, BMI is not well correlated with fat mass in children, especially at younger ages (Dietz and Robinson 1998; Pietrobelli et al. 1998). Current definition of obesity, based on age- and sex-specific BMI, may overpredict the level of fat mass and therefore overpredict the prevalence of obesity, particularly in younger children (Obarzanek 1993).

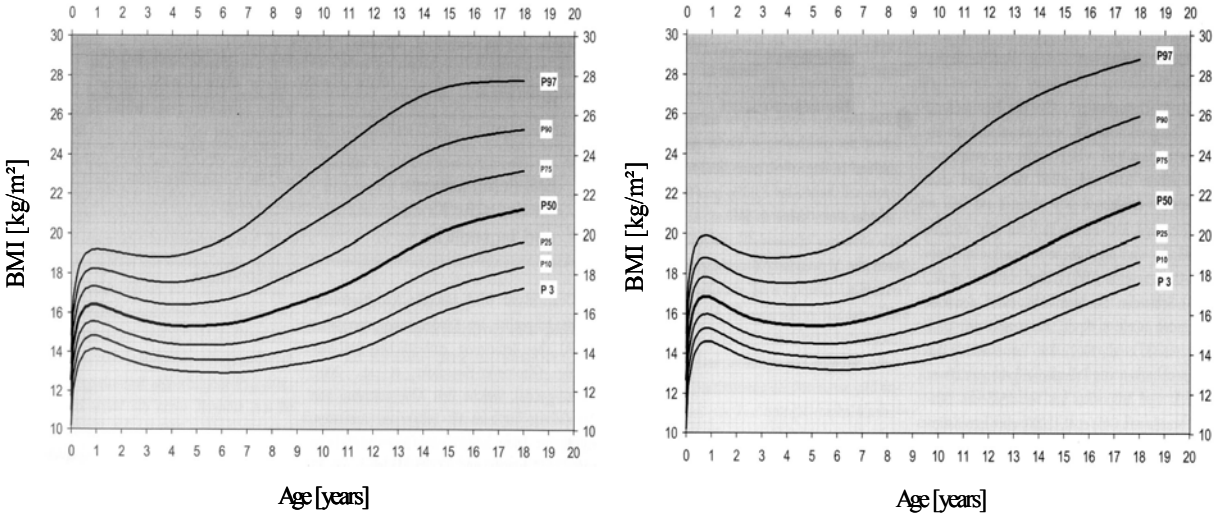

FIGURE 2. BMI percentile chart in German boys (right), and girls (left) aged 0-18 y (Kromeyer et al. 2001).

1.4.2. Body mass index standard deviation scores (BMI-SDS)

BMI-SDS is calculated according to the LMS method of (Cole and Green 1992) which describes the distribution of a measurement by its median (M), the coefficient of variation (S) and a measure of skewness (L). These three parameters are dependent on age. The formula to convert a child's BMI value to an exact BMI-SDS as follows:

$$\text{BMI-SDS} = [(\text{BMI}/\text{M})^{L} - 1]/(L \times S).$$

where L, M, S are the gender-specific values of L, M and S interpolated for the child's age (Schaefer et al. 1998). Depending on the reference data, for examples, the LMS constants are available at the CDC's website: www.cdc.gov/growthcharts for American children aged 2-20 years or in the guidelines of the Obesity Working Group in children and adolescents at the website www.a-g-a.de for German children aged 0-18 years. Two tables below present BMI percentile in German boys and girls (see next page).

1.4.3. Relationship between BMI-SDS and BMI percentiles

Smoothed percentile curves and z-scores for BMI are used to evaluate the growth of children. BMI-SDS or BMI z-scores have a direct relationship with percentiles, therefore BMI-SDS and percentiles are interchangeable. BMI-SDS values of -1.881, -1.645, -1.281, -1.036, -0.674, 0, 0,674, 1.036, 1.282, 1.645, 1.881 correspond with the BMI-for-age percentiles as follows: 3rd percentile, 5th percentile, 10th percentile, 15th percentile, 25th percentile, 50th percentile, 75th percentile, 85th percentile, 90th percentile, 95th percentile and 97th percentile (Kromeyer et al. 2001).

TABLE 1

BMI percentile and LMS values in German boys aged 0-18 years old (Kromeyer et al. 2001)[¶]

Age (y)	L	S	P3	P10	P25	P50 (M)	P75	P90	P97
0	1.31	0.10	10.20	11.01	11.81	12.68	13.53	14.28	15.01
0.5	-0.67	0.08	14.38	15.06	15.80	16.70	17.69	18.66	19.72
1	-1.05	0.08	14.58	15.22	15.93	16.79	17.76	18.73	19.81
1.5	-1.28	0.08	14.31	14.92	15.60	16.44	17.40	18.37	19.47
2	-1.45	0.08	14.00	14.58	15.25	16.08	17.03	18.01	19.14
2.5	-1.58	0.08	13.73	14.31	14.97	15.80	16.76	17.76	18.92
3	-1.67	0.09	13.55	14.13	14.79	15.62	16.59	17.62	18.82
3.5	-1.75	0.09	13.44	14.01	14.67	15.51	16.50	17.56	18.80
4	-1.80	0.09	13.36	13.94	14.60	15.45	16.46	17.54	18.83
4.5	-1.85	0.09	13.30	13.88	14.55	15.42	16.45	17.56	18.90
5	-1.88	0.09	13.24	13.83	14.51	15.40	16.46	17.61	19.02
5.5	-1.90	0.10	13.20	13.80	14.50	15.40	16.50	17.71	19.19
6	-1.92	0.10	13.18	13.79	14.51	15.45	16.59	17.86	19.44
6.5	-1.92	0.10	13.19	13.82	14.56	15.53	16.73	18.07	19.76
7	-1.92	0.11	13.23	13.88	14.64	15.66	16.92	18.34	20.15
7.5	-1.92	0.11	13.29	13.96	14.76	15.82	17.14	18.65	20.60
8	-1.91	0.11	13.37	14.07	14.90	16.01	17.40	19.01	21.11
8.5	-1.89	0.12	13.46	14.18	15.05	16.21	17.68	19.38	21.64
9	-1.87	0.12	13.56	14.31	15.21	16.42	17.97	19.78	22.21
9.5	-1.85	0.13	13.67	14.45	15.38	16.65	18.27	20.19	22.78
10	-1.83	0.13	13.80	14.60	15.57	16.89	18.58	20.60	23.35
10.5	-1.80	0.13	13.94	14.78	15.78	17.14	18.91	21.02	23.91
11	-1.77	0.14	14.11	14.97	16.00	17.41	19.24	21.43	24.45
11.5	-1.75	0.14	14.30	15.18	16.24	17.70	19.58	21.84	24.96
12	-1.72	0.14	14.50	15.41	16.50	17.99	19.93	22.25	25.44
12.5	-1.69	0.14	14.73	15.66	16.77	18.30	20.27	22.64	25.88
13	-1.66	0.14	14.97	15.92	17.06	18.62	20.62	23.01	26.28
13.5	-1.63	0.14	15.23	16.19	17.35	18.94	20.97	23.38	26.64
14	-1.61	0.14	15.50	16.48	17.65	19.26	21.30	23.72	26.97
14.5	-1.58	0.14	15.77	16.76	17.96	19.58	21.63	24.05	27.26
15	-1.55	0.14	16.04	17.05	18.25	19.89	21.95	24.36	27.53
15.5	-1.52	0.13	16.31	17.33	18.55	20.19	22.26	24.65	27.77
16	-1.49	0.13	16.57	17.60	18.83	20.48	22.55	24.92	27.99
16.5	-1.47	0.13	16.83	17.87	19.11	20.77	22.83	25.18	28.20
17	-1.44	0.13	17.08	18.13	19.38	21.04	23.10	25.44	28.40
17.5	-1.41	0.13	17.32	18.39	19.64	21.31	23.36	25.68	28.60
18	-1.39	0.13	17.56	18.63	19.89	21.57	23.61	25.91	28.78

[¶](M), median; (S), coefficient of variation; (L), measure of skewness.

TABLE 2

BMI percentile and LMS values in German girls aged 0-18 years old (Kromeyer et al. 2001)[¶]

Age (y)	L	S	P3	P10	P25	P50 (M)	P75	P90	P97
0	1.34	0.10	10.21	10.99	11.75	12.58	13.40	14.12	14.81
0.5	-0.03	0.08	13.86	14.55	15.29	16.16	17.08	17.95	18.85
1	-0.44	0.08	14.14	14.81	15.53	16.40	17.34	18.25	19.22
1.5	-0.71	0.08	13.94	14.59	15.32	16.19	17.16	18.11	19.15
2	-0.92	0.09	13.68	14.33	15.05	15.93	16.93	17.92	19.03
2.5	-1.07	0.09	13.46	14.10	14.82	15.71	16.73	17.76	18.92
3	-1.19	0.09	13.29	13.93	14.64	15.54	16.57	17.64	18.84
3.5	-1.30	0.09	13.16	13.79	14.51	15.42	16.46	17.56	18.81
4	-1.38	0.10	13.06	13.69	14.42	15.33	16.40	17.54	18.95
4.5	-1.46	0.10	13.00	13.64	14.37	15.31	16.41	17.58	18.97
5	-1.52	0.10	12.97	13.61	14.36	15.32	16.46	17.69	19.16
5.5	-1.58	0.10	12.94	13.60	14.36	15.35	16.53	17.83	19.40
6	-1.62	0.11	12.92	13.59	14.37	15.39	16.63	17.99	19.67
6.5	-1.65	0.11	12.93	13.62	14.42	15.48	16.77	18.21	20.01
7	-1.66	0.12	12.98	13.69	14.52	15.62	16.98	18.51	20.44
7.5	-1.65	0.12	13.06	13.80	14.66	15.81	17.24	18.86	20.93
8	-1.64	0.12	13.16	13.92	14.82	16.03	17.53	19.25	21.47
8.5	-1.61	0.13	13.27	14.06	15.00	16.25	17.83	19.65	22.01
9	-1.58	0.13	13.38	14.19	15.17	16.48	18.13	20.04	22.54
9.5	-1.54	0.13	13.48	14.33	15.34	16.70	18.42	20.42	23.04
10	-1.51	0.14	13.61	14.48	15.53	16.94	18.72	20.80	23.54
10.5	-1.47	0.14	13.76	14.66	15.74	17.20	19.05	21.20	24.03
11	-1.43	0.14	13.95	14.88	15.99	17.50	19.40	21.61	24.51
11.5	-1.39	0.14	14.18	15.14	16.28	17.83	19.78	22.04	25.00
12	-1.36	0.14	14.45	15.43	16.60	18.19	20.18	22.48	25.47
12.5	-1.33	0.14	14.74	15.75	16.95	18.56	20.58	22.91	25.92
13	-1.30	0.14	15.04	16.07	17.30	18.94	20.98	23.33	26.33
13.5	-1.27	0.14	15.35	16.40	17.64	19.30	21.36	23.71	26.70
14	-1.25	0.14	15.65	16.71	17.97	19.64	21.71	24.05	27.01
14.5	-1.23	0.14	15.92	17.00	18.27	19.95	22.02	24.35	27.26
15	-1.20	0.14	16.18	17.26	18.53	20.22	22.28	24.59	27.45
15.5	-1.18	0.13	16.40	17.49	18.76	20.45	22.50	24.77	27.57
16	-1.16	0.13	16.60	17.69	18.96	20.64	22.67	24.91	27.65
16.5	-1.13	0.13	16.78	17.87	19.14	20.81	22.82	25.02	27.69
17	-1.11	0.13	16.95	18.04	19.31	20.96	22.95	25.11	27.72
17.5	-1.09	0.13	17.11	18.20	19.47	21.11	23.07	25.20	27.74
18	-1.07	0.12	17.27	18.36	19.62	21.25	23.19	25.28	27.76

[¶](M), median; (S), coefficient of variation; (L), measure of skewness.

1.4.4. Special situation: indices used to assess nutritional status in neonates

As described above, BMI and BMI-SDS have been used to classify and evaluate the nutritional status of older children and adults. To study the body composition of neonates, the next three indices based on birth weight, birth length, gestational age at birth or body weight, length at time of study are mentioned.

1.4.4.1. Ponderal index (PI)

Rohrer's ponderal index is the most commonly used index of neonatal body proportionality. It has been used as an indicator of fetal growth status, especially to assess asymmetrical intrauterine growth retardation. PI is an indicator of wasting, newborns born with low PI (also known as "disproportionate" or thin) tend to experience more pronounced catch-up growth in childhood than those born with adequate ponderal indices (Khoury et al. 1990). PI relates birth weight to birth length: PI = 100 x birth weight (gram) divided by the cube of birth length (cm^3). High PI at birth is defined as PI above the 90th percentile, and normal PI at birth is defined as PI between 10th and 90th percentiles, low PI at birth is define as PI below 10th percentile (Lande et al. 2005).

1.4.4.2. Birth weight-for-gestational-age

Weight-for-gestational age at birth is often used to categorize an individual infant as having experienced normal, subnormal (small-for-gestational age or intrauterine growth retardation), or supranormal growth in utero. The classification most frequently used is: small-for-gestational age (SGA), appropriate-for-gestational age (AGA) and large-for-gestational age (LGA). The most commonly used cut-off points to distinguish between these three categories are based on percentiles of a distribution of birth-weight-for-gestational age derived from an accepted reference population; the 10th percentile is used most frequently as the cut-off between SGA and AGA, and the 90th percentile between AGA and LGA (Hediger et al. 1999)

The classification of a newborn as either SGA or LGA has implications for diagnosis, prognosis, surveillance, and treatment. SGA infants are more likely to have congenital anomalies (Khoury et al. 1988), the observation that an infant is growth-retarded often prompts a more careful physical examination or even laboratory tests such as karyotype determination to ascertain whether such an anomaly is present. The diagnosis of LGA can be

important for the individual infant. Large infants are at increased risk of birth trauma and of asphyxia secondary to obstructed labour (World Health Organization 1995).

1.4.4.3. Weight-for-length ratio (W/L ratio)

The W/L ratio is also used to assess the fetal growth. A low W/L ratio was correlated with perinatal morbidity and mortality, even in infants not small-for-gestational age (Williams and O'Brien 1997). W/L ratio correlated well with skinfold thickness in both full-term and preterm infants, this ratio is useful for evaluation of the nutritional status of intrauterine growth, and in the prediction of metabolic complications in both full-term and preterm newborns with abnormal intrauterine growth (Yau and Chang 1993).

1.5. Nucleotides

Nucleotides are biologically active, non-protein, nitrogenous compounds, which were first isolated from human milk in 1960 (Yu 1998). Large amount of nucleotides are present in human milk but present in a lower concentration in cow milk. Nucleotides are the structural units of nucleic acids like RNA and DNA; therefore nucleotides and their related metabolic products play a key role in many biological processes. As nucleotides are also essential compounds in energy transfer systems (that is, in ATP and GTP), they are an integral part of carbohydrate, lipid, protein and nucleic acid metabolism and modulators of important neonatal physiological functions (Carver 1995). Besides, nucleotides are components of three major coenzymes (NAD, FAD, CoA) and are metabolic regulators (Stryer 1988). In recent years, there has been an increasing interest in the role of dietary nucleotides in neonatal and infant nutrition. A large number of animal experiments together with several clinical studies on human infants have been conducted to investigate in particular the gastrointestinal and immunological effects as well as the effect on catch-up growth of dietary nucleotides. A comprehensive review of the literature was published in 1995 (Carver 1995).

2. Scope of this thesis

There is growing recognition of the need to measure body composition in sick children. First, in clinical settings, the rise in the prevalence of childhood obesity has increased the demand for accurate methods for determining body fatness in younger age groups. Second, measurement of body composition is important for optimum clinical care during hospitalization because the data on body composition are necessary for adjusting energy and fluid requirements during artificial nutrition. Third, measurements of body composition aid in the assessment and treatment of childhood growth disorders (Wells et al. 1999).

In hospital, the nutritional status of newborns, infants, children, and adolescents need to be precisely assessed. The nutritional status of children from 0-20 y can be classified based on age- and sex-specific BMI percentile (Himes and Dietz 1994). The limitation of the classification of the nutritional status by using BMI is that BMI cannot distinguish the weight due to fat mass or lean mass. With the advent of DXA and BIA, fat mass and %BF of an individual can be estimated. However, there is no widely accepted definition of obesity based on %BF; to define excess adiposity, age-and sex-specific %BF percentile were arbitrarily chosen (Lazarus et al. 1996; Schaefer et al. 1998). Literature on the performance of BMI in screening for excess adiposity is relatively sparse and no systematic evaluation appears to have been published (Lazarus et al. 1996). A number of study have been carried out in healthy children to investigate the relationship between BMI and %BF (Goran et al. 1996; Gutin et al. 1996; Lazarus et al. 1996; Daniels et al. 1997; Pietrobelli et al. 1998; Sampei et al. 2001; Tyrrell et al. 2001). In sick children, to our knowledge, this relationship has never been studied. The physiological status differs between sick and healthy children, it may lead to a different results. In chapter II the relationship between BMI-SDS and %BF is presented in a group of 393 children suffering from different kinds of diseases. Correlation, kappa agreement test, sensitivity and false-positive rates are used to assess this relation.

In clinical practice, it is necessary to have a rapid, safe, non-invasive, and precise method for use at bedside to measure body composition, especially for preterm babies. BIA is a method that meets these criterias (Nagano et al. 2000). %BF and TBW have been estimated from prediction equations (Kushner et al. 1992; Schmelzle and Fusch 2002). However, to the best of our knowledge, prediction equations for estimating FFM- an important component of body composition has not been yet developed in preterm babies. Protein energy malnutrition is still common in hospitalized paediatric patients (Hendrickse 1997). Protein is a main component

of FFM, preterm babies are particularly susceptible to loss of lean body mass. Therefore, attention is not only paid on the primary medical problems but also on the nutritional status of patients. The measurement of FFM should be an essential work of patient care, thereby an adequate nutrition support can be followed to improve the nutritional status of preterm babies. Chapter III presents the development of prediction equations for FFM in preterm babies and elucidate the role of BIA method applied for this population.

One of the most important body composition studies is to validate the published prediction equations and to test whether they are appropriate for use in a specific group. Crohn's disease is a chronic inflammatory disease of the intestine, patients with Crohn's disease have a decreased bone mineral density, weight loss and significant loss of FFM (Azcue et al. 1997; Boot et al. 1998; Tjellesen et al. 1998). The assessment of FFM for patients with Crohn's disease helps to quantify the degree of malnutrition and allows the clinicians to assess the extent of patient's nutritional depletion and provide patients with adequate nutrition supports during treatment process. Besides, the development of simple, accurate prediction equations using BIA specifically used for this population is needed for clinical practice. Only one study in adults with Crohn's disease correlated total body water with BIA (Royall et al. 1994). Chapter IV presents the results of the validation of some existing prediction equations derived from healthy children for children aged 7.3-16.9 years suffering from Crohn's disease and introduces a new prediction equation for estimating FFM in this population.

Another interesting research study involving body composition analysis is to compare the role of BIA with anthropometric method. BIA is said to have potential value as it can be used easily, quickly, and safely at patient's bedside, shows less inter-observer variation than do traditional anthropometric measurement (Schaefer et al. 1994). FFM or TBW is often estimated from prediction equations using BIA or anthropometric measurements as the predictors (Lukaski et al. 1985; Segal et al. 1985). To see the usefulness of BIA, it is necessary to justify the accuracy and precision of BIA-based predictive equations compared with those based on anthropometric measurements alone. In healthy subjects, there has been controversy about the predictive role of BIA-based and anthropometric-based predictors in estimating FFM: impedance index was reported to be better than anthropometric variable like body weight (Diaz et al. 1989; Sun et al. 2003); in contrast, some studies reported that height (or height^2) and weight were more significant predictors than were impedance index (Helenius et al. 1987; Gray et al. 1989). In a group of paediatric patients, BIA needs to be proved that it can be a method of choice to estimate FFM in comparison with anthropometric

method. Chapter V compares the predictive value of impedance index, weight, height, BMI for FFM in a group of patients aged 13.1 ± 3.3 y.

To assess the fetal growth of newborns, birth-weight-for-gestational age derived from an accepted reference population is used. According to birthweight percentile for gestational age and sex, newborns are classified as SGA, AGA, and LGA groups. Because this classification system is based on birthweight, it may not reflect well %BF. DXA measures precisely fat mass and %BF, then %BF cut-off points can be arbitrarily chosen to define low, medium and high fat. Weight-for-length ratio, ponderal index and BMI were considered to give a better reflection of soft tissue than birthweight percentile (Rohrer 1921). In the first days after birth, body fat is very important for the normal growth of newborns. The applicability of the classification depending on birthweight to predict %BF measured by DXA and the correlations between some anthropometric indices and fat mass measured by DXA in a population of term and preterm babies are presented in chapter VI.

Body composition measurement can be applied to evaluate the effects of nutrition intervention studies. Dietary supplementation of nucleotides in infant formulas is a kind of such studies. Recently, there have been a number of studies reporting the role of nucleotides in human nutrition (Carver 1995; Cosgrove et al. 1996). Traditionally, the composition of human milk is optimal for the growth and development of the infant during the first months of life, contains large amounts of nucleotides, and serves as the ‘gold standard’ in the research and development of infant formulas. Meanwhile, nucleotides in cow milk are present in a lower concentration and with a different composition compared with human milk. However, cow milk is the source of the vast majority of infant formulas, therefore an increasing number of infant formulas has been manufactured and marketed which are supplemented with nucleotides. Nucleotides can be synthesised endogenously and thus are not essential nutrients, during the period of rapid growth or after injury, dietary nucleotides may become essential nutrients (Rudloff and Kunz 1997). One study showed the positive effect of dietary nucleotide supplementation on growth in term small for gestational age infants (Cosgrove et al. 1996). Another study showed that nucleotide supplementation did not influence the growth of formula fed infants (Hawkes et al. 2006). Two studies showed the benefit of dietary intake of nucleotides on the function of the immune system (Carver et al. 1991; Pickering et al. 1998). In chapter VII the effect of dietary nucleotides on growth and body composition during the first four months of life in healthy term and preterm newborns is presented.

Chapter II

Body mass index versus percentage body fat measured by dual energy x-ray absorptiometry in sick children

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ABSTRACT

Objective: To measure the relationship between BMI standard deviation score (BMI-SDS) and percentage body fat (%BF) in sick children.

Design: Body weight, height and %BF of 393 in-patients aged from 4 mo to 18 y were measured. BMI was calculated and converted into BMI-SDS. %BF was measured by dual energy X-ray absorptiometry. Correlation between BMI-SDS and %BF was performed. The agreement between BMI and %BF in classifying nutritional status was evaluated using Kappa agreement test. Sensitivity and false-positive rates of BMI as the screening variable to define obesity were calculated.

