# Body Composition and Energy Utilization

*During the First Year of Life*

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# Body Composition and Energy Utilization

## *During the First Year of Life*

*Lichaamssamenstelling en energiegebruik tijdens het eerste levensjaar*

PROEFSCHRIFT
TER VERKRIJGING VAN DE GRAAD VAN DOCTOR
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Ter nagedachtenis aan mijn moeder

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# List of abbreviations

| | |
|---|---|
| BCM | Body cell mass |
| BF | Breast-fed |
| CV | Coefficient of variation |
| DEXA | Dual-energy X-ray absorptiometry |
| DLW | Doubly-labeled water |
| $D_2O$ | Deuterium enriched