Results: Correlations between %BF and BMI-SDS were 0.88 and 0.87; the kappa coefficients of agreement between BMI and %BF were 0.53 and 0.56 in boys and girls, respectively ($p < 0.0005$). The sensitivity of BMI in boys and girls were 68.4% and 54.1% when %BF cut-off points of 25% (boys) and 30% (girls) were needed to define obesity.

Conclusions: BMI agrees moderately with %BF in classifying nutritional status, BMI as screening variable has a moderate sensitivity in detecting obesity. Prediction of %BF from BMI should be used with caution, for clinical purposes, %BF needs to be estimated by other more precise methods.

KEY WORDS Body composition, nutritional status, obesity, body weight, sensitivity, correlation.

INTRODUCTION

The health of an individual is closely related to the nutritional status, in particular to the amount of body fat as well as lean body mass rather than to body weight alone (Taylor et al. 2002). Body composition refers to the amount of muscle mass, water, fat, bone, connective tissues, internal organs that comprise the body weight (Heyward and Stolarczyk 1996). Body mass index (BMI) is crude index of total body fat mass, and is calculated from body weight and length (Roche et al. 1981). It does not measure body fat directly but is internationally accepted to classify the nutritional status in children and adolescents (Himes and Dietz 1994; Wells et al. 2002). Recently, a national BMI reference data set in healthy German children aged 0 to 18 y was compiled. In the guidelines of the German Obesity Working Group, the BMI percentile values are proposed as cut-off points to classify the nutritional status in German children and adolescents (Kromeyer et al. 2001). Percentage body fat (%BF), in contrast, is measured accurately by more sophisticated methods like dual energy X-ray absorptiometry (DXA), underwater weighing, total body electrical conductivity, total body potassium (Fiorotto et al. 1987; Schaefer et al. 1994; Goran et al. 1998). However, in published literature there are no clear cut-off values of %BF to classify the nutritional status, i.e. normal, overnutrition and undernutrition (Lazarus et al. 1996). Some authors used different %BF cut-off points to identify children at risk of overweight and obesity as well as underweight (Williams et al. 1992; Dwyer and Blizzard 1996). In the clinical settings, monitoring body composition and classifying the nutritional status are important for providing adequate nutritional therapy and supporting the treatment process. Therefore, for a safe use of BMI to classify paediatric subjects, it is necessary to examine the relationship with %BF, which has not been done so far in sick children.

To investigate the relationship between %BF and BMI, usually one of the three following methods were applied. First, correlational analysis was used for making recommendations for prediction equations (Goran et al. 1996; Gutin et al. 1996; Daniels et al. 1997; Pietrobelli et al. 1998). Second, receiver operating characteristic analysis (ROC) was used for describing the performance of BMI as the screening variable while %BF as the reference variable to discriminate obesity from non-obesity (Lazarus et al. 1996; Sardinha et al. 1999; Mei et al. 2002). Third, kappa's agreement test measured the agreement between BMI and %BF in classifying the nutritional status (Sampei et al. 2001).

DXA is considered as a reference method for measuring human body composition because it

is safe and its results correlate well with direct chemical carcass analysis (Fusch et al. 1999). Fat mass obtained by DXA are highly correlated with other methods in children, adolescents and adults (Ogle et al. 1995; Lazarus et al. 1996). The aim of the study was therefore to examine the relationship between BMI and %BF measured by DXA in sick children with special respect to the ability of BMI as a tool to identify subjects at risk of obesity and the agreement between BMI and %BF in classifying the nutritional status.

SUBJECTS AND METHODS

Subjects, anthropometry and body composition measurement

Body composition data of 393 children who had been treated at the Children´s University Hospital in Greifswald between April 1998 and September 2003 were analysed in this study. Three different groups according to the causes for admission in the record chart were: a) patients with acute diseases (n = 76), b) patients with chronic diseases (n = 203), c) obese patients (n = 114). In the acutely ill group, %BF was measured during recovery period shortly before discharge from hospital. In the chronically ill and obese patients, if %BF was measured several times, only the first measurement at the beginning of the treatment was used. The number of patients in different chronic diseases was as follows: haematology and oncology (n = 71), Crohn's and celiac diseases (n = 45), asthma (n = 7), cystic fibrosis (n = 7), anorexia nervosa (n = 7), endocrinology (n = 31), autoimmunology (n = 11), and another diseases (n = 24). The study was approved by the Ethics committee of the Children's Hospital, University of Greifswald and the State Authority for Radiation Exposure and Control. Informed consent was obtained from all subjects and their parents.

Anthropometric measurements were taken by two trained technicians at the body composition research laboratory of the Children's Hospital. Subjects were required to wear only light clothing or underwear before measuring. For infants under 2 years of age, body weight was measured to the nearest 10 g by using a standard beam balance (Seca, Hamburg, Germany); body length was measured to the nearest 0.5 cm in the supine position by using a measuring board (Schaefer, Karlsruhe, Germany). For children over 2 years of age, standing height was measured to the nearest 0.5 cm with a wall-mounted stadiometer (Längenmesstechnik GmbH Limbach-O); body weight was measured to the nearest 0.1 kg with a Seca calibrated mechanical scale.

Because the distribution of BMI is age dependent, BMI-SDS (BMI standard deviation scores), which were adjusted for age, were used. Based on the new reference population from 34 422 healthy German children aged 0 to 18 y and the LMS transformation method by (Cole 1990), BMI was converted to BMI-SDS. The formula is as follows:

$$\text{BMI-SDS} = (\text{BMI}/\text{M})^{L} - 1)/(L*S)$$

where M is the median, S is the coefficient of variation of BMI at each age (every six months) and sex group whereas L is a skewness parameter in the distribution of BMI. Values for L, M, and S were derived from the study of Kromeyer et al. (2001).

%BF was measured by DXA using a whole body scanner (QDR 1500; Hologic, Waltham, MA, USA) with 5.67 version software (Hologic). Before each scan, shoes and metal objects were removed from the patients, patients were lying quietly in supine position during scans. The entire body of each subject was scanned, beginning at the top of the head and moving down the body to the feet. The whole body scan time took about 8-15 min depending on the length of subjects. The radiation dose from a scan is very low (< 0.05 mSv). Body fat was expressed in kilograms and %BF was expressed in %. Daily quality assurance tests recommended by the manufacturer were performed using a spine phantom. In the period from January 1999 to September 2003, the mean phantom lumbar spine bone mineral content of the DXA device used was 57.02 ± 0.19 gr (n = 724) and was close to the reference value of 57.14 ± 0.16 gr.

Cut-off values for nutritional status

For BMI, age- and sex-specific percentile values from the reference data set in German children and adolescents give cut-off values to classify the nutritional status. Following the accepted limits, subjects below the 10^{th} BMI percentile were classified as underweight, between the 10^{th} and 90^{th} BMI percentiles as normal weight, above the 90^{th} BMI percentile as overweight and obesity (Kromeyer et al. 2001).

To classify the limits for %BF, data on healthy German children aged from 4-14 y (n = 4629, boys: 2293; girls: 2336) were obtained from the Dortmund Nutritional and Anthropometric Longitudinally Designed Study (DONALD) run by the Research Institute of Child Nutrition, Dortmund, Germany. The DONALD Study is an ongoing open cohort study that has been collecting detailed information on diet, growth, development and metabolism between infancy

and adulthood since 1985 (Kroke et al. 2004). Similar to other cohort studies, the DONALD Study uses a convenient sampling scheme, which results in a selected, non-representative study sample characterised by a relatively high educational and socio-economic status (Kroke et al. 2004). However, the distribution of BMI among DONALD Study participants is similar to the German reference data (Buyken et al. 2005). DONALD Study participants' BF% is calculated using skinfold measurements and the equations of Brook (for body density) and Siri (for body fat) (Brook 1971). Due to the absence of a widely accepted definition of excess adiposity, the 10^{th} and 90^{th} age-and sex-specific percentiles of BF% from the DONALD Study were arbitrarily chosen to classify nutritional status. Underweight was defined as the %BF value below 10^{th} percentile, between the 10^{th} and 90^{th} percentile: normal weight, above the 90^{th} percentile: overweight.

Correlation between BMI-SDS and %BF, screening test

To assess the correlation between BMI-SDS and %BF, linear regression analysis was performed to develop simple linear regression equations in boys, girls and both sexes. To assess the performance of BMI in detecting obesity, analysis of a receiver operating characteristic (ROC) curve was done. The ROC curve is a plot of true-positive (sensitivity) against false-positive rates (1- specificity). Sensitivity and false-positive rate were described elsewhere (Lazarus et al. 1996). The range of %BF used as the reference variable was from 20 to 50% and 97^{th} percentile of BMI was set as the screening variable to define obesity. Valid screening variable should have sensitivity close to 100% and false-positive rates close to 0%.

Data processing and statistical analysis

Data were analyzed using SPSS, version 10.0 software (SPSS Inc, Chicago, USA). Results are presented as means and standard deviations. Student's t tests and Mann-Whitney U tests were used to compare variables between groups. Spearman rank correlation coefficients were calculated to assess the correlation between BMI-SDS and %BF. Kappa agreement test was used to measure the agreement between BMI and %BF in determining children's nutritional status. The level of statistical significance was set at $p \leq 0.05$.

RESULTS

Table 1 presents the median and mean %BF values of our subjects according to age group and those in children from the DONALD Study (Kroke et al. 2004) and Lazarus's study (Lazarus et al. 1996). Descriptive statistics are presented in **Table 2** for all subjects and groups classified by BMI percentiles. Body weight, body height, BMI and BMI-SDS were higher in boys than in girls ($p < 0.05$).

Figure 1 shows a significant relationship between %BF and BMI-SDS for boys and girls ($p < 0.0005$). The linear regression equation in boys: %BF = 7.4 x BMI-SDS + 24.4 ($r = 0.88$); in girls: %BF = 5.9 x BMI-SDS + 29.9 ($r = 0.87$); in both sexes: %BF = 6.5 x BMI-SDS + 27.4 ($r = 0.86$), which explained 74% to 77% of the variance in %BF.

Sensitivities and false-positive rates are shown in **Table 3** when BMI ≥ 97th percentile was used as the screening variable to define obesity. For the same %BF, BMI as the screening test gave higher true-positive and false-positive rates in boys than in girls. At the cut-off point of 25% body mass as fat for boys and 30% for girls used to define obesity by Williams et al. (1992), the true-positive rates in boys and girls were 68.4% and 54.1%, respectively, false-positive rates were zero. BMI ≥ 97th percentile provided the best trade-off between sensitivity and false-positive rates (maximizing the sum of sensitivity and specificity) at %BF values of 39% and 43% in boys and girls, respectively. At these two cut-off points, sensitivity and false-positive rate in boys were 89.5% and 10.4%; those in girls were 83.3% and 9.5%. The ROC curves of sensitivities versus false-positive rates for boys, girls from data in Table 2 are depicted in **Figure 2**. In general, as %BF increased, BMI ≥ 97th percentile yielded higher both true and false-positive rates in boys and girls.

The agreements between BMI and %BF in classifying the nutritional status for boys and girls are presented in **Table 4**. The kappa coefficient values showed that BMI agreed moderately with %BF. In boys, BMI identified correctly 30.8% (12/39) of the underweight children, 70.6% (36/51) of normal weight children and 87.1% (88/101) of the overweight and obese children. In girls, BMI identified correctly 66.7% (2/3) of the underweight children, 67.9% (74/109) of the normal weight children and 82.2% (74/90) of the overweight and obese children.

TABLE 1

Mean and median %BF values in children from our study, the DONALD Study and mean %BF from the study of Lazarus according to age[¶]

Subjects	Present study				DONALD Study				Lazarus's study		
	n	Age[§]	%BF[†]		n	Age[§]	%BF[*]		n	Age[§]	%BF[†]
			Median	Mean ± SD			Median	Mean ± SD			Mean ± SD
Boys	21	4.3 (1.3 to < 7.5)	23.2	28.5 ± 14.6	1191	5.4 (4.0 to < 7.5)	17.4	17.8 ± 3.7	23	6.0 (4.2-7.3)	14.1 ± 4.1
	31	9.3 (7.5 to < 10.5)	38.7	34.4 ± 12.6	645	8.9 (7.5 to < 10.5)	18.1	19.6 ± 6.2	24	8.9 (7.4-10.3)	17.1 ± 9.6
	58	11.7 (10.5 to < 13.0)	40.8	36.7 ± 13.3	454	11.2 (10.5 to < 13.0)	21.3	22.6 ± 7.5	24	11.7 (10.4-12.9)	23.5 ± 9.6
	81	15.0 (13.0 to < 17.0)	21.2	26.0 ± 15.7	376	14.3 (13.0 to < 17.0)	20.8	22.7 ± 7.4	25	14.7 (13.0-16.7)	16.9 ± 10.4
Girls	26	5.4 (0.4 to < 8.0)	25.3	28.2 ± 11.5	904	5.9 (4.5 to < 8.0)	16.5	17.4 ± 5.8	22	6.7 (4.6-8.0)	19.0 ± 7.2
	33	9.4 (8.0 to < 10.5)	35.5	33.6 ± 12.6	646	8.9 (8.0 to < 10.5)	19.8	20.9 ± 8.5	22	9.1 (8.1-10.2)	20.3 ± 8.6
	50	11.8 (10.5 to < 13.0)	33.1	34.4 ± 12.0	465	11.2 (10.5 to < 13.0)	22.3	23.5 ± 9.8	23	11.5 (10.3-13.1)	22.7 ± 7.4
	93	15.2 (13.0-18.0)	31.7	33.2 ± 11.3	366	14.3 (13.0 to< 17.0)	25.4	26.3 ± 8.8	22	15.1 (13.5-16.9)	28.2 ± 7.7

[¶]DONALD Study, Dortmund Nutritional and Anthropometric Longitudinally Designed Study.

[§]Mean of age, range in parentheses.

[*]%BF, percentage body fat measured by skinfold method.

[†]%BF, percentage body fat measured by DXA.

TABLE 2

Body composition characteristics in all children, boys, girls and the groups classified by BMI percentile[¶]

	All children	Boys	Girls	BMI < 10th percentile	BMI from 10th to 90th percentile	BMI ≥ 90th percentile
	(n = 393)	(n = 191)	(n = 202)	(n = 54)	(n = 165)	(n = 174)
Age (y)	12.0 ± 3.6	11.9 ± 3.6	12.1 ± 3.6	12.0 ± 4.6	12.4 ± 3.7	11.7 ± 3.1
Weight (kg)[*]	54.4 ± 24.0	57.4 ± 25.4	51.5 ± 22.4	34.8 ± 14.2	44.2 ± 15.4	70.2 ± 23.5
Height (m)[**]	1.52 ± 0.22	1.54 ± 0.23	1.50 ± 0.20	1.5 ± 0.3	1.52 ± 0.22	1.55 ± 0.17
$\%BF_{DXA}$ (%)	31.9 ± 13.5	30.9 ± 15.0	32.9 ± 11.8	17.8 ± 6.4	23.9 ± 9.3	43.9 ± 7.1
BMI (kg/m^2)[**]	22.4 ± 6.8	23.0 ± 6.9	21.8 ± 6.7	14.9 ± 1.7	18.4 ± 2.4	28.5 ± 5.5
BMI-SDS[**]	0.70 ± 1.8	0.87 ± 1.8	0.53 ± 1.8	-2.2 ± 1.0	-0.1 ± 0.7	2.4 ± 0.6

[¶]Mean ± SD; n in brackets, $\%BF_{DXA}$, percentage body fat measured by dual-energy X-ray absorptiometry; BMI-SDS, body mass index standard deviation score; BMI percentile, calculated from the German reference data.

[*]Significant difference between two sexes, $p < 0.05$ (Student's t tests).

[**]Significant difference between two sexes, $p < 0.05$ (Mann-Whitney U tests).

TABLE 3

True-positive and false-positive rates using BMI ≥ 97th percentile according to German reference data as the cut-off point to define obesity at various %BF values by sex[¶]

	Boys (n =191)		Girls (n = 202)		Both sexes (n = 393)	
	($n = n_1 + n_2$) $n_1 = 88$; $n_2 = 101$		($n = n_1 + n_2$) $n_1 = 59$; $n_2 = 143$		($n = n_1 + n_2$) $n_1 = 139$; $n_2 = 254$	
%BF	True-positive rate	False-positive rate	True-positive rate	False-positive rate	True-positive rate	False-positive rate
20	63.5	0	34.7	0	47.0	0
21	64.5	0	35.3	0	47.8	0
22	66.7	0	36.6	0	49.5	0
23	66.7	0	38.8	0	51.1	0
24	67.8	0	41.3	0	53.3	0
25	68.4	0	43.4	0	54.9	0
26	70.8	0	44.4	0	56.5	0
27	72.5	1.2	46.1	0	58.2	0.6
28	74.5	1.2	49.2	0	61.1	0.6
29	76.7	1.1	51.3	0	63.3	0.6
30	78.2	1.1	54.1	0	65.7	0.5
31	78.2	1.1	55.2	1.0	66.5	1.1
32	78.4	4.3	58.6	1.0	68.4	2.5
33	79.2	4.2	60.0	1.9	69.6	3.0
34	80.6	5.1	61.3	1.8	71.0	3.4
35	83.0	6.8	63.2	3.5	73.1	5.0
36	82.8	7.7	65.1	4.2	74.1	5.8
37	83.5	8.5	65.4	5.0	74.7	6.6
38	84.0	10.9	70.7	4.7	77.6	7.6
39	89.5	10.4	73.9	6.0	82.1	8.1
40	90.3	12.6	77.4	7.9	84.3	10.0
41	90.9	16.0	80.4	9.6	86.1	12.5
42	93.3	18.3	83.3	9.5	88.6	13.6
43	92.9	20.7	83.3	9.5	88.2	14.8
44	96.2	21.0	80.9	13.5	89.0	17.1
45	95.7	24.8	79.5	15.2	87.8	19.8
46	95.0	27.8	84.6	16.0	89.9	21.7
47	94.4	29.7	88.2	17.3	91.4	23.2
48	93.1	32.7	96.2	19.3	94.5	25.7
49	95.0	35.7	94.7	22.4	94.9	28.8
50	93.8	37.1	94.1	23.2	93.9	30.0

[¶]n_1, number of children who were defined as having a BMI above the 97th percentile; n_2, number of children who were defined as having a BMI below the 97th percentile.

TABLE 4

Kappa agreement test between BMI and %BF in classifying the nutritional status[¶]

	Boys				Girls			
	%BF[§]				%BF[§]			
BMI[†]	< 10th percentile	10th to 90th percentile	≥ 90th percentile	Total	< 10th percentile	10th to 90th percentile	≥ 90th percentile	Total
< 10th percentile	**12**	8	0	20	**2**	30	2	34
10th to 90th percentile	27	**36**	13	76	1	**74**	14	89
≥ 90th percentile	0	7	**88**	95	0	5	**74**	79
Total	39	51	101	**191**	3	109	90	**202**

[¶]Kappa coefficient value: 0.53 (boys); 0.56 (girls), $p < 0.0005$.

[§]%BF cut-offs using data from the DONALD Study: below 10th percentile: underweight; 10th - 90th percentile: normal weight; above 90th percentile: overweight and obese.

[†]BMI cut-offs calculated from the German reference data: below 10th percentile: underweight; 10th - 90th percentile: normal weight; above 90th percentile: overweight and obese.

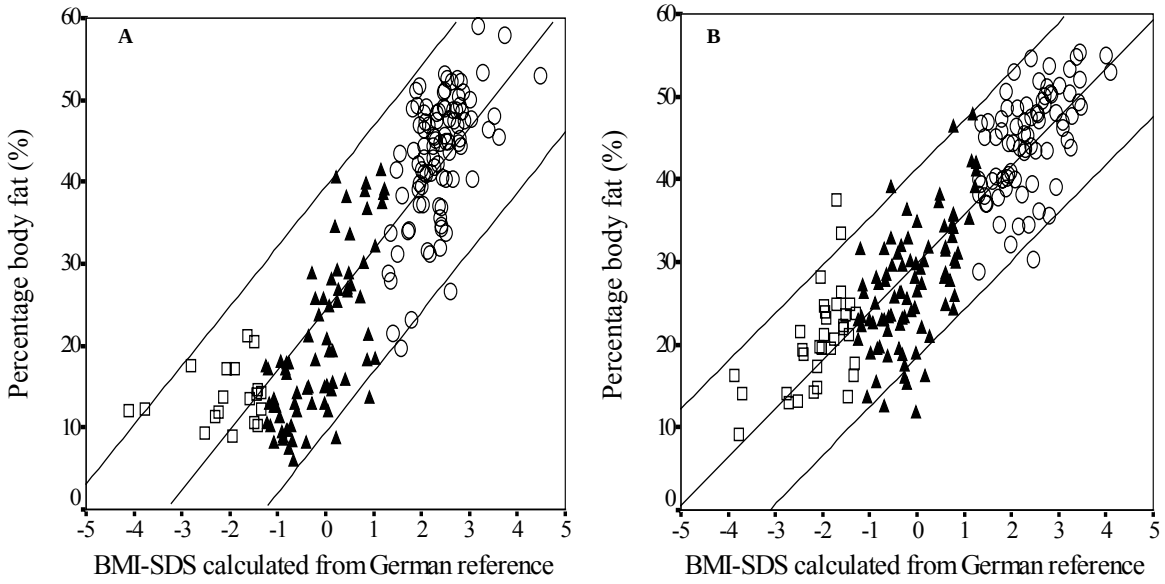

FIGURE 1. Relationship between BMI-SDS and %BF for boys (A), for girls (B). Different symbols illustrate the groups classified by BMI percentiles: underweight group (□), the normal weight group (▲) and the overweight and obese group (O). The lines indicate regression line and 95% CI for individual measurements.

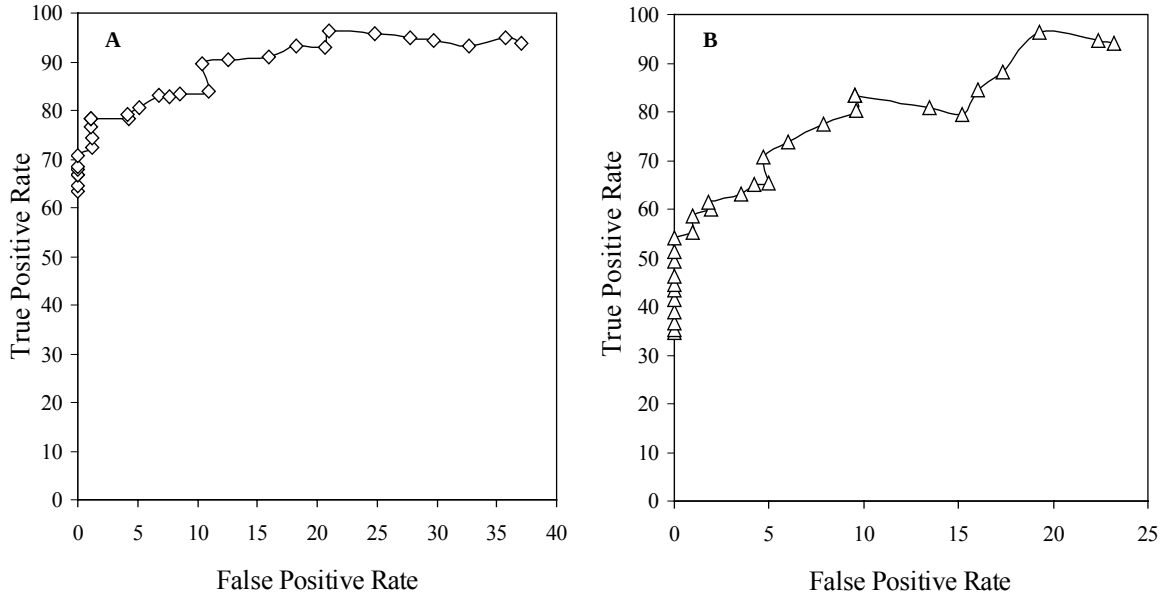

FIGURE 2. Receiver operating characteristic curves for boys (A), for girls (B) comparing BMI with %BF by DXA.

DISCUSSION

The results of this study suggest that BMI-SDS was correlated well with %BF. However, due to the large interindividual variation, BMI-SDS and %BF had only a moderate agreement in classifying the nutritional status. At some %BF cut-off points used to define obesity, BMI as the screening variable had moderate sensitivities in detecting obese subjects. Our study presents sensitivity and false-positive rates at various absolute %BF values as the reference test, this means that at each given %BF cut-off point it is possible to know the screening performance of BMI separately for boys and girls.

The present study includes a large number of paediatric patients suffering from different diseases with extremely high and low %BF and wide age range. In clinical practice, in order to monitor the treatment progress, the nutritional status and body composition of patients need to be routinely and precisely assessed. This is the reason to investigate the paediatric patients in this study.

Previous studies in healthy children showed the correlation between actual BMI and %BF measured by DXA ranging from 0.5 to 0.83 (Goran et al. 1996; Gutin et al. 1996; Daniels et al. 1997; Pietrobelli et al. 1998). However, the interpretation should be cautious when predicting a specific individual's %BF from BMI (Goulding et al. 1996; Pietrobelli et al. 1998; Schaefer et al. 1998). Two more recent studies correlating %BF with BMI-SDS in healthy children showed correlations of 0.87-0.88 that matched our results (Tyrrell et al. 2001; Field et al. 2003).

Because BMI is age-dependent, the use of BMI-SDS is more meaningful than BMI alone. The nutritional status of two children with an identical BMI value but different ages cannot be compared, unless their BMI value is converted into BMI-SDS or BMI percentile. The advantage of age- and sex-specific BMI-SDS over absolute BMI is that it adjusts for the normal changes in body composition and fatness that take place throughout childhood, thus making children at different ages comparable with each other.

To examine the performance of BMI as the screening variable for obesity, Lazarus et al. (1996) used DXA-derived %BF $\geq 85^{th}$ age- and sex-specific percentile as the reference variable to define excess adiposity, and BMI $\geq 95^{th}$ age- and sex-specific percentile of the NHANES reference curves as the screening variable, while Schaefer et al. (1998) used the skinfold-derived %BF $\geq 85^{th}$ age- and sex-specific percentile as the reference variable to

define excess adiposity, and BMI ≥ 95th age-and sex-specific percentile of self produced curves as the screening variable. Sensitivity and false-positive rates were: 39%/1% and 31%/1%, respectively (Lazarus et al. 1996; Schaefer et al. 1998). At the false-positive rate of 1.1% for both sexes shown in table 2, the sensitivity from our results was higher than the above values. This may be partly explained by using different cut-off values.

BMI or BMI-SDS does not distinguish between weight gain associated to muscle gain or weight gain associated to fat and it also does not reflect body fat distribution (He et al. 2001). In many cases the increase in %BF does not correspond with an increase in BMI (Goulding et al. 1996). BMI is a poor predictor of body fatness, both in infancy and childhood. It should only be used as the screening variable to identify subjects at risk for obesity rather than to precisely predict %BF (Schaefer et al. 1998). The Kappa analysis classification of overweight children was not so bad, but it did get worse for normal and underweight children, perhaps because BMI is not generally used in the definition of underweight in children. That partly explains the moderate agreement between BMI and %BF in classifying the nutritional status in our study. Sampei et al. (2001) tested the agreement between BMI and %BF in 10- and 11 year old Japanese adolescents, and gave the kappa coefficient value of 0.49, which was lower than our results. Correlational analysis measures the degree of correlation between BMI and %BF, while ROC analysis and kappa test described more detailed the nature and extent of misclassifications.

The limitation of our study is that the %BF percentile cut-off points in children of 4 and 14 year old from the DONALD Study were used to define nutritional status for children below 4 and above 14 y, respectively. This may cause a bias, because ideally, each age group should have its own cut-off points to classify the nutritional status. However, obesity was also defined by a single 85th %BF percentile for an age range from 2-3 y (Lazarus et al. 1996). This supports our method which used %BF cut-off points in children with a narrow age range to classify the nutritional status for children with a wider age range. Another limitations are: the results may be sample-specific and the number of underweight girls is small.

In summary, BMI is well correlated with %BF in sick children. BMI as the screening variable for detecting obesity has moderate sensitivities at some absolute %BF cut-off points. BMI agrees moderately with %BF in classifying the nutritional status. For clinical purposes, when %BF needs to be estimated, more precise methods than BMI should be used.

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Chapter III

Body composition of preterm infants measured during the first months of life: bioelectrical impedance provides insignificant additional information compared to anthropometry alone

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ABSTRACT

Background: Detailed knowledge of body composition in preterm neonates during their later postnatal period may be important for the treatment process. However, little consideration has been given to test whether bioelectrical impedance analysis (BIA) is a useful bedside method to predict fat-free mass (FFM).

Objective: To assess whether BIA is a bedside method to measure FFM in preterm neonates.

Design: FFM of 118 white subjects (51 males, 67 females), mean gestational age of 30.1 ± 3.1 weeks and birth weight of 1.26 ± 0.47 kg were measured at gestational age of 38.6 ± 3.8 weeks and actual body weight of 2.6 ± 0.54 kg using dual energy X-ray absorptiometry (FFM_{DXA}). Weight (W), height (Ht) and bioelectric impedance (I) measurements were collected. Multiple regression analysis was performed to develop prediction equations to estimate FFM with impedance index (Ht^2/I, cm^2/Ω) **a**nd W (kg) as predictor variables. Bootstrap analysis was performed for validating the derived prediction equations.

Results: Correlations between FFM_{DXA} and weight were 0.96, 0.98 and 0.97 in boys, girls and both sexes, respectively. Those between FFM_{DXA} and Ht^2/I were: 0.73, 0.81, and 0.79. Equations used to predict FFM (kg) were for boys: $FFM = 0.05\ Ht^2/I + 0.68\ W + 0.40$ ($R^2 = 0.919$) and for girls: $FFM = 0.04\ Ht^2/I + 0.71\ W + 0.29$ ($R^2 = 0.957$).

Conclusions: In preterm neonates, weight is a more effective predictor of FFM than impedance index. The study provides a bedside procedure for estimating FFM, mainly based on anthropometric parameters rather than BIA.

KEY WORDS Dual energy X-ray absorptiometry, correlation, impedance index, body composition, nutritional status.

INTRODUCTION

In clinical practice, preterm neonates need special nutritional care. Neonatologists need to know their body composition status to evaluate the treatment progress (Schmelzle and Fusch 2002). Appropriate food and energy intake are selected and adjusted for better physical growth based on the body composition measurements. The assessment of a rapid, safe, noninvasive, convenient and adequately accurate method to measure body composition in this population is useful during postnatal period. There is only few data about the precise body composition in preterm infants in the first months of life. With the introduction of dual energy X-ray absorptiometry (DXA)- one of the most frequently used reference methods for measuring human body composition (Heyward and Stolarczyk 1996), it is possible to accurately and precisely measure changes in lean mass, fat mass and bone mineral mass in infants (Rigo et al. 2001).

Anthropometric measurements like body weight and height are easily recorded at field sites or in clinical settings. Bioelectrical impedance analysis (BIA) is a quick, inexpensive, safe and noninvasive method for measuring body composition in children (Nagano et al. 2000; Pietrobelli et al. 2003). BIA is based on the assumption that the body is a cylinder of constant length and cross-sectional area, the impedance (Z) to an electric current through the body is directly related to the length of the conductor (L) and inversely related to its cross-sectional area (A): $Z = pL/A$, where p is volume resistivity in Ω-cm and is a constant (Heyward and Stolarczyk 1996). Multiplying both sides of the equation by L/L gives: $Z = pL^2/AL$, where AL is equal to volume (V). Substituting gives $Z = pL^2/V$ or $V=pL^2/Z$. On the basis of this relationship, the volume (V) of a conductor is proportional to L^2/Z, which is expressed as $V = pL^2/Z$. Considering V as the total body water (TBW), and knowing that the water content of the fat-free mass (FFM) is about 73%, BIA could be used to estimate FFM by applying a predictive equation based on the measured bioelectrical impedance of the subject. The pediatric prediction equations for FFM contain predictors: impedance index that is expressed as height (Ht, in cm^2) divided by impedance (I, in Ω) in combination with body weight (Houtkooper et al. 1992; Kushner et al. 1992), with age and sex (Deurenberg et al. 1991; Goran et al. 1993; Schaefer et al. 1994). Such prediction equations have been developed for children at different stages of maturation, from different ethnic backgrounds or with specific medical problems (Deurenberg et al. 1990; Kushner et al. 1992; Kim et al. 1994; Horlick et al. 2002). Some previous studies were carried out to provide bedside methods to calculate

percentage body fat or total body water in infants (Kushner et al. 1992; Schmelzle and Fusch 2002). To the best of our knowledge, prediction equations for FFM in preterm neonates are not available.

In this study we aim to characterise body composition using DXA, BIA and simple anthropometric measurements in a group of preterm infants in the first months of life. Additionally, we intend to test whether BIA is a useful bedside method in this population.

SUBJECTS AND METHODS

Subjects

Fifty-one male and sixty-seven female white preterm neonates, with birthweight below 2500 g treated at the division of neonatology, Children's Hospital, University of Greifswald were recruited in the study. In the first days of life preterm neonates were given a mixture of parenteral and enteral nutrition. From the total requirement, the orally given portion was subtracted and the remainder was parenterally administered. The parenteral nutrition consisted of lipid infusion and mixed infusion containing glucose, amino acids, electrolytes, vitamins, and trace elements. Body composition was measured before discharge from hospital as part of a nutritional study. Exclusion criteria were major congenital, chromosomal or metabolic anomalies and multiple births other than twins. The study protocol was approved by the University Ethical Committee and the State Authority for Radiation Exposure and Control. Written and informed consents were obtained from the parents.

Methods of body composition measurement

DXA, BIA and anthropometric measurements were obtained in a quiet, warm room in the morning after the infants had been fed. The infants were wrapped in cotton blankets without clothing or diaper, and DXA measurement was performed when the infants were sleeping. After this procedure, anthropometric and BIA measurements were assessed.

Anthropometry

Anthropometric measurements were collected by a technician of the body composition laboratory. Body weight of the neonates was measured without clothes to the nearest 10 g by

using a standard beam scale (Seca, Hamburg, Germany). Recumbent length was measured to the nearest 0.5 cm by using a measuring board (Schäfer, Karlsruhe, Germany).

Bioelectrical impedance analysis

Whole-body impedance was measured by the same technician at 50 kHz using a single frequency impedance instrument (Bodystat 1500, Bodystat Ltd, UK). The instrument performs automatically its own calibration check each time a measurement is taken. Two electrodes (Bodystat Electrode Pads) were attached to each dorsal right hand and dorsal right foot whilst the infants were lying in a supine position. Ten repeated bioelectrical impedance readings were then measured, the average of these readings was recorded. Impedance index as the predictor variable (calculated as height in cm square divided by bioelectrical impedance in Ω) was calculated for each subject to develop prediction equations for estimating FFM. Coefficient of variation for bioelectrical impedance measurements was 0.8%.

Dual energy X-ray absorptiometry (DXA)

Whole-body scans were performed with the Hologic QDR 1500 (Waltham, MA, USA) in a single-beam mode. The scans were analyzed by using a Hologic modified infant whole-body software version 5.67. Results from DXA include body skeletal area (cm^2), bone mineral content (g), fat mass (g), lean mass (g), and percentage body fat (%). FFM is the sum of bone mineral content and lean mass (FFM_{DXA}). The data are given as raw values without applying device-specific conversion equation. Scan time took 8 minutes; dose exposed in each scan was safe and posed no risk (Lohman 1996). Daily quality control scans were performed with the use of an anthropometric spine phantom to ensure the normal function of the machine.

Statistical analysis

Data were analyzed by using SPSS, version 10.0 software (SPSS Inc; Chicago). Results are presented as means ± SDs. The level of statistical significance was set at $P \leq 0.05$. Pearson's correlation coefficient (r) was used to measure the association between FFM_{DXA} and each of the following variables: impedance index, body weight and age. Student's t-test was used to compare mean variables between boys and girls. Multiple regression analysis was performed to generate equations to predict FFM with BIA and anthropometric measurements as independent variables and FFM measured by DXA as dependent variable. Residual sum of squares (RSS), SEEs were calculated to describe the precision of the predictions equations.

To evaluate the prediction equations, the difference between predicted and measured values was calculated (predicted-measured), and the percentage difference was calculated (predicted-measured)/measured. The Bland-Altman plots were used to evaluate the limits of agreement between FFM predicted by equations and FFM_{DXA} (Bland and Altman 1986). To validate the derived equations, 1000 sets of bootstrap samples for boys, girls and both sexes were drawn from original data set. Mean R^2 and 95% CI values between predicted FFM and FFM_{DXA} were calculated from above bootstrap samples (Manly 1997).

RESULTS

The mean gestational age at birth, gestational age at study and other body composition characteristics are shown in **Table 1**. There were no significant differences in gestational age at birth, gestational age at study (as defined in Table 1), weight, height, $\%BF_{DXA}$ between boys and girls ($P > 0.05$). The lean, fat-free masses, impedance index, BMC, bioelectrical impedance were significantly higher in boys than in girls. **Figure 1** depicts the time course of body composition measured by DXA. Body weight, lean mass, fat mass, BMC, and %BF increased with gestational age at study.

The correlation coefficient r values between FFM_{DXA} with each of the independent variables: impedance index, weight and gestational age at study were: 0.73, 0.96, and 0.63 (in boys); 0.81, 0.98, and 0.75 (in girls); and 0.79, 0.97, and 0.69 (for both sexes), respectively. Obviously, body weight was the strongest predictor of FFM compared with impedance index and gestational age at study.

Three equations (no 1-3) for determining FFM from BIA are presented in **Table 2**. Inclusion of age, together with body weight and impedance index, gave higher R^2 values of prediction equations for FFM in boys (0.935) and in both sexes (0.947), but not in girls. R^2 values of the models with impedance index and body weight as the predictors were higher than those derived from models with body weight as the predictor alone: 0.942/0.939 for both sexes; 0.919/0.916 for boys; 0.957/0.956 for girls. The mean differences and mean percentage differences between FFM predicted from equations and FFM_{DXA} are shown in Table 2. Mean differences ranged from -20 g in girls (range: -170 g to 240 g) to -4 g in boys (range: -320 g to 300 g) and percentage differences ranged from -0.81% in girls (range: -7.1% to 12.7%) to 0.10% in boys (range: -11.2% to 14.2%).

TABLE 1

Characteristics of the study population[1)]

	Boys (n = 51)	Girls (n = 67)	Both sexes (n = 118)
Gestational age at birth (wk)	29.5 ± 3.1	30.6 ± 3.1	30.1 ± 3.1
Birth weight (kg)	1.21 ± 0.47	1.28 ± 0.46	1.26 ± 0.47
Gestational age at study (wk)[2)]	39.3 ± 4.5	38.1 ± 3.2	38.6 ± 3.8
Weight at study (kg)	2.72 ± 0.54	2.50 ± 0.53	2.60 ± 0.54
Height at study (cm)	46.2 ± 3.1	45.3 ± 2.8	45.7 ± 3.0
$\%BF_{DXA}$ (%)	13.4 ± 4.2	13.7 ± 3.8	13.6 ± 3.9
LM_{DXA} (kg)	2.38 ± 0.44	2.16 ± 0.40	2.26 ± 0.43[b)]
BMC_{DXA} (g)	37.9 ± 10.1	34.2 ± 9.3	35.8 ± 9.8[a)]
FFM_{DXA} (kg)	2.40 ± 0.4	2.20 ± 0.4	2.30 ± 0.4[b)]
Impedance (Ω)	713.6 ± 98.9	748.3 ± 84.4	733.3 ± 92.2[a)]
Impedance index (cm^2/Ω)	3.06 ± 0.6	2.80 ± 0.5	2.92 ± 0.6[b)]

[1)]Mean ± SD; $\%BF_{DXA}$, percentage body fat measured by DXA; LM_{DXA}, lean mass measured by DXA; BMC_{DXA}, bone mineral content measured by DXA; FFM_{DXA}, fat-free mass measured by DXA.

[2)]Gestational age at study: gestational age at birth plus postnatal days of life until study.

[a)]Significant difference between the sexes, $P < 0.05$.

[b)]Significant difference between the sexes, $P < 0.01$.

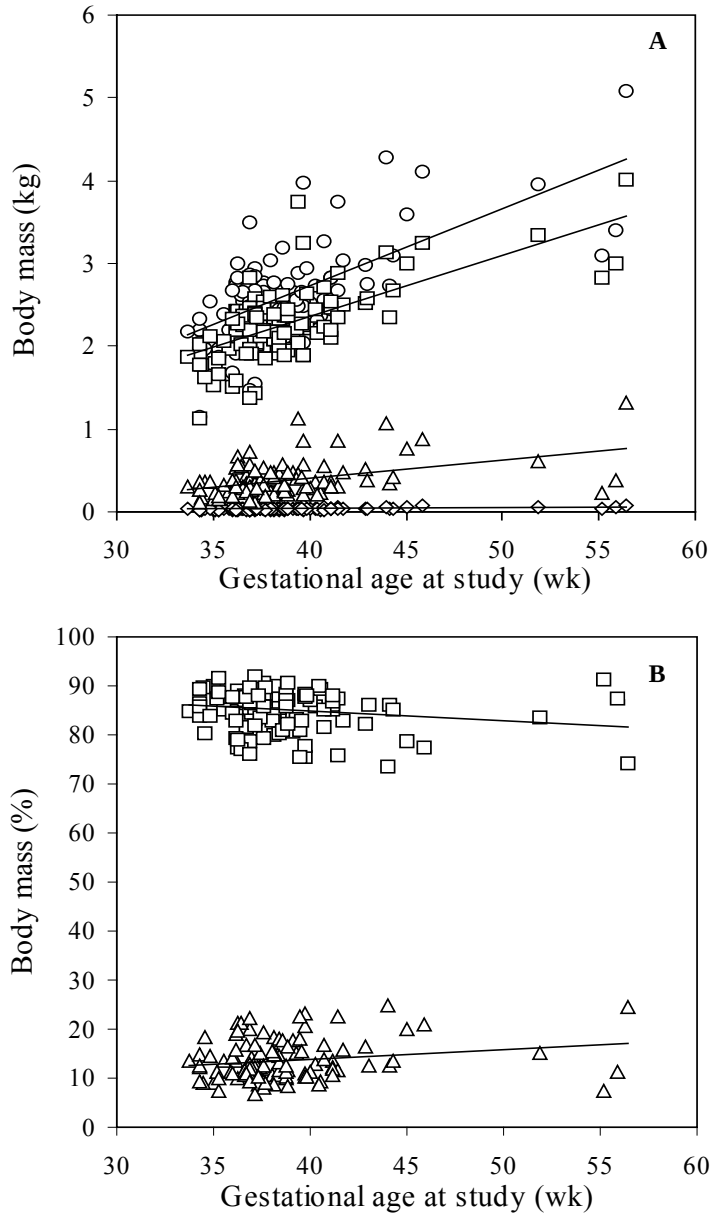

FIGURE 1. (**A**) Plots of gestational age at study versus subject's body composition characteristics: (○; body weight), (□; lean mass), (△; Fat mass), (◇; Bone mineral content) (in kg). (**B**) Plots of gestational age at study versus: lean mass (□) and body fat (△) (in percentage).

TABLE 2

The prediction equations for estimating FFM and differences between FFM predicted by prediction equations and that measured by DXA[1)]

Subjects	Equation	R^2	P	Difference[2)]		Percentage difference[3)]	
				Mean (kg)	95% CI	Mean (%)	95% CI
Boys	1. FFM (kg) = 0.05 Ht^2/I + 0.68 W + 0.40	0.919	< 0.0005	-0.004	-0.036 to 0.028	0.10	-1.20 to 1.42
Girls	2. FFM (kg) = 0.04 Ht^2/I + 0.71 W + 0.29	0.957	< 0.0005	-0.02	-0.042 to -0.001	-0.81	-1.78 to 0.17
Boys and girls	3. FFM (kg) = 0.05 Ht^2/I + 0.69 W + 0.31	0.942	< 0.0005	-0.04	-0.061 to -0.024	-1.62	-2.40 to -0.84

[1)]Ht^2/I, impedance index (cm^2/Ω); W, body weight (kg); the impedance "I" was measured by a single frequency impedance instrument (Bodystat 1500, Bodystat Ltd, UK).

[2)]Difference = predicted - measured.

[3)]Percentage difference = 100 x (predicted – measured)/measured.

Table 3 shows the residual sum of squares and SEE from the regression analysis of the relation between FFM_{DXA} and FFM predicted by weight alone or weight combined with impedance index. In boys, residual sum of squares (RSS) of the prediction equation with body weight and impedance index as the predictors was 0.61 kg, whereas the RSS value for only body weight as the predictor rised to 0.63 kg. This demonstrated an improvement of precision (1.77%) using the impedance index additionally to body weight. In girls, the values were 0.44, 0.46, 2.38%; in both sexes were: 1.08, 1.15, 3.03%, respectively. When a subgroup of infants with a study age of below 42 weeks was used for calculation of FFM (n = 107), the impedance index also did not give higher precisions additionally to body weight (data not shown).

TABLE 3

Precision gained by bioelectrical impedance analysis

Subjects	Correlations	Residual sum of squares (RSS)	Standard error of estimate (SEE)	Gained by BIA[3)]
		(kg)	(kg)	(%)
Boys (n=51)	$FFM_{weight+BIA}$ vs. FFM_{DXA}[1)]	0.61	0.111	1.77%
	FFM_{weight} vs. FFM_{DXA}[2)]	0.63	0.113	
Girls (n=67)	$FFM_{weight+BIA}$ vs. FFM_{DXA}	0.44	0.082	2.38%
	FFM_{weight} vs. FFM_{DXA}	0.46	0.084	
Both sexes (n=118)	$FFM_{weight+BIA}$ vs. FFM_{DXA}	1.08	0.096	3.03%
	FFM_{weight} vs. FFM_{DXA}	1.15	0.099	

[1)]Correlation between FFM calculated by weight combined with impedance index as the predictors and FFM measured by DXA.

[2)]Correlation between FFM calculated by weight alone as the predictor and FFM measured by DXA.

[3)]Precision gained by impedance index.

Validation of prediction equations by bootstrap sampling method gave mean R^2 of 0.918 ± 0.02 (95% CI: 0.916, 0.919) in males; 0.951 ± 0.02 (95% CI: 0.950, 0.953) in females; and 0.943 ± 0.02 (95% CI: 0.942, 0.945) in both sexes. The Bland and Altman plots for comparing the differences between predicted and criterion values plotted against the mean of the predicted and criterion values are shown in **Figure 2**.

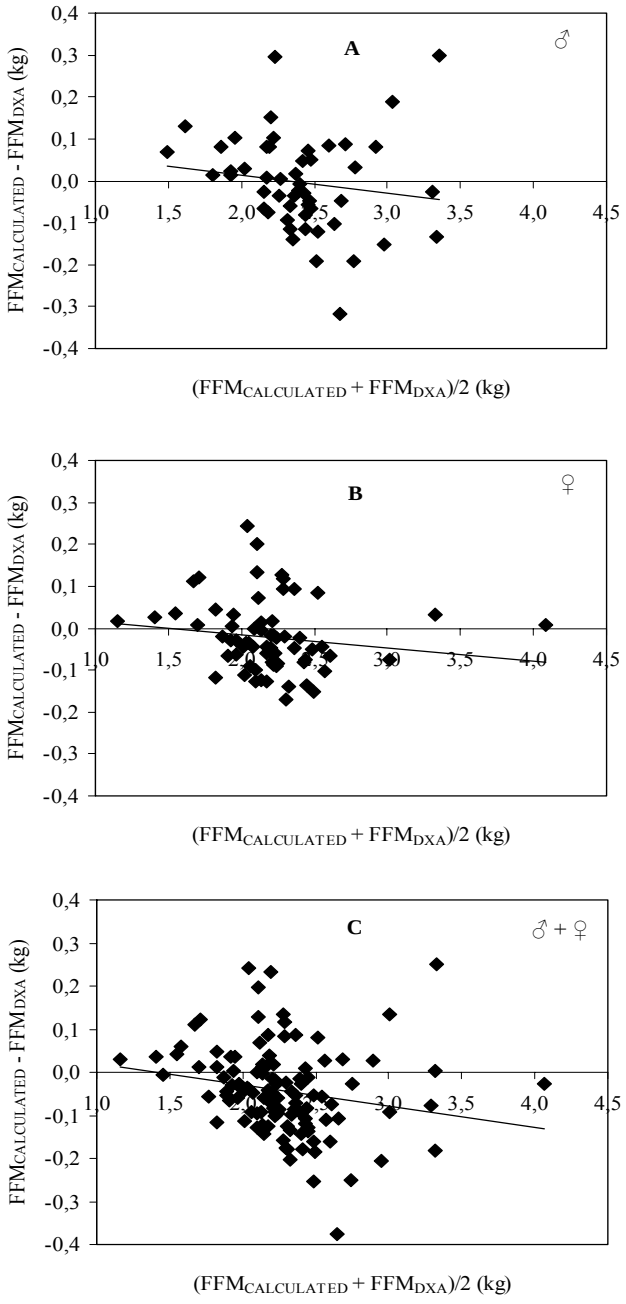

FIGURE 2. Bland-Altman plots for comparing the FFM predicted from equations calculated for boys (A), for girls (B), for both sexes (C) with FFM measured by DXA.

DISCUSSION

This study aims to assess body composition characteristics and test whether BIA is a useful method in preterm neonates at a mean study age of 38.6 gestational weeks. The results of the study show that impedance index and body weight correlate well with FFM_{DXA}, and age correlates less well with FFM_{DXA}. Among three predictor variables, body weight has the best correlation with FFM_{DXA}. Addition of impedance index provides only a small improvement in the accuracy of the prediction equations. It is possible that BIA is not useful in clinical routine for preterm neonates in the first months of life.

FFM changes according to the development of growing children. In developing prediction equations for FFM of a population with wide age range, FFM should be estimated not only by impedance index and body weight but also by age. Though the observed age range extends more than 10 weeks, the influence of age on the predictive accuracy of equations is negligible. Therefore, our prediction equations did not include age as the predictor for estimating FFM. This is in accordance with the findings reported by Houtkooper et al. (1992): inclusion of age as the predictor did not significantly improve the predictive accuracy of their BIA equations.

Our findings are consistent with the results of Hammami et al. (2003). Their study in 73 healthy neonates with birth weights 3354 ± 316 g, gestational ages 39.5 ± 1.2 wk showed that weight was significantly correlated with all DXA measurements. They confirmed that body weight was the best physiologic predictor of overall body composition. Jackson et al. (1988), Van Loan and Mayclin (1987) observed that standard anthropometric measurements were more powerful predictors of FFM than was bioelectrical impedance in a broad sample of adult subjects. However, our outcome was contradictory with the comment of Lohman et al. (1992) that believed the impedance index is a relatively stronger predictor of FFM than body weight.

In this study, DXA was used as a reference method to measure FFM. Studies in animals have shown a linear relation between lean and fat mass measured by DXA and those obtained by chemical carcass analysis (Picaud et al. 1996; Brunton et al. 1997). DXA is a fast and noninvasive method compared to other methods like hydrostatic weighing which is not appropriate in neonates. DXA has a wide application for assessing BMC and body composition in preterm and term neonates (Rigo et al. 1998; Lafeber 1999). The trend lines in Figure 1 show that lean mass, fat mass, and BMC increased with gestational age at study. Our data are comparable to those of other studies using the DXA technique for newborns

(Lapillonne et al. 1997; Koo et al. 2004). Small-for-gestational age infants with 38-39 weeks of gestational age at birth reported by Lapillonne el al. (1997) had smaller body weight (2.286 kg), BMC (27.0 g), and lean mass (1.81 kg) than those of our subjects. In 74 appropriate-for-gestational age infants with 35.9 weeks of gestational age at birth, Koo et al. (2004) reported mean lean mass (2.131 kg) and %BF (11.9%), which were smaller than our data.

BIA is said to have great potential value as it can be used easily, quickly and safely at the patient's bedside and show less inter-observer variation than do traditional anthropometric measurements (Schaefer et al. 1994). Pietrobelli et al. (2003) supported the use of BIA method for predicting fat-free mass in children. Though this might be true for measurement in adults and pediatric patients, our results show that the contribution of the impedance index to the accuracy of the prediction equations in preterm infants was only small. Our study lacks a second group of preterm neonates to cross validate the prediction equations. Bootstrap sampling method was used to validate the prediction formulas. High mean R^2 values from bootstrap method indicated that three equations were appropriate enough for estimating FFM in preterm neonates. The main goal is to achieve a good growth of lean mass. When infants are crossing the age-and sex-specific percentile, it must be decided whether energy intake should be either reduced or increased. If the physician can realize that lean mass growth is inadequate despite normal weight gain, calorie intake must be changed.

Malnutrition in hospitalized pediatric infants is not uncommon and results in poor growth and reduced or delayed mental and psychomotor development (Hendrickse et al. 1997; Lucas et al. 1998). Initial nutritional assessment followed by adequate nutritional support should become an essential practice in the clinical management of pediatric intensive care patients. Some prediction equations have been developed using BIA for measuring body composition in term-born infants (Kushner et al. 1992; Tang et al. 1997). Kushner et al. (1992) developed the prediction equation for total body water in a group of various ages from infancy to adulthood: impedance index was the strongest predictor of TBW, explaining 99% of the variance in TBW. Tang et al. (1997) used BIA in neonates receiving intensive care in the first week after birth to develop prediction equations for estimating TBW. Tang et al. (1997) found that body weight on the day of study was the best single predictor of TBW, $r = 0.995$. Our study may be the first to present equations for predicting FFM in preterm-born infants. One could consider if our equations can be applied with other impedance-meters than the brand

used. To the best of our knowledge, there is no study reporting whether the BIA data obtained by Bodystat are identical with those of other devices. Our study was restricted to white preterm neonates of less than 4 months of age; therefore the results cannot be extrapolated to nonwhite or older white subjects.

In conclusion, a bedside method for measuring body composition of preterm neonates was assessed. The study provides prediction equations for determining FFM and presents the relationship between FFM_{DXA} and impedance index, body weight, and age. The strongest predictor of FFM is body weight but not impedance index; age has the lowest correlation with FFM_{DXA}. During the first months of life, FFM of preterm neonates can be predicted mainly from anthropometry rather than impedance index.

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Chapter IV

The use of bioelectrical impedance analysis and anthropometry to measure fat-free mass in children and adolescents with Crohn's disease

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ABSTRACT

Objective: To investigate the precision of published prediction equations for fat-free mass (FFM) from bioimpedance measurements in children with Crohn's disease using dual-energy X-ray absorptiometry (DXA) as an in-vivo gold standard.

Design: FFM of 49 white boys and girls aged 7.3-16.9 y suffering from Crohn's disease was measured by DXA. Body weight, height, and bioimpedance measurements were also collected. FFM measured by DXA (FFM_{DXA}) was compared with FFM predicted by the only five published prediction equations available for children and adolescents (Deurenberg, Schaefer, Lorenzo and Pietrobelli). An equation was developed for predicting FFM and was validated using bootstrap method.

Results: When correlating predicted FFM with FFM_{DXA}, Schaefer's equation showed the highest R^2 (0.950), the smallest SEE (2.05 kg), the smallest percentage error (0.28%). Our prediction equation for estimating FFM was: $FFM = 0.652\ Ht^2/Z + 0.0385\ Wt + 0.586\ Age - 0.327$, $R^2 = 0.951$, $SEE = 2.08$, $p < 0.0005$, where Ht^2/Z is the impedance index in cm^2/Ohm, Wt is body weight in kg, age in year. R^2 value from bootstrap method was 0.950 ± 0.01 (95% CI: 0.927, 0.968), indicating an acceptable validation of the derived formula.

Conclusions: The formula of Schaefer is the best for predicting FFM. The present study provides a new prediction equation for estimating FFM in children with Crohn's disease that may be used in clinical settings where more sophisticated body composition measuring equipments are not available.

KEY WORDS Dual energy X-ray absorptiometry, nutritional status, prediction equation, body composition, bootstrap method.

INTRODUCTION

Prediction equations based on bioelectrical impedance analysis (BIA) have been widely used for measuring body composition in healthy children but their validation has not been much tested in the population of malnourished or sick children (Royall et al. 1994). The validation of published prediction equations and the development of simple and accurate body composition measuring models for evaluating and monitoring growth and nutritional status in children is essential for clinical and field studies (Horlick et al. 2002).

Crohn's disease is a chronic inflammatory disease of the intestines (Katznelson et al. 2003). It primarily causes ulcerations of the small and large intestines, but can affect the digestive system anywhere from the mouth to the anus, leading to a wide variety of clinical symptoms. Children suffering from Crohn's disease have a decreased bone mineral density (Boot et al. 1998), weight loss, nutritional depletion (Jahnsen et al. 2003), and significant loss of fat-free mass (FFM) (Azcue et al. 1997; Tjellesen et al. 1998). The assessment of FFM as well as that of fat mass for Crohn's patients is very important. It quantifies the magnitude of malnutrition and allows the clinicians to assess the extent of patient's nutritional depletion and provide them adequate nutrition supports during treatments.

BIA is a quick, easy, low-cost and noninvasive method that has been proposed as an alternative to laboratory-based techniques of measuring body composition in children (Kushner et al. 1992). It uses the conductivity of the human body exposed to a weak alternating current at 50 kHz. Conductivity is correlated to body water pool and is expressed as the impedance or resistance. Impedance is a function of resistance and reactance, where $Z = \sqrt{R^2 + X_c^2}$. Resistance (R) is a measure of pure opposition to current flow through the body; reactance (Xc) is the opposition to current flow caused by capacitance produced by the cell membrane (Kushner et al. 1992). Resistance index (RI) has been well correlated to FFM, however it is also common to take impedance index (ZI) to predict FFM (Horlick et al. 2002). Prediction equations for FFM use BIA-derived variables (impedance in ohms) in combination with weight, height, age, sex (Deurenberg et al. 1990; Schaefer et al. 1994; de Lorenzo et al. 1998; Pietrobelli et al. 2003). Dual-energy X-ray absorptiometry (DXA) has been used extensively in pediatric practice for determining FFM, fat mass, lean mass (Ellis et al. 1997; Wells et al. 1999) and recognised as a reference method for body composition research (Gutin et al. 1996). Its results correlate well with direct chemical analyses and there is a good

agreement between percentage body fat estimated by hydrodensitometry and by DXA (Going et al. 1993; Svendsen et al. 1993). BIA is a valid bedside technique for the estimation of FFM provided that the equation used for calculating FFM is appropriate for the study population (Kyle et al. 2002b). The published prediction equations were often derived and cross-validated in healthy children, so the precision and accuracy may be lower in the presence of acute or chronic diseases. Therefore, the development of a population-specific prediction equation is needed. Previous reports suggested that population-specific prediction equations might be necessary to some investigators (Arpadi et al. 1996; Reilly et al. 1996). In fact, such equations are required for their specific patient group (Bedogni et al. 1996).

To the best of our knowledge, BIA-based prediction equations for FFM are only available for healthy children or adults suffering from Crohn's disease. A study in adults suffering from Crohn's disease has been published correlating total body water with BIA (Royall et al. 1994). The first aim of the present study was to validate five published prediction equations from four previous studies in determining FFM (**Table 1**) by using DXA as the reference method in a group of children with Crohn's disease. The second aim was to develop a new, simple prediction equation from the population under study to predict FFM and validate it with the bootstrap method.

TABLE 1

Prediction equations validated in the present study[¶]

Author	Study population	Formula
Deurenberg	Boys and girls 7-25 y, n = 246	FFM (kg) = 0.488 (Ht^2/Z) + 0.221 Wt + 0.1277 Ht - 14.7
Schaefer	Boys and girls 3-19 y, n = 112	FFM (kg) = 0.65 (Ht^2/Z) + 0.68 Age + 0.15
de Lorenzo	Boys and girls 7.7-13 y, n = 35	FFM (kg) = 0.588 (Ht^2/Z) + 0.211 Wt + 2.33
Pietrobelli	Boys 7-14 y, n = 50	FFM (kg) = 0.6375 (Ht^2/Z) + 5.9913
	Girls 7-14 y, n = 25	FFM (kg) = 0.7597 (Ht^2/Z) + 3.5853

[¶]Ht^2/Z, Impedance index (body height in cm^2 divided by mean impedance in ohm); Wt, body weight (kg); Ht, body height (cm); Age (y).

SUBJECTS AND METHODS

Subjects

In the period from July-2003 to August-2005, a total of 49 white children aged 7.3-16.9 y (16 boys and 33 girls) suffering from Crohn's disease treated in the Children's Hospital, University of Greifswald, Germany were enrolled in the study. University Ethical Committee approved the study protocol. State Authority for Radiation Exposure and Control accepted the use of DXA for measuring body composition in this patient group. Written and informed consent were obtained from all subjects and their parents.

Procedures used to measure body composition

All measurements were performed at the Body Composition Unit of the Children's Hospital, Greifswald University, Germany.

Body weight was measured to the nearest 0.1 kg with a Seca calibrated scale while children wearing hospital gowns. Standing height was measured to the nearest 0.5 cm using a wall-mounted stadiometer (Längenmeßtechnik GmbH Limbach-O). Two skilled technicians performed the anthropometric measurements for all subjects.

The single frequency impedance instrument (Bodystat 1500, Bodystat Ltd, U.K) was used to measure whole-body impedance at 50 kHz on the right side of the body. Two electrodes were placed on each dorsal right hand and dorsal right foot while subjects were lying in a supine position. The whole body bioimpedance was measured 10 times; the mean value was calculated and used for the calculation of the impedance index. The impedance index (Ht^2/Z) was expressed as body height (Ht) in cm^2 divided by the mean impedance (Z) in Ω.

Whole-body scans were performed with the Hologic QDR 1500 (Waltham, MA) in a single-beam mode. The scans were analyzed using Hologic whole-body software version 5.67 while subjects lying comfortably in supine position. Results from DXA include bone mineral area (cm^2), bone mineral content (BMC) (g), fat mass (g), lean mass (g), percentage body fat (%); FFM (g) was expressed as total body weight minus fat mass. Metallic objects (buckles, watches, zips) were removed before scans were performed.

Daily quality control scans for bone mineral measurements were performed with the use of an anthropometric spine phantom to ensure the correct function of the scan machine. In the

period from January 2000 to October 2004, mean phantom lumbar spine BMC was found to be 57.01 ± 0.19 gr (n = 692) and was close to the reference value (BMC: 57.14 ± 0.16 gr).

Statistical analysis

Data were analyzed by using SPSS, version 10.0 software (SPSS Inc; Chicago). Results are presented as means ± SD. Student's t-test was used to compare characteristics between boys and girls. Because DXA provides a 3-compartment model, which was validated and considered as a reference method (Svendsen et al. 1993; Ellis et al. 1994), FFM measured by DXA was chosen as the criterion variable to compare with those from published equations (Table 1). The level of statistical significance was set at $p \leq 0.05$.

The following criterias were used to select the best model in predicting FFM: The Pearson's correlation coefficients, SEE, percentage prediction errors, and the Bland-Altman limits of agreement. The Pearson's correlation coefficients and SEE were calculated from the relationship between FFM predicted by the published equations and FFM_{DXA}. Percentage prediction error was computed [(predicted−observed)/observed] for each subject and then averaged for each published equation. The methods of Bland and Altman (1999) were used to evaluate the 95% limits of agreement [mean error ± (2 x the SD of the differences)] between FFM predicted from published equations and FFM measured by DXA. Since DXA is the reference method, i.e. FFM_{DXA} is closer to the true value, FFM_{DXA} was put on the abscissa in the Bland-Altman plot. Therefore, in this case the difference between predicted FFM and FFM_{DXA} is plotted against FFM_{DXA}. The most appropriate model was the one that maximized correlation, gave the smallest SEE, minimized percentage prediction errors, and showed the best limits of agreement (Horlick et al. 2002).

Multiple regression analysis was performed to develop a prediction equation for FFM with impedance index, body weight, and age as the predictors. The derived formula was validated using the bootstrap method (Manly 1997). 1000 sets of bootstrap samples with a sample size of 49 were randomly drawn from the original data set, then mean R^2 and 95% CI values between FFM_{DXA} and FFM measured by the derived formula from all samples were calculated.

RESULTS

Age and body composition characteristics of sixteen boys and thirty-three girls with Crohn's disease are presented in **Table 2**. No differences were found for all variables between boys and girls, except for percentage body fat, which was higher in girls than in boys ($p < 0.001$). The mean FFM_{DXA} was 32.67 ± 9.11 kg, those predicted by the prediction equations of Deurenberg et al. (1990), Schaefer et al. (1994), de Lorenzo et al. (1998) and Pietrobelli et al. (2003) are shown in the last column of **Table 3**.

TABLE 2

Body composition characteristics of the study population[¶]

	Boys (n = 16)	Girls (n = 33)	All children (n = 49)
Age (y)	12.6 ± 2.9	13.4 ± 2.4	13.2 ± 2.6
Weight (kg)	43.3 ± 13.3	44.6 ± 10.7	44.1 ± 11.5
Height (cm)	157.5 ± 19.7	156.1 ± 12.5	156.6 ± 14.9
$\%BF_{DXA}$ (%)[§]	17.1 ± 7.8	27.6 ± 6.3	24.2 ± 8.4
LM_{DXA} (kg)	33.8 ± 11.7	29.9 ± 6.6	31.2 ± 8.7
BMC_{DXA} (kg)	1.48 ± 0.59	1.55 ± 0.44	1.53 ± 0.49
FFM_{DXA} (kg)	35.3 ± 12.3	31.4 ± 7.0	32.7 ± 9.1
Impedance (Ω)	696 ± 139	731 ± 147	720 ± 144
Impedance index (ht^2/Ω)	38.6 ± 14.9	35.0 ± 9.5	36.2 ± 11.5

[¶]Mean ± SD; n in brackets; $\%BF_{DXA}$, percentage body fat measured by DXA; $LM_{DXA,}$ lean mass measured by DXA; BMC_{DXA}, bone mineral content measured by DXA; FFM_{DXA}, fat-free mass measured by DXA.

[§]Significant difference between the two sexes, $p < 0.001$.

TABLE 3

Correlation and difference between FFM predicted by each equation and FFM measured by DXA[¶]

Published methods	R^2	SEE[$] (kg)	Error (kg)[§]			Percentage error (%)[‡]			Predicted FFM (kg)
			Mean	Lower limit	Upper limit	Mean	Lower limit	Upper limit	
Deurenberg et al. (1990)	0.949	2.08	0.02	-4.38	4.42	-0.39	-14.59	13.81	32.70 ± 9.67
Schaefer et al. (1994)	0.950	2.05	-0.06	-4.16	4.04	0.28	-13.32	13.88	32.61 ± 8.62
de Lorenzo et al. (1998)	0.930	2.42	0.23	-4.57	5.03	1.05	-13.75	15.85	32.91 ± 9.02
Pietrobelli et al. (2003)[†]	0.890	3.08	-2.41	-8.61	3.79	-6.62	-23.60	10.40	30.26 ± 7.91

[¶]Mean ± SD; R^2, correlation between FFM predicted by published equations and FFM_{DXA}.

[$]SEE, standard error of estimate from the correlation between FFM predicted by equations and FFM_{DXA}.

[§]Error = predicted-observed, and the 95% limits of agreement for error from the correlation between FFM predicted by equations and FFM_{DXA}.

[‡]Percentage error = [(predicted-observed)/observed] and the 95% limits of agreement for percentage error from the correlation between FFM predicted by equations and FFM_{DXA}.

[†]Results are calculated by using the two equations of Pietrobelli et al.

There were strong correlations between FFM predicted by each model and FFM_{DXA}. Mean errors between FFM predicted by the equations of Deurenberg et al. (1990), Schaefer et al. (1994) and de Lorenzo et al. (1998) and FFM_{DXA} were not significantly different from zero. The lower and upper limits of agreement for the model of Schaefer et al. (1994) were -4.16 to 4.04 kg, which means that 95% of subjects could be expected to have predicted and criterion values that agree to within ≈ 4.0 kg (13%). The widest limits of agreement for the error (-8.61 to 3.79 kg), and for the percentage error (-23.6 to 10.4%) were found in the model of Pietrobelli et al. (2003).

The best model for predicting FFM was that of Schaefer et al. (1994), because it gave the highest correlation, the lowest SEE, the lowest ranges between upper and lower limits of agreement for error and percentage error.

The Bland and Altman approach for evaluating the difference between each of four models for determining FFM and the corresponding reference method are shown in **Figure 1**. Positive correlation is found in Figure 1A (Deurenberg), negative correlation are found in Figure 1B (Schaefer), Figure 1C (de Lorenzo) and Figure 1D (Pietrobelli).

The prediction equation for FFM in our group of children with Crohn's disease is as follows:

$$FFM = 0.652\ Ht^2/Z + 0.0385\ Wt + 0.586\ Age - 0.327$$

$$R^2 = 0.951,\ SEE = 2.08\ (p < 0.0005)$$

where Ht^2/Z is the impedance index in cm2/Ω, Wt is body weight in kg, age in year. Both impedance index and body weight were correlated well with FFM, the Pearson's correlation coefficients were 0.965 and 0.896, respectively.

The above formula was validated using the bootstrap sampling method. It gave a mean R^2 value ± SD between the measured and predicted FFM of 0.950 ± 0.01 (95% CI: 0.927, 0.968), indicating an acceptable validation of the derived formula. **Figure 2** shows the Bland-Altman plot for comparing FFM_{DXA} with FFM predicted by our own prediction equation. The error derived from our equation was -0.008 ± 2.0 kg, the limits of agreement were: -4.008 to 3.992 kg. The percentage error derived from our equation was 0.24 ± 6.7 %, the limits of agreement were: -13.16 to 13.64%.

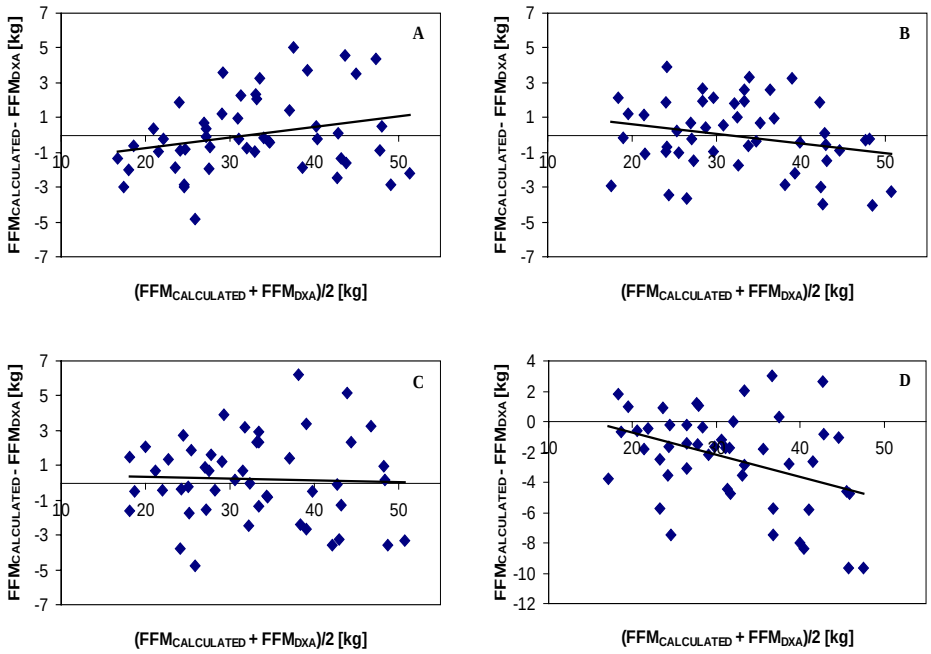

FIGURE 1. The Bland and Altman analysis to compare FFM predicted by published prediction equations with FFM measured by DXA. Panel A: model of Deurenberg; panel B: model of Schaefer; panel C: model of de Lorenzo; panel D: model of Pietrobelli.

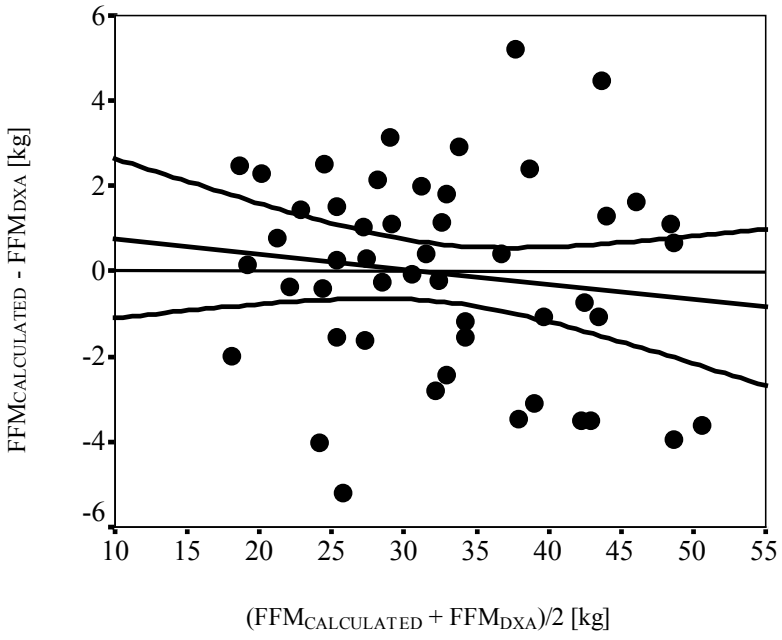

FIGURE 2. The Bland and Altman analysis to compare FFM predicted by our own prediction equation with FFM measured by DXA.

DISCUSSION

The main purpose of this study is to validate published prediction equations for FFM in a group of children with Crohn's disease. The model of Schaefer et al. (1994) gives the best formula whereas the model of Pietrobelli et al. (2003) shows the worst equations for predicting FFM. With the small percentage errors and small SEE (derived from the correlation between FFM predicted and FFM_{DXA}), three prediction equations of Deurenberg, Schaefer, and de Lorenzo were appropriate enough in predicting FFM for the whole group of children with Crohn's disease. However, the observed wide limits of agreement indicate that BIA-based prediction equations are not appropriate for the evaluation of body composition in individual persons.

The inability to estimate precisely body composition with simple, inexpensive, and easily applied techniques is a limitation to clinical investigations in nutrition. Therefore, Royall et al. (1994) compared calculation of FFM by five methods to determine whether the currently used equations for total body water (TBW) as assessed by BIA were applicable to 19 malnourished patients with Crohn's disease. His study showed that BIA overestimated TBW compared with the reference method in malnourished children. Finally, a prediction equation for TBW was developed using $height^2$/resistance and body weight as independent variables.

Unlike the equation of Royall et al. (1994) for TBW, our prediction equation was developed for estimating FFM. It showed a SEE value of 2.08 kg, which was little higher than those from validated prediction equations: 1.87 kg, 1.98 kg and 1.0 kg in formulas of Deurenberg et al. (1990), Schaefer et al. (1994) and de Lorenzo et al. (1998), respectively. The accuracy and precision of the BIA method are affected by instrumentation and subject factors. The body's physiological status of individuals may differ from person to person due to healthiness or illness. The published equations validated in this study were developed in healthy subjects with different sample sizes and body composition measuring instruments. The bioimpedance analyzer (BIA 101, RJL Systems) was used by Deurenberg et al. (1990) and Lorenzo et al. (1998). The body composition analyzer (Holtain Ltd) was used by Schaefer et al. (1994). The device Human-Im BIA (DS Medigroup, Milan, Italy) was used by Pietrobelli et al. (2003). Our subjects were ill and older than their study subjects; and the equation was developed using the single frequency impedance instrument (Bodystat 1500, Bodystat Ltd, U.K). This might explain the differences in FFM estimated from the models and the higher SEE from our prediction equation in the presence of the chronic disease like Crohn.

The model of Pietrobelli et al. (2003) used impedance index as the predictor for FFM whereas the other authors (Deurenberg et al. 1990; Schaefer et al. 1994; de Lorenzo et al. 1998) used weight, height or age additionally to predict FFM. FFM predicted by the Pietrobelli's equations was less precise than FFM predicted from other equations and FFM_{DXA} (table 3). It may indicate that the predictive accuracy of a prediction equation is influenced by factors like weight, height or age other than impedance index alone.

The application of existing equations to predict FFM in specific subgroups was an area of particular interest to previous investigators (Schaefer et al. 1994; Arpadi et al. 1996; Reilly et al. 1996). The choice of using the full BIA-based or simple BIA-based models depends on the investigators. The simple models contain only impedance index and body weight as the predictors while the full models contain additional variables like age, sex, race, disease states. The study of Horlick et al. (2002) showed that the full models eliminated the problem of bias for subgroups. Our new prediction equation including impedance index, weight, and age as the predictors gave the smaller percentage error, narrower ranges between upper and lower limits of agreement for error and percentage error compared to those of Schaefer et al. (1994). The equation was validated by the statistical bootstrap analysis- a simple and reliable method for validating prediction equations in case there is no second sample to cross-validate the derived prediction equation (Schmelzle and Fusch 2002). It all suggests that our equation is better than other equations validated for predicting FFM.

With more advantages over the anthropometric technique, BIA-based prediction equations are now the promising measures to evaluate the nutritional status of healthy and ill individuals (Kyle et al. 2002a). It is particularly necessary for the clinical settings where the more accurate body composition measuring equipments like DXA, magnetic resonance imaging, computer tomography scans may be not available. A previous study showed that BIA can detect changes in FFM of from 3% to 5% in more than 95% of cases, supporting the use of BIA in clinical practice (Kotler et al. 1996).

The size of R is much larger than Xc (at a 50 kHz frequency) when measuring whole body impedance. Therefore, R is a better predictor of FFM and TBW than Z (Lohman 1989). Similarly, the resistance index ($Height^2/R$), instead of impedance index ($Height^2/Z$), is often used in many BIA equations to predict FFM or TBW. However, Z and R are often used interchangeably because Xc is ordinarily very small relative to Z (< 4%) (Baumgartner 1996). Because some devices only give figures for impedance but not for resistance, we therefore

validated only published prediction equations for FFM using impedance index as the predictor. Those using resistance index as the predictor for FFM were not validated in our study (Cordain et al. 1988; Houtkooper et al. 1992; Goran et al. 1993; Suprasongsin et al. 1995; Sun et al. 2003).

This validation of prediction equations determining FFM in children with Crohn's disease showed that the model of Schaefer et al. is the best to predict FFM. Our study provides a new, simple prediction equation for FFM in a group of children with Crohn's disease which was validated by the bootstrap method. However, for a further application of this formula it should be tested in other groups of Crohn's patients.

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Chapter V

Impedance index or standard anthropometric measurements, which is the better variable for predicting fat-free mass in sick children

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ABSTRACT

Objective: To compare the predictive value of impedance index (ZI, height^2/impedance) with anthropometric measurements for estimating fat-free mass (FFM).

Design: FFM of 120 white pediatric children (46 males, 74 females), aged 2.5-18 y was measured by using dual energy X-ray absorptiometry. Weight, height, mid-upper arm circumference (MUAC), skinfold thickness (biceps, triceps, subscapular, suprailiac), and bioelectrical impedance were also obtained. Stepwise multiple regression analysis and residual plots were performed to determine the most significant variables to predict FFM.

Results: The single best predictor of FFM was ZI, which explained 96.2% of the variance in FFM (r = 0.981, SEE = 2.15 kg). Addition of weight to the model containing ZI increased the explained variance of FFM to 96.6% (r = 0.983, SEE = 2.03 kg). BMI and MUAC were the poorest predictors of FFM: r = 0.422, SEE = 10.2 kg and r = 0.621, SEE = 8.93 kg, respectively.

Conclusions: Impedance index is a more significant predictor of FFM than other anthropometric measurements. The predictive accuracy of bioelectrical impedance analysis-based prediction equations for FFM was improved by addition of weight.

KEY WORDS Bioelectrical impedance analysis, body composition, body weight, dual energy X-ray absorptiometry, predictive value.

INTRODUCTION

Determining fat-free mass (FFM) by bioelectrical impedance analysis (BIA) is based on the principle that the human body is regarded as a cylindrical conductor and the impedance (Z in ohm) of a conductor is related to its length (L in cm), cross-sectional area (A in cm squared), and applied signal frequency (Kushner et al. 1992). This relationship is expressed as $Z = pL/A = pL^2/AL = pL^2/V$, where p is volume resistivity in ohm-cm and is a constant, and AL is equal to volume (V), in other way: $V = pL^2/Z$ (Lukaski et al. 1985). In the 1960s of the last century, Thomasset et al. (1962) and Hoffer et al. (1969) tested this hypothesis in a biological system and demonstrated that the volume of total body water (TBW)- a component of FFM was proportional to L^2/Z. Thus BIA could be used to estimate FFM by applying a predictive equation based on the measured bioelectrical impedance of the subject.

Since then, a number of prediction equations for estimating FFM have been developed. Several investigators have demonstrated that the impedance index (ZI, $height^2$/impedance) yielded larger correlation coefficients than body weight or height when used as predictors of TBW, densitometrically determined FFM (Lukaski et al. 1985; Segal et al. 1985). On the other hand, some investigators have observed that anthropometric variables can be stronger predictors of TBW than BIA (Jackson et al. 1988; Diaz et al. 1989). Deurenberg et al. (1991) reported that the prediction of FFM by the BIA method gave little or no advantage over simpler anthropometric techniques that use weight, height, sex and age.

Prediction equations for estimating fat mass and FFM using skinfold thickness (SKF) as well as mid-upper arm circumference (MUAC) have been published (Brook 1971; Houtkooper et al. 1992). However, to the best of our knowledge, there is no study done in sick children that compares the predictive value of body weight, height, MUAC, SKF, and ZI for the estimation of FFM. Moreover, because there is heterogeneity in physiological status between sick and healthy children, i.e. the water content of FFM varies during childhood and during different diseases. Thus, there is a need to further compare and confirm the role of BIA and anthropometric methods for estimating body composition in sick children.

Dual energy X-ray absorptiometry (DXA) is an in-vivo reference method for studying body composition, which has been adapted for use in children, adolescents, and adults (Sardinha et al. 1999; Bray et al. 2001; Avesani et al. 2004). We therefore designed this study to determine whether ZI or anthropometric measurements has the better predictive effect on FFM measured by DXA in a group of pediatric patients.

SUBJECTS AND METHODS

Subjects

Anthropometric, BIA and DXA measurements were obtained in 120 white children (46 males and 74 females). All children were ill and needed in-patient treatment at the University Children's Hospital. Forty-one subjects (15 males and 26 females) were diagnosed as suffering from acute illness, which was defined as an onset of diseases one to seven days prior to hospital admission. Seventy-nine subjects (31 males and 48 females) were diagnosed as suffering from chronic illness which was defined as a disease state that included one or more pathologies lasting for more than the last three months and needed continuous medical treatment. The main chronic diseases the children suffered belonged to hematology, oncology, Crohn's and celiac diseases, asthma, cystic fibrosis, anorexia nervosa, endocrinology and autoimmunology. For subjects with acute illness, FFM was measured during the recovery period shortly before discharge from hospital. For subjects with chronic illness, patients with clinical apparent oedema were excluded from the study; furthermore, if FFM was measured more than one time, only the first measurement was used. The study was approved by the University Ethical Committee and the State Authority for Radiation Exposure and Control. Written informed consent was obtained from at least one parent of each subject.

Procedure for measurements

For all subjects weight, height, skinfold thickness, mid-upper arm circumference (MUAC), BIA and FFM were determined on the same day at the body composition laboratory of the University Children's Hospital.

Anthropometry

Measurement of body weight, height was performed by two trained observers according to standard techniques (Cameron 1984). With the subjects wearing hospital gowns, their body weight was measured to the nearest of 0.1 kg by using a Seca calibrated mechanical scale. Standing height was measured without shoes to the nearest of 0.5 cm by using a wall-mounted stadiometer (Längenmesstechnik GmbH Limbach-O).

A skilled technician of the body composition laboratory performed the skinfold thickness measurements. Biceps, triceps, suprailiac, and subscapular skinfold thicknesses were measured in triplicate on the left side of the body under standard conditions by using a

standard skinfold caliper (Holtain Ltd, Croswell, Crymych, United Kingdom) with a constant pressure of 10 g/mm^2. The biceps and triceps SKF were determined midway between the acromion and olecranon at the anterior and posterior surface of the arm, respectively. The suprailiac SKF was measured one cm above the superior anterior rim of the iliac crest. The subscapular SKF was measured one cm caudally and medially to the scapular angle. MUAC was measured with a precision of one mm on the left arm, at the mid-point between the olecranon and acromion.

DXA scans

FFM of the subjects were performed immediately preceding anthropometric measurements by using a whole-body densitometer (Hologic QDR 1500, Hologic Waltham, MA) operated in the pencil-beam mode. All scans were analyzed by using a whole-body software (version 5.67; Hologic) developed in conjunction with the manufacturer which was described elsewhere (Fusch et al. 1999). Scans were performed while the subjects were wearing light indoor clothing and no metal objects. The typical scan duration was 8-15 min depending on the height of the subject. With our densitometer, the radiation exposure during a whole body scan is < 0.05 mSv. FFM in kg was evaluated as the sum of lean body mass (kg) and bone mineral content (kg). Daily quality control scans for bone mineral measurements were performed with the use of an anthropometric spine phantom to ensure the correct function of the scan machine. For ethical reasons, duplicate scans were not performed. The CV for the repeated measurement of FFM was 0.4% (Fusch et al. 1999); the interassay CV reported by Carr et al. (1999) for lean mass was 1.3%.

Bioelectrical impedance analysis

Whole-body impedance (Z) was measured at 50 kHz using a single frequency impedance instrument (Bodystat 1500, Bodystat Ltd, U.K). The subjects lay in supine position comfortably on a stretcher with limbs not touching the body. Electrodes were placed at the middle of the dorsal surfaces of the right hand and foot, respectively, proximally to the metacarpal-phalangeal and metatarsal-phalangeal joints and medially between the distal prominence of the radius and the ulna at the wrist and between the medial and lateral malleoli at the ankle. The distance between the electrodes was at least 3 cm (Schaefer et al. 1994). Whole-body impedance was measured either in the morning after breakfast or in the afternoon after lunch. Bioimpedance was recorded as the mean of ten consecutive measurements made

in immediate succession. The impedance index was calculated as height in centimeters squared divided by whole-body impedance in Ohm. The CVs (%) on 10 consecutive measurements of a subject for bioelectrical impedance without changing electrode placement was 0.8%.

Statistical analysis

Data are presented as means ± SDs. Linear and stepwise multiple-regression analyses were performed to determine the most significant variables to predict FFM and to yield the lowest SEE. The mean residuals (FFM predicted - FFM measured), the 95% limits of agreement [mean residuals ± (2 x the SD of the mean residuals)] were calculated and residual plots between predicted and measured FFM were drawn to compare the role of ZI with other anthropometric variables for predicting FFM. Statistical calculations were performed by using SPSS for Windows (Version 10.0; SPSS Inc, Chicago), and a p-value < 0.05 indicated a significant difference.

RESULTS

Age, anthropometric, DXA and BIA measurements are shown in **Table 1**. Stepwise linear regression was performed for 120 subjects by using weight, height, BMI, height^2, MUAC, sum of 2 or 4 SKFs, and impedance index (ZI) as the independent variables while FFM as the dependent variable. Except for the sum of SKF, other variables were identified as significant independent predictors of FFM ($p < 0.001$) (**Table 2**). ZI was the strongest predictor identified, explaining 96.2% of the variance in FFM. The derived equations for predicting FFM, the residual between FFM predicted from each equation and FFM measured by DXA (FFM_{DXA}) are also presented in table 2.

Weight, height and height^2 accounted only for 75.3%, 81.7% and 83.9% of FFM variation, respectively. When only either weight or height was included in the regression model, SEEs were higher than that derived from the model containing only ZI. Even when height^2 and weight were included in the regression model, the correlation was still lower than that derived from the model with ZI as the predictor alone. BMI and MUAC were identified as the two poorest predictors of FFM.

TABLE 1

Subject characteristics[§]

	Mean ± SD	Range
Age (y)	13.1 ± 3.3	(2.5, 18.0)
Weight (kg)	46.5 ± 14.9	(16.0, 81.8)
Height (cm)	156 ± 19	(98, 194)
Fat-free mass (kg)	33.8 ± 11.2	(12.0, 62.8)
Percentage body fat (%)	24.8 ± 10.7	(5.8, 51.3)
MUAC (mm)	22.4 ± 3.3	(16.0, 31.0)
Sum of 4 skinfold thicknesses (mm)	43.7 ± 22.4	(15.9, 115.3)
Sum of triceps and subscapular (mm)	21.5 ± 10.7	(9.4, 56.3)
Impedance (ohm)	688 ± 112	(480, 990)
Impedance index (cm^2/ohm)	37.4 ± 12.4	(13.1, 69.9)

[§]Data are means ± SD, range in parentheses; fat-free mass was measured by DXA; percentage body fat was measured by DXA; MUAC, mid-upper arm circumference; sum of 4 skinfold thicknesses: biceps + triceps + suprailiac + subscapular.

TABLE 2

The impact of independent variables, equations for predicting FFM, the residual and 95% limits of agreement between FFM predicted and FFM_{DXA}[§]

				Residual (kg)		
Predictor	Equations	r	SEE (kg)	Mean ± SD, (range)	Lower limit	Upper limit
Weight	FFM = 0.651 Wt + 3.586	0.868	5.56	-0.005 ± 5.5 (-13.2, 13.8)	-11.0	10.9
Height	FFM = 0.540 Ht - 50.646	0.904	4.79	-0.07 ± 4.8 (-15.3, 11.5)	-9.7	9.5
$Height^2$	FFM = 0.002 Ht^2 - 11.331	0.916	4.48	-0.07 ± 4.5 (-14.5, 12.0)	-9.1	8.9
$Height^2$ + weight	FFM = 0.001 Ht^2 + 0.262 Wt - 9.283	0.937	3.90	-6.17 ± 4.1 (-19.3, 2.9)	-14.4	2.0
$Height^2$/impedance	FFM = 0.880 ZI + 0.994	0.981	2.15	-0.009 ± 2.1 (-6.5, 4.9)	-4.2	4.2
BMI	FFM = 1.344 BMI + 8.960	0.422	10.2	-0.001 ± 10.1 (-27.5, 22.1)	-20.2	20.2
MUAC	FFM = 2.111 MUAC - 13.613	0.621	8.93	0.006 ± 8.9 (-23.1, 28.9)	-17.8	17.8

[§]FFM, fat-free mass in kg; Wt, weight in kg; Ht, height in cm; ZI, impedance index in cm^2/Ohm; MUAC, mid-upper arm circumference in mm.

The equation using ZI as the predictor was better (95% limits of agreement: -4.2 and 4.2 kg) for estimating FFM than those using either only weight, height, BMI, MUAC or the combination of weight and sum of SKFs as the predictors. The best prediction equation combining ZI and weight for the estimation of FFM was:

$$FFM\ (kg) = 0.786\ ZI + 0.093\ Wt + 0.194,\ r = 0.983,\ SEE = 2.03\ (1)$$

where ZI is impedance index (cm^2/Ohm), Wt is body weight (kg). Mean residual, range, the 95% lower and upper limits of agreement derived from FFM predicted by the equation 1 and FFM_{DXA} were: 0.005 ± 2.0 kg; -5.4 to 5.4 kg; -3.9 and 4.0 kg, respectively.

However, the combination of either 2 SKFs (triceps + subscapular) or 4 SKFs (biceps + triceps + suprailiac + subscapular) and weight gave similar results: i.e. r values, SEEs, and limits of agreement from the relationship between FFM_{DXA} and FFM predicted by the equations. The equation combining weight and sum of 2 SKFs for predicting FFM was:

$$FFM\ (kg) = Wt\ [(1- (0.859\ \Sigma 2SKF + 5.555)/100],\ r = 0.981,\ SEE = 2.20\ kg\ (2)$$

where Wt is body weight (kg), Σ2SKF is the sum of triceps and subscapular in mm. Mean residual value, range, the 95% lower and upper limits of agreement derived from FFM predicted by the equation 2 and FFM_{DXA} were: 0.42 ± 2.4 kg; -8.1 to 5.2 kg; -4.4 and 5.2 kg, respectively.

The plots of residual value against FFM_{DXA} shown in **Figure 1** illustrate the predictive value of ZI relative to other anthropometric variables for estimating FFM. It has been shown that the equation using ZI and weight as the predictors (figure 1B) for FFM had the smallest limits of agreement compared with other equations.

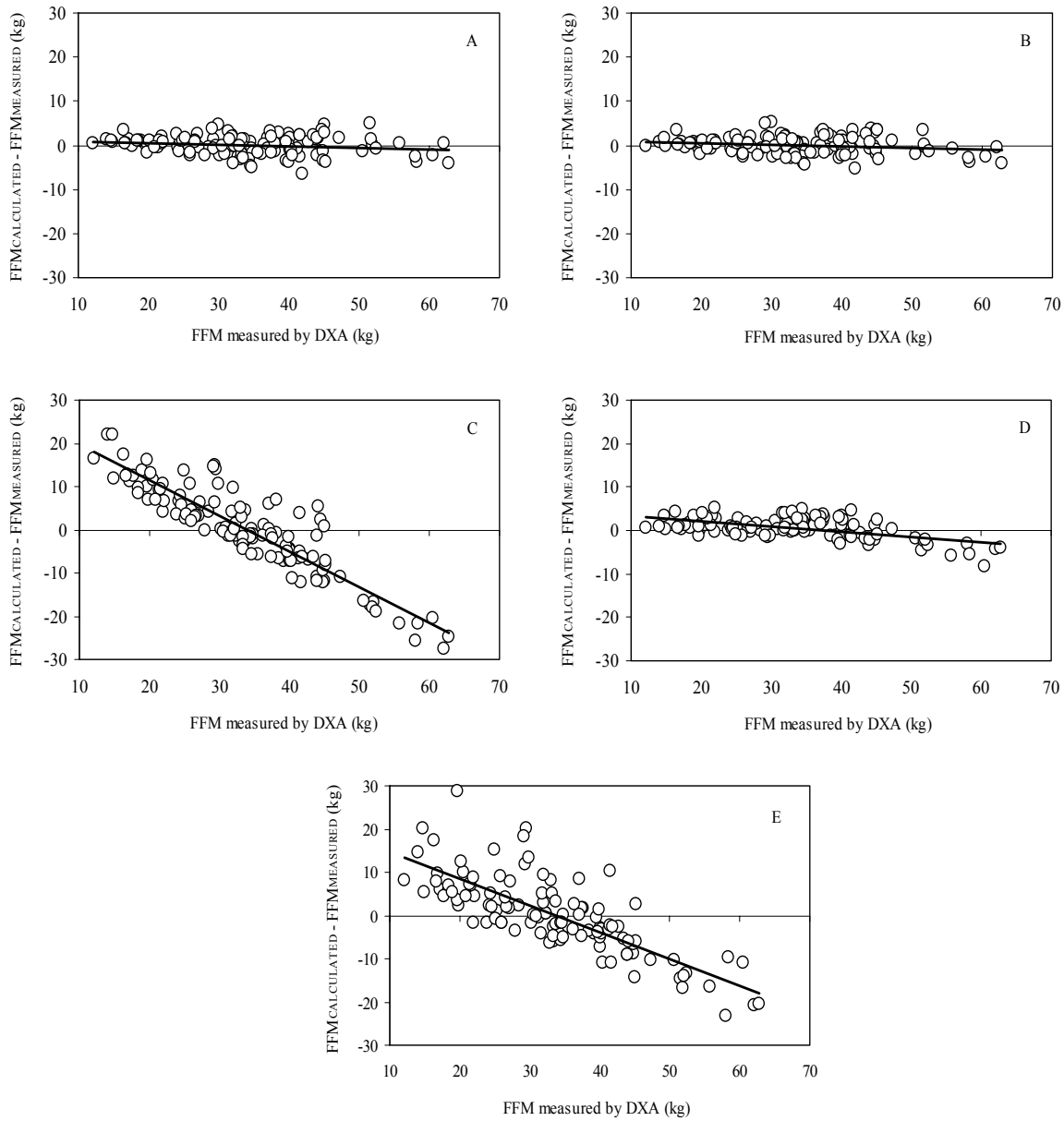

FIGURE 1. Residual plots for comparing FFM measured by DXA with FFM predicted from impedance index (A); impedance index plus weight (B); body mass index (C); weight plus sum of triceps and subscapular SKFs (D); mid-upper arm circumference (E).

DISCUSSION

The present study was designed to compare the predictive value of impedance index (ZI) and standard anthropometric measurements in a group of pediatric patients. Our results showed that, in comparison with other anthropometric measurements, ZI is the better predictor of FFM. Addition of weight improves the predictive accuracy of the BIA prediction equations for FFM.

BIA is a safe, inexpensive, portable, rapid and easy method to measure body composition. Similarly, measuring body weight, and height is also a simple procedure to assess the nutritional status. Both require some operator training. However, it takes less training to do a bioimpedance measurement compared to skinfold thickness or MUAC measurements. The accurate and precise body composition results depend on the regression equations using BIA or body weight, height, and other anthropometric parameters as the predictors. To see the usefulness of BIA, it is necessary to justify the accuracy and precision of BIA-based predictive equations compared with those based on anthropometric measurements alone.

ZI was reported to be the best single predictor of FFM by multiple-regression analysis in a number of studies (Kushner and Schoeller 1986; Lukaski et al. 1986; Diaz et al. 1989; Fjeld et al. 1990; Deurenberg et al. 1991; Schaefer et al. 1994). A recent study in a sample of healthy white and black subjects reported that ZI was the better single independent variable than body weight for estimating FFM (Sun et al. 2003). Several authors observed that the formulas for estimating FFM in children were improved when body weight was included in the equation along with ZI (Houtkooper et al. 1992; Goran et al. 1993; Suprasongsin et al. 1995).

In contrast, some studies reported that height (or height^2) and weight were more significant predictors than were ZI (Helenius et al. 1987; Van Loan and Mayclin 1987; Jackson et al. 1988; Diaz et al. 1989; Gray et al. 1989). This controversy is due to the fact that the selection of subjects for analysis was different from one study to another and might reduce the importance of ZI. Diaz et al. (1989) investigated in a small group of young adults whose weights and heights were smaller than those in other validation studies involving adults, they concluded that after weight and height were entered into the prediction equations, ZI contributed less than 5% to the prediction of FFM. Helenius et al. (1987) studied in a group of overweight middle-aged men and women and they found that ZI did not contribute to the estimation of percent body fat when added to selected anthropometric variables. Studying in a

group of adults with 75% of whom were obese, Gray et al. (1989) also reported that weight and height^2 were stronger predictors than ZI.

Interestingly, our results showed that height gave higher r value and smaller SEE in comparison to weight. However, Kushner et al. (1992) reported that weight was identified as the better predictor than height for TBW- a component of FFM. It may be explained by the fact that subjects of the two studies are different.

The smaller limits of agreement (absolute values) derived from ZI than those derived from either any independent anthorpometric parameter (table 2) or the combination of different anthropometric variables (height^2 plus weight; weight plus sum of SKFs) suggested that ZI was the best predictor for FFM. Our study pointed out that although the sum of SKFs did not correlate with FFM (data not shown), but inclusion of weight and the sum of SKFs in the model gave a good estimate of FFM compared to FFM_{DXA}, indicating that FFM can be well predicted from the formulas using weight and sum of SKFs. The correlation, SEE, and the limits of agreement from the relationship between FFM_{DXA} and FFM predicted by the equations using the sum of 4 SKFs or the sum of 2 SKFs were nearly the same. It suggested that the sum of 4 SKFs was not better compared to the sum of triceps and subscapular SKFs when combined with weight to predict FFM.

Our study was carried out in sick children suffering from chronic and acute diseases. The difference in physiological status in sick children compared with healthy subjects may influence the outcome and therefore lead to the different results. Though a number of studies were done to investigate the role of ZI and anthropometric variables in predicting FFM, to the best of our knowledge, this study may be the first of its kind that was done so far in sick children. The study includes a large, heterogenous data set with a wide range in weight, height and age. It allows us to statistically compare the predictive value of ZI and some anthropometric measurements for estimating FFM.

We used DXA as a reference technique to measure FFM. This method provides a direct measurement of body composition with high accuracy and precision compared with those of other noninvasive methods, i.e. bioelectrical impedance analysis, deuterium dilution, total body electrical conductivity, underwater weighing. Animal studies have shown a linear relationship between lean and fat mass measured by DXA and by chemical carcass analysis (Brunton et al. 1997; Fusch et al. 1999). Easy and simple performance of DXA in the

laboratory allows us to get the body composition data of all study subjects; this work is more complicated when done with underwater weighing. For a single method, DXA is usually regarded as a good reference one to measure body composition. In comparison with the 4-compartment model that combines several methods to estimate a body composition characteristic, DXA might underestimate or overestimate the true value (Wong et al. 2002). However, the 4-compartment model is too complicated to be used in clinical routine.

We chose FFM as the dependent variable because FFM is considered to be directly correlated with health and life expectancy (Shephard 1994). It is an important predictor of survival in some clinical diseases and malignancies (Hill 1992). Measurement of FFM is important for optimum clinical care during hospitalization because the size of the FFM is an important index of energy and fluid requirements during artificial nutrition (Wells et al. 1999).

We conclude that ZI is a stronger predictor than anthropometric variables for predicting FFM in sick children. Inclusion of weight improves the predictive accuracy of the impedance index-based prediction equation and gives the best estimate of FFM. FFM can also be well predicted by using skinfold thickness and weight. Finally, this study supports that BIA method is better than anthropometry in measuring body composition.

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Chapter VI

Birth weight categorization according to gestational age does not reflect percentage body fat in term and preterm newborns

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ABSTRACT

Objective: To prove the applicability of the small-for-gestational age (SGA), appropriate-for-gestational age (AGA) and large-for-gestational age (LGA) classification depending on birth weight to predict percentage body fat (%BF) measured by dual energy X-ray absortiometry (DXA) in term and preterm infants.

Design: Data of 159 healthy term and preterm neonates (87 boys and 72 girls) with a gestational age at delivery of 38.4 weeks from two longitudinal studies were analyzed. Anthropometry and body composition data were assessed within the first 10 days after birth. Correlations between anthropometric parameters and fat mass measured by DXA were calculated. Prevalences of observations with low, middle and high %BF measured by DXA were compared between SGA, AGA and LGA groups, according to sex and gestational age.

Results: In term infants, 42.9% of the newborns with less than 10% body fat were classified to be AGA; 9.9% of all AGA newborns had less than 10% body fat. For the whole group, among the ratios investigated weight-length ratio (r = 0.82) showed the best correlation to fat mass measured by DXA. %BF at the time of study was higher in girls (14.75%) than in boys (11.95%).

Conclusions: Traditional classification based on birth weight centiles does not reflect %BF in term and preterm newborns.

KEY WORDS Dual energy X-ray absorptiometry, neonates, weight-length ratio, body composition, ponderal index, body fat.

INTRODUCTION

The adequacy of intrauterine growth has been assessed in neonates by birth weight percentile, with small-for-gestational age (SGA) infants defined as less than the 10^{th} birth weight percentile, appropriate-for-gestational age (AGA) infants defined as between 10^{th} and 90^{th} birth weight percentile and large-for-gestational age (LGA) infants defined as greater than the 90^{th} percentile for gestational age, sex and race (Thomas et al. 2000). Infants classified as SGA (small) are often considered as having grown under unfavorable intrauterine conditions leading to underweight mostly due to lack of accumulation of fat mass. LGA infants are often considered as being macrosomic, leading to "too much" fat mass. Fetal nutritional status at birth may be linked with neonatal and later morbidity or even mortality (Williams et al. 1982). However there is doubt whether non-AGA classified growth is always an indicator of abnormal nutritional status. Similar to later life, growth below 10^{th} percentile may also occur in the presence of normal fat mass when the subject is well proportioned but only short. On the other hand, gestational age specific weight-for-length charts currently do not exist, infants may have subnormal fat mass but may be regarded as AGA because they have a bigger body. To our knowledge, it is not known whether the more "classic" classifications do really reflect body fat. Thus, there is an interest to prove the applicability of the classification depending on birth weight to predict percentage body fat (%BF) measured by dual energy x-ray absorptiometry (DXA).

Body composition of infants and children born at the extremes of birth weight are often measured by DXA (Lapillonne et al. 1997a; Koo et al. 2004). DXA provides data on fat mass, fat-free mass, bone mineral content and %BF. DXA is a safe and quick method for measuring body composition and requires little co-operation from the patients. It is considered as the best technical choice for pediatric use for measuring body composition in clinical settings (Lapillonne et al. 1997b). In addition, it has recently been shown that fat mass measured by DXA correlates well with chemically determinated total fat mass (Koo et al. 1995; Picaud et al. 1996; Fusch et al. 1999; Rigo et al. 2000).

Weight-for-length ratio (weight in grams/length in centimeters), ponderal index [(birth weight in grams/crown-heel length in centimeters^3) x 100] and body mass index (Quetelet index) [weight kg/crown-heel length in m^2] were suggested to give a better reflection of soft tissue mass than birth weight percentile (Rohrer 1921). Body fat at birth is the most important source of energy in the first days of the newborn's life. With the accurate measurement of fat

mass by DXA it is possible to give a more detailed estimation of the newborn's nutritional status.

The first aim of this study was to compare the SGA, AGA and LGA classification depending on birth weight percentiles with fat mass measured by DXA in a group of term and preterm infants. The other aims were to investigate the body composition characteristics and the correlations between some anthropometric indices and fat mass measured by DXA in this population.

SUBJECTS AND METHOD

Subjects

In this study data of 159 healthy preterm and term newborn infants were analyzed. Subjects were recruited from two longitudinal studies in Greifswald (Germany) and Berne (Switzerland) and were fed mother's milk or formula (fortified or preterm formula until body weight reached 2000 g). Anthropometry and DXA measurements were assessed within the first 10 days after birth. Preterm and term infants were measured within the mean age after birth of 7.2 ± 2.1 days and 4.1 ± 1.6 days, respectively. In order to assess healthy infants, exclusion criteria for both studies were as follows: major congenital anomalies, major chromosomal anomalies, metabolic anomalies such as hypoglycemia and hypocalcemia: (plasma glucose concentration < 2.5 mmol/l, plasma ionized calcium concentration < 1.12 mmol/l), high-order multiple births, arterial blood pH below 7.00, 10-minute APGAR score below 7. Gestational age in completed weeks was determined from the first day of the mother's last menstrual cycle and the date of delivery and confirmed by antenatal ultrasonic measurement of the biparietal diameter. Prematurity is defined by a gestation age of less than 37 weeks. All infants were of Caucasian origin. Both studies were approved by the University Ethical committees. Written informed consent was given by all parents.

Procedure of measurement

DXA was obtained in a quiet and warm room usually after oral feeding. If children were still sleeping after taking off their clothes, they were swaddled into cotton blankets without additional clothing or diaper and DXA-measurement was performed immediately. In most cases, performance could be done without moving artifacts after an acceptable time of

calming. If movement artifact was noted the scanning procedure was interrupted, and the scan was repeated when infants was calmed.

Anthropometry

Birth weight and weight on the day of examination of the naked infants were measured to the nearest 10 g using a standard beam balance (Seca, Hamburg, Germany). Accuracy was confirmed using calibrated weights of known mass. Birth length and length on the day of study were measured in triplicate to the nearest 0.5 cm using a tape measure. In a previous study, the length made with the tape measure was compared with that obtained by using a measuring board (Schaefer, Karlsruhe, Germany). The mean (± SD) difference between the 2 measurements (0.02 ± 0.55 cm) was not significant (Schmelzle et Fusch 2002).

Dual energy X-ray absorptiometry

The basic theory and methodology for DXA have been described elsewhere (Koo et al. 1995). The babies were measured on a whole body scanner (QDR 1500, Hologic, Waltham, MA) operated in a single beam mode. The X-ray tube is pulsed between high (140 kVp) and low (70 kVp) voltage at a rate of 50 Hz to produce dual energy X-ray beams. A detector mounted above the infant measured the transmitted intensity on a pixel-by-pixel basis. External calibration was performed with a step phantom with known equivalent amounts of fat and lean tissue.

Daily quality control scans were performed using a manufacturer-supplied anthropometric spine phantom with a known amount of calcium hydroxyapatite embedded in a cubical epoxy block. For ethical reasons, duplicate measurements were not performed.

All scans were performed on a pediatric platform with the baby lying in a supine position using the pediatric whole body scanning procedure. The pediatric platform filters the low-energy beam to improve system linearity in small subjects and to reduce the radiation dose. Scan time was about 8 minutes. All scans were analyzed with a modified infant whole body software (version 5.67) with separate drift corrections for both x-ray energy levels. This is not the case in the version originally supplied by Hologic. Due to the smaller sample size of neonatal patients this correction improves the quality (Fusch et al. 1999). This modification was implemented by the manufacturer as a result of a pilot study, in which we investigated the performance of our DXA system for the measurement of small bodies (data not published).

In a previous animal study we report that CV for the repeated measurement of FM was 4.6% and fat mass measured by DXA was highly correlated with total fat by chemical analysis (r^2 = 0.961, the slope and intercept of the regression equation were: 0.763 and 52.4 g, respectively), (Fusch et al. 1999). Similar results were found by others (Picaud et al. 1996; Rigo et al. 2000). Use of DXA fat mass therefore gives an accurate estimation of chemical measures of body fat.

Children of both studies were measured with the same DXA-device and software, since the device was moved from Berne to Greifswald in 1998.

Statistical analysis

All values are expressed as mean ± SD. Due to the absence of the standard definition of nutritional status based on %BF, we divided arbitrarily %BF into 3 categories: < 10%, 10-20%, and > 20%. T-test was used to detect the difference in %BF between boys and girls, between term and preterm infants. Differences in gestational age at birth, age at study, anthropometric and body composition measurements among SGA, AGA, and LGA groups were assessed by one-way analysis of variance (ANOVA), followed by the Bonferroni post hoc comparisons to investigate pairwise differences between individual groups. Linear regression analysis was performed to show relationship between the different anthropometric and body composition parameters which were measured on the day of study.

RESULTS

A total of 159 neonates were included in the study. 35 children measured were born under 37 weeks of gestational age. Mean gestational age at delivery was 38.4 ± 2.9 weeks. 26 children (16.4 %) were SGA (< 10^{th} centile), 15 children (9.4 %) LGA (> 90^{th} centile) using actual age- and sex- related centile charts (Weller and Jorch 1993). The demographic, anthropometric, and body composition data are given in **Table 1**. Significant differences could not be seen between the SGA, AGA and LGA groups concerning gestational age at birth and age at study ($p > 0.05$). All anthropometric and body composition parameters showed statistically significant differences between the SGA, AGA and LGA groups at a level of $p < 0.05$, except for the weight loss which was not different between SGA and AGA groups.

TABLE 1

Subject characteristics of the whole group, preterm infants, term infants, and infants born SGA, AGA and LGA[¶]

	All children (n=159)	Preterm infants (n=35)	Term infants (n=124)	SGA (n=26)	AGA (n=118)	LGA (n=15)
Gestational age at birth (wk)	38.4 ± 2.9	33.9 ± 1.9[§]	39.7 ± 1.4	38.2 ± 2.7	38.3 ± 3.0	39.2 ± 1.8
Age at study (d)	4.8 ± 2.2	7.2 ± 2.1[§]	4.1 ± 1.6	5.0 ± 2.4	4.7 ± 2.2	4.7 ± 2.1
Birth weight (g)	3140 ± 850	2120 ± 720[§]	3420 ± 640	2320 ± 660[$]	3150 ± 680[†]	4430 ± 630[‡]
Weight at study (g)	3050 ± 790	2100 ± 650[§]	3320 ± 600	2280 ± 590[$]	3080 ± 660[†]	4180 ± 610[‡]
Weight at study - birth weight (g)	-85 ± 119	-24 ± 110[§]	-101 ± 116	-36 ± 115	-74 ± 105[†]	-248 ± 107[‡]
Birth length (cm)	49.3 ± 4.0	44.3 ± 4.3[§]	50.7 ± 2.6	46.1 ± 4.8[$]	49.7 ± 3.5[†]	52.3 ± 2.5[‡]
Length at study (cm)	49.5 ± 3.9	44.6 ± 3.9[§]	50.8 ± 2.6	46.3 ± 4.1[$]	49.8 ± 3.5[†]	52.7 ± 3.0[‡]
BMI (kg/m^2)	12.2 ± 1.9	10.2 ± 1.6[§]	12.8 ± 1.5	10.4 ± 1.4[$]	12.2 ± 1.5[†]	15.0 ± 1.7[‡]
FM_{DXA} (g)	440 ± 290	200 ± 140[§]	510 ± 290	210 ± 100[$]	430 ± 190[†]	980 ± 510[‡]
$\%BF_{DXA}$ (g)	13.2 ± 5.7	8.5 ± 3.9[§]	14.6 ± 5.4	8.6 ± 3.1[$]	13.1 ± 4.3[†]	22.3 ± 8.2[‡]
LBM_{DXA} (g)	2600 ± 580	1910 ± 540[§]	2800 ± 410	2080 ± 520[$]	2650 ± 520[†]	3170 ± 360[‡]
BMC_{DXA} (g)	54.5 ± 18.9	32.8 ± 14.6[§]	60.6 ± 15.0	39.2 ± 16.0[$]	54.5 ± 15.8[†]	81.1 ± 16.7[‡]

[¶]Mean ± SD; BMI, body mass index; FM_{DXA}, fat mass measured by DXA; LBM_{DXA}, lean body mass measured by DXA; BMC_{DXA}, bone mineral content measured by DXA.

[§]Differs from term infants, $p < 0.001$.

[$]Differs from AGA, $p < 0.05$.

[†]Differs from LGA, $p < 0.05$.

[‡]Differs from SGA, $p < 0.05$.

The ages at study were not statistically different between boys and girls in SGA, AGA and LGA subgroups (data not shown) ($p > 0.05$, T-test). For the whole group, 87 boys and 72 girls were studied, boys showed lower %BF (11.95%) than girls (14.75%) ($p < 0.01$). Anthropometric and body composition measurements were found to be significantly lower in preterm than those in term infants ($p < 0.001$).

There was no significant difference in ponderal index between boys and girls (2.42 vs. 2.50) ($p > 0.05$) at time of study. Length was significantly higher in boys than in girls for the LGA (boys 54.9 cm vs. girls 51.6 cm) ($p = 0.037$) but not for the SGA and AGA subgroup.

Strong associations among all anthropometric factors are summarized in **Table 2**. Weight-length-ratio ($r = 0.82$) showed the best correlation to DXA fat mass among the ratios studied, better than the parameters using cubed (Ponderal index, $r = 0.61$) or squared (BMI, $r = 0.79$) length. Weight alone showed a correlation coefficient of 0.82.

TABLE 2

Correlation matrix between anthropometric parameters[¶]

	W	L	FM	PI	BMI	W/L	BW%	%BF
W	1.0							
L	0.90	1.0						
FM	0.82	0.63	1.0					
PI	0.61	0.25	0.61	1.0				
BMI	0.91	0.68	0.79	0.88	1.0			
W/L	0.99	0.84	0.82	0.73	0.97	1.0		
BW%	0.67	0.50	0.62	0.55	0.67	0.68	1.0	
%BF	0.74	0.58	0.96	0.60	0.75	0.76	0.55	1.0

[¶]W, weight at date of measurement; L, length at date of measurement; FM, fat mass measured by DXA; PI, ponderal index; BMI, body mass index; W/L, weight-length ratio; BW%, birthweight percentile; %BF, percentage body fat measured by DXA.

Table 3 shows the number and percentage of observations which were classified by birth weight percentiles and by percentage body fat in only term infants (n = 124). The subgroup with less than 10% body fat contains 57.1% SGA, but also 42.9% AGA infants. Subjects with more than 20% fat mass were classified to be AGA (42.9%, 6 infants) or LGA (57.1%, 8 infants).

TABLE 3

Percentage of observations classified by birth weight percentile and percentage body fat in term infants[¶]

Groups according to %BF	Groups according to birth weight percentile			Total
	SGA	AGA	LGA	
< 10%	(12)	(9)	(0)	(21)
	57.1%	42.9%	0%	100%
	60.0%	9.9%	0%	16.9%
10-20%	(8)	(76)	(5)	(89)
	9.0%	85.4%	5.6%	100%
	40.0%	83.5%	38.5%	71.8%
> 20%	(0)	(6)	(8)	(14)
	0%	42.9%	57.1%	100%
	0%	6.6%	61.5%	11.3%
Total	(20)	(91)	(13)	(124)
	16.1%	73.4%	10.5%	100%
	100%	100%	100%	100%

[¶]SGA, small-for-gestational age group; AGA, appropriate-for-gestational age group; LGA, large-for-gestational age group; %BF, percentage body fat; absolute number of infants in brackets.

Figure 1 and 2 show the relative distribution of subjects classified by different %BF cut-off points on the birth weight for gestational age charts in boys (n = 87) and girls (n = 72), respectively. There was an overlap on %BF among SGA, AGA, and LGA groups in these two figures.

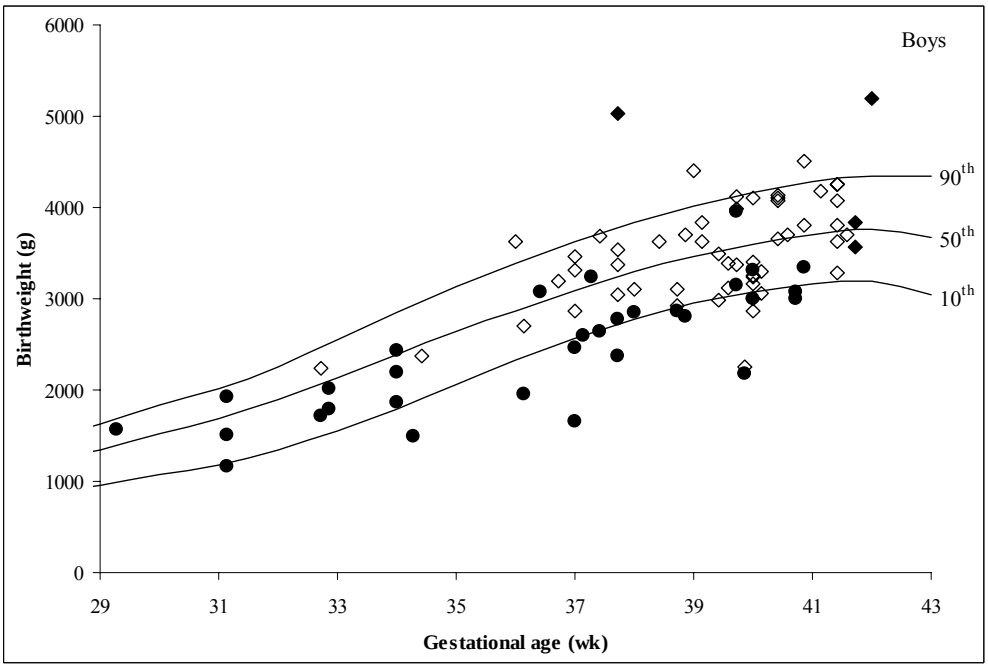

FIGURE 1. Distribution of subjects with percentage body fat below 10% (●), between 10-20% (◇), above 20% (◆) on the birth weight for gestational age chart in term and preterm boys.

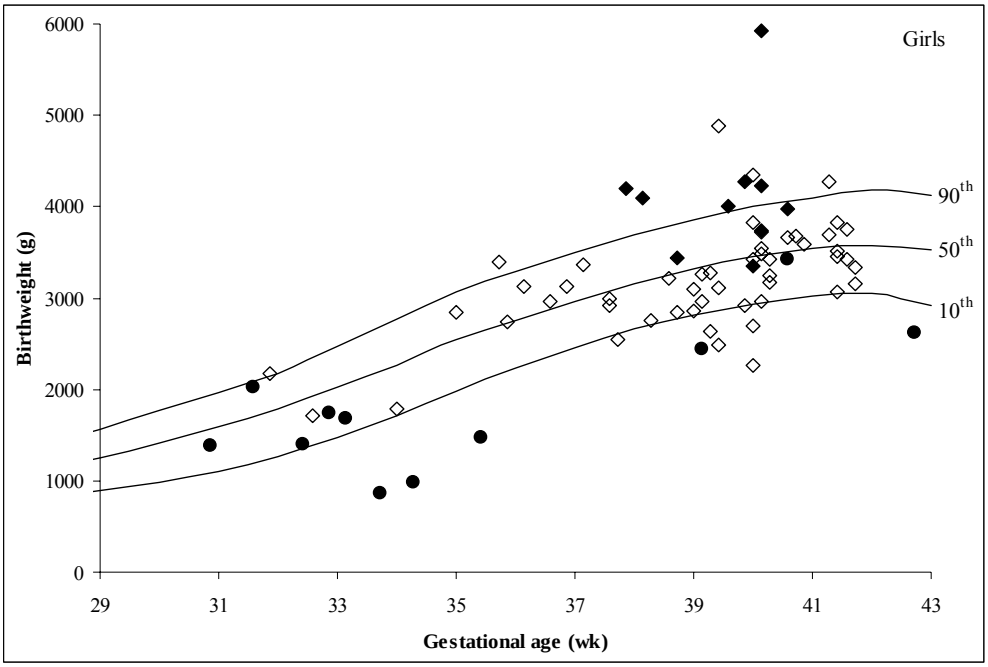

FIGURE 2. Distribution of subjects with percentage body fat below 10% (●), between 10-20% (◇), above 20% (◆) on the birth weight for gestational age chart in term and preterm girls.

DISCUSSION

The present study showed that the classification of the nutritional status based on birth weight percentile does not precisely reflect %BF in healthy term and preterm infants. The weight for length ratio shows the best correlation with fat mass measured by DXA. Higher %BF is found in females than in males as well as in term than in preterm infants.

The new feature of our study is that intrauterine growth using age-and sex-matched percentiles was compared with the nutritional status using %BF measured by DXA. In addition, the correlations between anthropometric parameters with absolute fat mass were calculated. %BF in male and female infants as well as in term and preterm babies was assessed.

We see a large overlap of %BF between the three growth groups SGA, AGA and LGA: infants born AGA had %BF both lower than 10% and higher than 20%; infants born SGA or LGA had %BF between 10 and 20%. However, the observed overlap is considered to reflect the real condition of the infant's nutritional status. This indicates that there are SGA infants of normal body composition with %BF supposed to be 10-20%, they are simply too "small", but the relative amount of body fat reflects a normal fetal growth and nutritional status. Similarly, there are LGA infants with %BF in normal range, they are simply too "large", but are also considered to have a normal fetal growth according to this genetic potential.

According to age- and sex-matched birth weight percentile chart, infants born AGA are not considered as a group with higher risks of morbidity and mortality when compared with SGA and LGA groups (Hediger et al. 1998). In our study, among subjects with %BF less than 10%, there were more than 40% infants born AGA. The results state clearly that infants born AGA with low nutritional status i.e. fat mass less than 10% could not be detected by birth weight percentiles.

Concerning the body composition characteristics, our study shows that weight, length, fat mass, %BF, lean mass, bone mineral content of SGA infants were significantly lower than those observed in AGA and LGA infants. This finding is consistent with that derived from a smaller study which assessed body composition using DXA in 20 SGA and 90 AGA infants (Lapillonne et al. 1997a). The feeding regime may influence the %BF of subjects. Despite that body composition of preterm infants was measured 3 days later after birth than term infants, %BF in preterm infants was significantly lower than those in term infants. Body composition

was measured nearly at the same time for both boys and girls; however, %BF was higher in girls than in boys. It may indicate that %BF are accumulated in utero during pregnancy more in girls than in boys as well as more in term than in preterm infants. Longitudinal body composition data of newborn infants after birth show that during weight loss water and solids were lost from the body in proportion to their presence in the body (vd Wagen et al. 1985). Therefore it can be assumed that %BF does not change during postnatal adaptation. Moreover, even if this would have been the case the data of this study would correlate fetal growth classification with the starting point after postnatal adaptation is completed-as the basis of further growth and nutritional needs. Butte el al. (2000a) used DXA to measure body composition and reported that human females have greater fat mass than males at 6 and 9 months of age. Lapillonne et al. (1997a) pointed out that preterm infants showed lower fat mass compared to term ones. There might be little differences due to the different device models used by Lapillonne et al. (1997a) and Butte et al. (2000a), but the tendency of the results is similar and supports our findings. The greater amount of body fat in LGA infants compared with SGA and AGA infants explains why there were no LGA infants in the group with body fat below 10% as well as there were no SGA infants in the group with body fat above 20% in our study (Hammami et al. 2001; Koo et al. 2004). It also explains the higher percentage of observations with body fat above 20% compared to that with body fat lying between 10-20% in the LGA group (61.5 % vs. 38.5%).

For the correlations between anthropometric indices and absolute fat mass, we found that, compared with BMI and ponderal index, the ratio of W/L correlated better with fat mass measured by DXA. This finding is confirmed by the study using other methods to estimate fat mass like that of de Bruin et al. (1995), in which they found that ponderal index did not correlate well with fat mass estimated by total-body electrical conductivity method ($r^2 = 0.1$). A poor correlation with whole-body fat mass and fat-free mass has been described for Quetelet`s index and skinfold thickness in early infancy by Davies and Lucas. (1989, 1990). Interestingly the correlation between a single anthropometric parameter and %BF is weaker than the correlation between each anthropometric parameter with absolute fat mass.

In this study, DXA was used to measure body composition. A number of validation studies have been done to allow the use of DXA in neonates with good precision and accuracy (Braillon et al. 1992; Koo et al. 1995; Picaud et al. 1996; Fusch et al. 1999). DXA has been used in neonatology for the evaluation of bone mineralization and body composition (Butte et al. 2000a; Schmelzle and Fusch 2002; Koo et al. 2004; Lapillonne et al. 2004). However,

DXA for precise assessment of nutritional status is not available in many clinical settings, skinfold method is a means of estimating fat mass, when more detailed knowledge about fat mass is needed at bedside, this method can be used (Schmelzle and Fusch 2002).

In newborns, to our knowledge, the cut-off points used to define low or high %BF have been not identified. We therefore defined arbitrarily cut-off points to classify %BF. A possible limitation of our study is the use of these cut-off points, because it may lead to the errors in calculating results. Therefore, it is better to choose the cut-off points that link with mortality and morbidity of the neonates. Further studies need to be implemented to identify these cut-offs. Another limitation of the study is that two cohorts measured at different places at different times could influence our results. But both populations are of Caucasian origin, the acceleration is not a question of years but of decades or centuries.

We summarize that the classification based on birth weight percentiles alone only gives a rough estimation of nutritional status of term and preterm newborns. To assess the individual or collective risk for babies with low %BF, longitudinal studies should be performed. Between classic anthropometric ratios and absolute fat mass the best correlation was found for W/L ratio. Girls show higher %BF than boys.

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Chapter VII

The effect of dietary nucleotides on growth and body composition during the first four months of life

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ABSTRACT

Background: There has been an increasing interest in the role of dietary nucleotides in neonatal and infant nutrition.

Objective: To compare effects on growth and body composition in a nucleotide supplemented formula-fed study group (NF) versus a standard formula (SF) and a breast-fed control group (MM).

Design: Partly randomized, partly double-blinded prospective study. 112 healthy term and preterm newborns (48 girls, 64 boys), breast- or formula-fed infants were randomized to receive a formula with or without supplemented nucleotides. Longitudinal measurement of weight, length, mid arm and head circumference, body composition (lean body mass, percentage body fat) using dual energy x-ray absorptiometry and skinfold thickness at the age of 0, 2, and 4 months were collected.

Results: Weight gain was higher in the SF group during the last 2 months than in the NF group ($p < 0.05$, t-test), caused by a higher accretion of lean mass ($p < 0.01$, t-test). Weight gain during the last 2 months ($p < 0.05$) and gain of lean mass during the total 4 months ($p < 0.01$) was higher in the SF group than in the groups nourished with NF or breast-milk. Progress of head circumference was higher in infants fed SF than in infants of other groups ($p < 0.05$, ANOVA). Gain of bone mineral content was higher in breast fed than in formula-fed infants during the first 2 months ($p < 0.05$).

Conclusions: Dietary nucleotides fail to show the positive effects on growth and body composition in healthy term and preterm infants.

KEY WORDS Nutrition, infants, nucleotide, dual energy x-ray absorptiometry.

INTRODUCTION

Human milk is believed to be the best and natural nutrition during the first months of life. In cases where breast-feeding cannot be performed, formula feeding usually is a safe alternative. However, commercially available formulas are usually prepared on a cow milk basis and, though continuous efforts have been made to adapt their nutritional composition to that of breast milk, the micronutrient composition may still be different.

Nucleotides are said to be semi-essential nutrients that may be necessary for DNA formation, are intermediates in many biosynthetic processes, participate in energy transfer reactions (ATP, GTP), are components of three major coenzymes (NAD, FAD, CoA), and are metabolic regulators (Stryer 1988). Nucleotides are found in breast milk, but are only in few amounts present in cow milk and usually not supplemented in standard term formulas (Kobata et al. 1962; Gil and Uauy 1995). However, it has been hypothesized that increased amounts may be needed during periods of rapid growth i.e. during the first 6 months of life (Cosgrove 1996). This need may be even more pronounced in infants that suffer from intrauterine growth retardation due to intrauterine malnutrition/placental insufficiency-infants that usually show postnatal "catch-up" growth. It has been shown that in these infants dietary intake of nucleotides may be a limiting factor for growth and that dietary supplementation may have a beneficial effect (Cosgrove 1996). Additionally it has been shown that the function of the immune system may be positively influenced by additional dietary intake of nucleotides (Carver et al. 1991; Pickering et al. 1998).

Based on these findings it was therefore the aim of the current study to assess the impact of dietary supplementation of nucleotides on postnatal growth using a prospective, partly randomised and partly double-blind trial. For this purpose two groups of infants receive either nucleotide supplemented or nucleotide free but otherwise identical formula. Both groups are additionally compared with a group of exclusively breast fed infants.

SUBJECTS AND METHODS

Study design

The study was a partially randomized double-blind prospective trial (breast-fed group not randomized and blinded). The two formula groups (nucleotide supplemented vs. standard,

formula content of nucleotides see below) were randomized using a block design with a block size of 2, i.e. one of two subsequent formula-fed infants randomly obtained nucleotide supplemented formula, the other infants the standard formula. Primary outcome measure was the comparison of anthropometric growth data between the two formula groups. Secondary outcome measure was the growth data of breast-fed infants, those were used as control group.

The subjects entered the study after careful clinical examination and after obtaining written informed parental consent. No other diet or weaning was allowed during the whole study period. Anthropometric measurements (length, weight, skinfold thickness, DXA) were performed on three occasions: during neonatal screening, at 2 and at 4 months of age. Time points of measurements were defined as postnatal days in order to standardize the period of the feeding regimen: t_0: 0-5 days post partum, t_1: 55-65 days post partum, t_2: 115-125 days post partum. The study protocol was approved by the local Ethical Committee as well as by the Federal Bureau of Radiation Safety.

Subjects

Infants were either born at the maternity of our university hospital or, if they were born at surrounding district hospitals, were enrolled in the study until day 5 of life. Gestational age was determined using either menstrual period of the mother or according to the findings obtained by early prenatal ultrasound. Subjects were classified SGA when the birth weight was below the 10^{th} percentile using specific charts for sex and gestational age (Weller and Jorch 1993).

In order to study healthy infants, subjects with the following criteria were not included in the study: major congenital anomaly, major chromosomal anomaly, mechanical ventilation, gestational age not known, conditions which affect fetal and neonatal growth (e.g. maternal diabetes, hypertension, abnormalities of the placenta, maternal use of drugs), metabolic anomalies, infants born from higher-order multiple births (but no exclusion of twins), arterial cord blood pH < 7.00 and 10 minutes APGAR score < 7.

One hundred and twelve healthy newborn infants were consecutively included in the study. Ninety-six were born at term (37-42 week of gestation), sixteen infants were born between 34 and 36 weeks of gestational age. All infants were exclusively orally fed either with breast milk or with formula.

Diet

The infants were fed ad libitum with one of the following diets: own mother milk (MM), commercially available formula containing nucleotides (NF) and formula identical however without nucleotides (SF). Nucleotide contents of NF are as follows: 5'- CMP 2.3 mg/100 kcal (1.6 mg/100 ml), 5'-UMP 1.0 mg/100 kcal (0.7 mg/100 ml), 5'-AMP 0.9 mg/100 kcal (0.6 mg/100 ml), 5'-GMP 0.4 mg/100 kcal (0.3 mg/100 ml), 5'-IMP 0.2 mg/100 kcal (0.1 mg/100 ml).

The formulas were produced as one single charge and shelf life was checked at 4, 8, 12, 15 and 18 months. No formula feeding was allowed in the breastmilk group. Infants initially fed human milk could be included in one of the two formula groups, if breast-feeding ended up to day 5. Weaning could only be started from 4 months on. The mothers were asked to keep a diary during the whole study period. Milk intake was recorded every day and remarks on illness, drug use etc. were made.

Anthropometry and DXA

Length was measured in triplicate to the nearest 0.5 cm using a tape measure, in 20 children additional measurements were done using a measuring board (Schäfer, Offenburg, Germany). Weight was measured to the nearest 10g using a standard beam balance (Seca, Hamburg, Germany).

Head circumference was measured in triplicate to the nearest 0.1 cm using a tape measure. Triceps, biceps, suprailiacal and subscapular skinfolds were measured in triplicate under standard conditions on the left side of the body using a standard Holtain skinfold caliper (Holtain Ltd., Crymych, UK) operating with a constant pressure of 10 g/mm^2. Triplicate measurements were performed and the mean was used. The majority of the measurements were obtained by one observer (H.R.S) with the help of a well-trained medical technical assistant for holiday cover.

The basic theory and methodology for DXA have been described elsewhere (Koo et al. 1995). The babies were measured on a whole body scanner (Hologic QDR 1500, USA) operated in a single beam mode. The x-ray tube is pulsed between high (140 kV) and low (70 kV) voltage at a rate of 50 Hz to produce dual energy x-ray beams. A detector mounted above the infant measured the transmitted intensity on a pixel-by-pixel basis. External calibration was

performed with a step phantom with known equivalent amounts of fat and lean tissue.

Daily quality control scans were performed using a manufacturer-supplied anthropometric spine phantom with a known amount of calcium hydroxyapatite embedded in a cubical epoxy block.

All scans were performed on an infant platform with the baby lying in a supine position. The infant platform filters the low-energy beam to improve system linearity in small subjects and to reduce the radiation dose. All infants were measured during postprandial sleep without additional sedation. Total scan time was about 8 minutes. All scans were analyzed with the manufacturer-supplied infant whole body software (version 5.67).

Statistics

Results are presented as mean ± SD. As growth velocity for length and weight is nearly linear from 34 weeks to term the influence of gestational age is compensated by representing the growth data as differences between the study time points. Differences between the formula groups were tested using t-test, differences between the three groups were tested using ANOVA (SPSS for Windows, version 10.0). Differences in frequencies were tested using the chi square test. Level of statistical significance was set to 0.05.

RESULTS

Table 1 shows the distribution of the subjects included in the study for the three study periods with respect to gestational age as well as to intrauterine growth retardation (SGA). There were no differences between both, the SF and NF group. Also, the numbers of drop-outs at the different time points were not different. The MM group, however, consisted mainly of infants born at term and - in this respect - its composition was statistically different from both formula groups ($p < 0.001$).

TABLE 1

Number of subjects in the three study groups at three time points [total number/number of preterm infants][†]

n [all/preterm]	t_0			t_1			t_2		
Group	SF	NF	MM	SF	NF	MM	SF	NF	MM
SGA	6/3	7/2	10/0	2/1	6/2	8/0	1/1	5/2	8/0
Normal	19/4	21/5	49/2	15/4	16/4	35/1	12/4	13/4	32/1
Total	25/7	28/7	59/2	17/5	22/6	43/1	13/5	18/6	40/1

[†]SF, standard formula; NF, nucleotide supplemented formula; MM, mother's milk; SGA, small-for-gestational age; t_0, 0-5 days post partum; t_1, 55-65 days post partum; t_2, 115-125 days post partum.

Table 2 reveals that starting conditions and time points at the different study days were not different between both formula groups. However, due to the higher proportion of term infants in the MM group, gestational age ($p < 0.01$), birthweight and length ($p < 0.05$) were significantly higher when compared with the formula groups. Study intervals, however, were not different between all three groups.

TABLE 2

Gestational age, weight, length, and head circumference at birth; age corrected for gestational age at t_0, t_1, and t_2 expressed in mean ± SD†

	NF	SF	t-test	MM	ANOVA
Gestational age [weeks]	38 ± 2	38 ± 3	NS	40 ± 2	$p < 0.01$
Weight [g]	3040 ± 620	2940 ± 760	NS	3380 ± 580	$p < 0.05$
Length [cm]	49.5 ± 3.5	49.5 ± 3.5	NS	51.5 ± 2.5	$p < 0.05$
Head circumference [cm]	33.9 ± 1.8	34.3 ± 3.9	NS	35.1 ± 1.7	NS
Age corrected at t_0 [days]	-7 ± 14	-8 ± 18	NS	2 ± 10	$p < 0.001$
Age corrected at t_1 [days]	55 ± 18	53 ± 21	NS	62 ± 10	n.d.
Age corrected at t_2 [days]	109 ± 16	111 ± 27	NS	122 ± 10	n.d.

†NF, nucleotide supplemented formula; SF, standard formula; MM, mother's milk; n.d., not done; t_0, 0-5 days post partum; t_1, 55-65 days post partum; t_2, 115-125 days post partum.

Table 3 shows the longitudinal data of anthropometry and body composition: The growth data obtained in the formula groups compare favourably with those obtained in the MM control group. Over the whole study period differences in gain of weight, LBM and fat mass were observed between both formula groups, but they failed to reach statistical significance (all $p > 0.08$). However, during the second observation period the gain of body weight and lean mass was significantly higher in the SF group when compared to the NF group ($p < 0.05$ and < 0.01, respectively). The DXA data indicate that the higher weight gain in the SF group is most probably not caused by an increased gain in fat mass: for all three groups it is found that- over the whole study period- 55% of the weight gain is due to the accretion of lean mass. Moreover, accretion of lean mass in the formula fed infants is higher than that observed in the MM group. There is no difference between the formula groups for the gain of skinfold thickness, arm circumferences, length and head circumference. However, there is a small but statistically significant higher gain of head circumference in the formula groups when compared with the MM control group.

Analysis of subgroup data (term, preterm, SGA, AGA/LGA children) did not reach statistical significance (data not shown). Accretion of BMC in the first two months was significantly higher in the MM group than in the formula groups ($p < 0.05$). Within the formula groups, the

SF group showed tend to a higher BMC gain throughout the whole study period, but the difference did not reach statistical significance.

TABLE 3

Longitudinal anthropometric data (weight, length, head circumference, right and left mid arm circumference), body composition from DXA measurement (LBM, FM, BMC), skinfold thickness measurements (sum of 4 skinfolds) from birth to the age of 2 months (t_1-t_0), from the age of 2 months to the age of 4 months (t_2-t_1), and from birth to the age of 4 months (t_2-t_0) expressed in mean ± SD†

		NF group		SF group		t-test	MM group		ANOVA
		n	Mean ± SD	n	Mean ± SD		n	Mean ± SD	
Weight gain [g]	t_1-t_0	21	1870 ± 520	17	2020 ± 610	NS	43	2100 ± 610	NS
	t_2-t_1	17	1540 ± 400	13	1920 ± 570	$p < 0.05$	40	1500 ± 430	$p < 0.05$
	t_2-t_0	18	3460 ± 600	13	3960 ± 950	NS	40	3530 ± 750	NS
Length gain [cm]	t_1-t_0	21	8.5 ± 2.0	17	7.5 ± 3.5	NS	43	8.5 ± 2.0	NS
	t_2-t_1	17	5.0 ± 2.0	13	6.0 ± 1.5	NS	40	5.5 ± 2.0	NS
	t_2-t_0	18	14.5 ± 2.0	13	13.5 ± 4.5	NS	40	13.5 ± 2.0	NS
LBM gain [g]	t_1-t_0	22	1140 ± 270	17	1220 ± 320	NS	40	1040 ± 300	NS
	t_2-t_1	18	730 ± 320	11	1080 ± 310	$p < 0.01$	34	670 ± 370	$p < 0.01$
	t_2-t_0	18	1930 ± 430	11	2280 ± 540	NS	35	1710 ± 440	$p < 0.01$
FM gain [g]	t_1-t_0	22	800 ± 450	17	780 ± 430	NS	40	1000 ± 390	NS
	t_2-t_1	18	750 ± 350	11	970 ± 490	NS	34	860 ± 400	NS
	t_2-t_0	18	1550 ± 520	11	1920 ± 680	NS	35	1830 ± 610	NS
BMC gain [g]	t_1-t_0	22	22 ± 14	17	23 ± 13	NS	40	30 ± 12	$p < 0.05$
	t_2-t_1	18	31 ± 15	11	36 ± 15	NS	34	29 ± 13	NS
	t_2-t_0	18	52 ± 17	11	63 ± 20	NS	35	59 ± 16	NS
Head circumference gain [mm]	t_1-t_0	21	4.7 ± 1.2	17	5.1 ± 0.9	NS	41	4.5 ± 0.8	NS
	t_2-t_1	17	2.9 ± 0.5	13	3.0 ± 0.6	NS	40	2.6 ± 0.5	$p < 0.05$
	t_2-t_0	18	7.6 ± 1.5	13	8.1 ± 1.5	NS	39	7.0 ± 0.9	$p < 0.05$
Arm circumference right gain [cm]	t_1-t_0	19	2.2 ± 1.0	13	2.1 ± 1.1	NS	40	2.1 ± 1.0	NS
	t_2-t_1	17	1.4 ± 0.7	13	1.7 ± 1.3	NS	40	1.4 ± 0.6	NS
	t_2-t_0	15	3.7 ± 0.9	9	3.7 ± 0.9	NS	38	3.6 ± 1.0	NS
Arm circumference left gain [cm]	t_1-t_0	19	2.1 ± 1.0	13	2.1 ± 1.0	NS	40	2.2 ± 1.0	NS
	t_2-t_1	17	1.4 ± 0.8	12	1.8 ± 1.3	NS	40	1.4 ± 0.7	NS
	t_2-t_0	15	3.6 ± 0.9	9	3.9 ± 1.7	NS	39	3.8 ± 1.7	NS
Skinfold thickness gain [mm]	t_1-t_0	16	8.0 ± 4.0	11	7.6 ± 6.1	NS	29	10.0 ± 6.6	NS
	t_2-t_1	14	4.4 ± 5.0	9	6.6 ± 8.6	NS	35	3.4 ± 4.1	NS
	t_2-t_0	14	12.7 ± 5.2	7	14.0 ± 10.5	NS	28	13.3 ± 6.1	NS

†NF, nucleotide supplemented formula; SF, standard formula; MM, mother's milk; LBM, lean body mass; FM, fat mass; BMC; bone mineral content.

DISCUSSION

The SF group showed a higher growth velocity and a faster accretion of lean mass compared with the other groups. Progress of head circumference was higher in infants fed with SF than in infants of other groups. Higher body weight gain in formula-fed compared to breast-fed infants has been found, this finding is in line with that from other studies (Dewey et al. 1992; Agostoni et al. 1999; Butte et al. 2000b; Fomon 2004).

Although dietary nucleotides have been suggested to have beneficial gastrointestinal and immunological effects (Brunser et al. 1994; Pickering et al. 1998; Vasquez-Garibay et al. 2004), most studies reported no effect of nucleotide supplementation on physical growth in healthy term infants (Carver and Walker 1995; Carver 2003; Makrides et al. 2004; Hawkes et al. 2006). Only one study in term infants with small-for-gestational age showed that nucleotides were associated with better catch-up growth (Cosgrove et al. 1996). Nucleotides can be synthesized endogenously and thus are not essential nutrients. Dietary nucleotides seem not to be essential for growth under normal condition. However, nucleotides are considered as semi-essential nutrients in certain conditions such as in the presence of prematurity, fetal growth retardation, intestinal injury and limited nutrient intake. This condition may require larger amounts of nucleotides that cannot be provided by endogenous production. The most likely explanation for the observation from this study is that their subjects were intrauterine growth retarded, therefore they might get the benefits from the supplementation of nucleotides.

Faster growth could not be shown in our population receiving nucleotide supplemented food, indicating that nucleotides seem not to be semi-essential and do not result in significant benefits on the physical growth for a normal healthy population of young infants. The number of preterm and SGA subgroups in our study were too small to differentiate the effect of nucleotides for these groups.

It could be shown that the higher growth velocity of infants fed with non-supplemented formula is a consequence of a higher lean mass accretion. Higher lean mass is not known to be associated with a higher risk for adiposity. No negative effects could be shown from a faster growth, especially if the growth is based on lean mass accretion, which is said to be associated with positive effects like a better ventilatory function (Lazarus et al. 1997).

It could be shown that breast-fed infants show a higher gain in BMC than the formula-fed

groups in the first two months of life. This could help to understand the well established observation that in preterm children supplementation of calcium showed no higher accretion of BMC than in breast fed babies (Faerk et al. 2000). Poor calcium absorption has been described for formula fed children (Bronner et al. 1992), and the reason for reduced calcium absorption is supposed to be dependent on the fatty acid position in triacylglycerides. In human milk, the palmitic acid is placed in the sn-2 position, whereas in formulas it is often placed in sn-1 or sn-3 position. A significant higher calcium and fat excretion in stools have been found in formula-fed infants compared with human milk-fed infants (Quinlan et al. 1995). Low bone mass increases the fracture risk independently from age (Kanis 1994).

The most commonly used and practical method to assess growth and well-being of preterm infants is weight gain, because weight can be measured accurately, rapidly, frequently, and without expensive, sophisticated instrumentation (Katrine 2000). With the sophisticated equipments like DXA, the components of weight gain can be determined: i.e. lean mass or fat mass. We used DXA to assess in vivo body fat because DXA provides a direct measurement of body composition with high accuracy and precision compared to other non-invasive methods based on direct and indirect measurements. A linear relationship of DXA lean and fat mass with data obtained by chemical carcass analysis has been shown in several animal studies (Koo et al. 1995; Picaud et al. 1996; Brunton et al. 1997; Fusch et al. 1999; Rigo et al. 2000). Because DXA tends to overestimate fat mass systematically, equations have been developed which can fully convert DXA and chemical carcass analysis (Koo et al. 1995; Picaud et al. 1996; Brunton et al. 1997; Fusch et al. 1999; Rigo et al. 2000).

As a conclusion we report that weight gain and gain in lean mass was higher in the SF group than in the groups nourished with NF or breast-milk. Nucleotide supplementation in infant formula did not result in a higher growth velocity in early infancy of a normal neonatal population. Progress of head circumference was higher in children fed with SF than in infants of other groups. Gain in BMC was higher in breast fed than in formula-fed infants during the first 2 months of life.

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Summary

This thesis mentions the validation and comparison of several methods for measuring body composition and assessing nutritional status in a population of paediatric patients. The thesis also deals with the effects of infant formulas supplemented with nucleotides on the growth and body composition of healthy term and preterm babies.

1. BMI has been shown to be the well-known index for classifying the nutritional status in adults (World Health Organization 1995). Recently, BMI has also been used as an indicator to classify the nutritional status in children from 0 to 20 years of age (Must et al. 1991; Rolland-Cachera et al. 1991; Cole et al. 1995; Lindgren et al. 1995; Luciano et al. 1997). The relationship between actual BMI value and %BF has been reported in healthy children in a number of studies (Goran et al. 1996; Gutin et al. 1996; Daniels et al. 1997; Pietrobelli et al. 1998), but not in a population with paediatric patients suffering from different kinds of diseases. Three methods to assess the relationship between BMI-SDS and %BF in sick children have been used: 1) the correlational analysis; 2) receiver operating characteristic analysis; 3) Kappa agreement test. The results showed that BMI-SDS correlates well with %BF. However, due to the large interindividual variation, BMI-SDS and %BF have a moderate agreement for the classification of nutritional status. Although the correlation between BMI-SDS and %BF is high, the prediction of %BF from BMI-SDS should be used with caution; for clinical purpose, if %BF needs to be estimated, more precise body composition method should be used. At 25% body weight as fat in boys and 30% body weight as fat in girls used to define obesity, BMI > 97^{th} percentile as the screening variable for identifying obese children give the sensitivity of 68.4% in boys and 54.1% in girls, respectively.

2. In preterm infants, it has been shown that there are no prediction equations available for estimating FFM. BIA is a quick, inexpensive, safe and non-invasive method, shows less inter-observer variation for measuring body composition than anthropometry (Schaefer et al. 1994); and has been used widespreadly in children (Nagano et al. 2000; Pietrobelli et al. 2003). Therefore, investigating the role of bioelectrical impedance in prediction equations for FFM and developing new prediction equations for FFM in preterm infants are necessary. Our results showed that bioelectrical impedance provides only little additional information on FFM compared to anthropometry. In more details: body weight is the stronger predictor of FFM than impedance index, this is consistent with the result of other study (Van Loan and

Mayclin 1987; Jackson et al. 1988), but is contradictory with those of other author (Lohman 1992); addition of impedance index provides little improvement in the predictive accuracy of the prediction equations for FFM. The study also indicated that age correlates less well with FFM as measured by DXA when compared with body weight and impedance index. The prediction equations developed for estimating FFM have high R^2 values, which were derived from bootstrap sampling method. It proves the acceptable validation of the formulas.

3. It has been shown that the BIA-based prediction equations have been widely used to estimate body composition in healthy children, but their validation has not been tested in the population of malnourished or sick children (Royall et al. 1994). Thus, the validation of published equations and the development of simple and accurate formulas for evaluating and monitoring growth, nutritional status of children are essential for clinical settings. The validation of published prediction equations for FFM in children with Crohn's disease showed that three formulas of Schaefer, Deurenberg and Lorenzo provide estimates of FFM which are similar to FFM_{DXA}. Only formulas of Pietrobelli underestimate FFM in comparison with FFM_{DXA}. The model of Schaefer is the best to predict FFM in children with Crohn's disease. However, when the formulas were used to calculate FFM value in each individual, the published prediction equations gave estimates of FFM that were considerably different from FFM_{DXA}. The study pointed out that the accuracy of a prediction equation is influenced by factors like body weight, height and age other than impedance index alone. This study used statistical bootstrap technique to validate the prediction equation for estimating FFM, this technique is simple and reliable for validating prediction equations (Schmelzle and Fusch 2002).

4. Previous studies show contradictory opinions about the role of impedance index and anthropometric measurements in predicting FFM. In healthy subjects, some authors suppose that impedance index is the better predictor of FFM than body weight, height (Lukaski et al. 1985; Segal et al. 1985). On the other hand, some other authors suppose that anthropometric measurements have better predictive value for FFM than impedance index (Jackson et al. 1988; Diaz et al. 1989). In patients, such statement has not been elucidated. Due to the difference in the physiological status between healthy and sick subjects, this study was therefore implemented to confirm the role of impedance index and anthropometric measurements in predicting FFM in a group of paediatric patients aged 13.1 ± 3.3 y. The results of study showed that impedance index has a stronger correlation with FFM than body

weight, body height, and body height squared. It indicates that in older sick children, the BIA method provides more information than anthropometry in estimating FFM. Another conclusion from this study is that addition of weight improves the predictive accuracy of BIA-based prediction equations for FFM. The study supports the use of BIA in measuring body composition of patients.

5. Although birthweight percentile has long been used to classify the fetal growth of newborn babies, it is not known whether this classification system reflects %BF in term and preterm newborns. Some anthropometric indices like weight-length ratio, ponderal index, and BMI are supposed to give better reflection of soft tissue mass than birthweight percentile (Rohrer 1921). The fifth study of this thesis was therefore carried out to compare intrauterine growth using age-and sex-matched percentiles with the nutritional status using %BF measured by DXA and to investigate the body composition and the correlations between some anthropometric indices and fat mass in a group of term and preterm infants. The results indicated that traditional classification based on birthweight centiles does not reflect %BF. Female subjects show higher %BF than male subjects. The best correlation was found between weight-length ratio and absolute fat mass measured by DXA. Correlation between anthropometric parameters and %BF is weaker than correlation with absolute fat mass. Ponderal index - the most commonly used index of neonatal body is found not to be more highly correlated to fat mass compared with weight-length ratio.

6. Nucleotides are semi-or conditionally essential nutrients and play vital roles in many biological processes. Nucleotides may become essential under certain conditions: the presence of diseases, prematurity, fetal growth retardation, intestinal injury, and periods of limited nutrient intake or rapid growth. Nucleotides have beneficial effects upon the immune system, small intestinal growth and development, lipid metabolism, and hepatic function (Carver 1995). Based on the positive characteristics of nucleotides, the last study of the thesis was undertaken to investigate the effects of dietary nucleotides on growth and body composition during the first four months of life in healthy term and preterm infants. The results showed that weight gain and gain in lean mass are higher in the standard formula group than in the groups nourished with nucleotides or breast milk. Nucleotide supplementation in infant formula does not result in a higher growth velocity in early infancy of a normal neonatal population.

Conclusion

1. Relationship between BMI and percentage body fat (%BF)

1. In sick children, BMI-SDS correlates with %BF ($r = 0.86$, $p < 0.0005$). However, due to the large interindividual variation, BMI-SDS and %BF have a moderate agreement for the classification of nutritional status in the individual subject.

2. Although the correlation between BMI-SDS and %BF is high, the prediction of %BF from BMI-SDS should be used with caution. For clinical purpose, if %BF needs to be estimated, more precise body composition methods like DXA, underwater weighing should be used.

3. Using 25% body weight as fat in boys and 30% body weight as fat in girls to define obesity, BMI > 97^{th} percentiles as the screening variable for identifying obese children give the sensitivity of 68.4% in boys and 54.1% in girls, respectively.

2. Use of bioelectrical impedance analysis in preterm infants aged near term

1. Impedance index and body weight correlate well with fat-free mass measured by dual-energy X-ray absorptiometry (FFM_{DXA}); age correlates less well with FFM_{DXA}.

2. Body weight is the stronger predictor of FFM than impedance index.

3. Addition of impedance index provides only little improvement in the predictive accuracy of the prediction equations for FFM.

3. Validation of published prediction equations for FFM in children with Crohn's disease

1. For the whole group, three prediction equations of Schaefer, Deurenberg and Lorenzo provided estimates of FFM, which were similar to FFM measured by DXA. Only those of Pietrobelli underestimated FFM in comparison with FFM_{DXA}. The model of Schaefer is the best to predict FFM in children with Crohn's disease.

2. When calculating an individual’s value, published prediction equations gave estimates of FFM, which were considerably different from measured FFM.

3. The accuracy of a prediction equation with impedance index as the predictor is improved by the addition of body weight.

4. This study provides an equation used specifically for a group of children with Crohn's disease. The bootstrap sampling method supports the use of the derived prediction equation.

4. Comparison of the role of impedance index and anthropometric measurements in predicting fat-free mass (FFM) in a group of paediatric patients aged 13.1 ± 3.3 y

1. Impedance index is the better predictor of FFM than body weight, body height or body height^2.

2. Addition of weight improves the predictive accuracy of the BIA-based prediction equations in estimating FFM.

3. Our study supports the use of bioelectrical impedance analysis in measuring body composition of patients.

5. Birthweight percentiles and percentage body fat in term and preterm newborns

1. Traditional classification based on age-and sex-matched birthweight centiles does not precisely reflect %BF in term and preterm newborns.

2. Female subjects show higher %BF than male subjects.

3. The best correlation is found between W/L ratio and absolute fat mass measured by DXA. Correlation between anthropometric parameters and %BF is weaker than correlation with absolute fat mass. Ponderal index - the most commonly used index of neonatal body is found not to be more highly correlated to fat mass compared with W/L ratio.

6. Effects of dietary nucleotides on growth and body composition in healthy term and preterm infants during the first four months of life

1. Dietary nucleotides show no positive effects on growth and body composition in healthy term and preterm infants.

2. Standard formula-fed infants show a higher weight gain and a faster accretion of lean mass compared with nucleotide supplemented and breast-fed subjects.

3. Progress of head circumference is higher in infants fed standard formula than in those fed breast milk or nucleotide-supplemented formula.

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Eidesstattliche Erklärung

Hiermit erkläre ich, daß ich die vorliegende Dissertation seslbständig verfaßt und keine anderen als die angegebenen Hilfsmittel benutzt habe.

Die Dissertation ist bisher keiner anderen Fakultät vorgelegt worden.

Ich erkläre, daß ich bisher kein Promotionsverfahren erfolglos beendet habe und daß eine Aberkennung eines bereits erworbenen Doktorgrades nicht vorliegt.

Greifswald, den 13 März 2006 Unterschrift

Nguyen Quang Dung

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Sonstiges

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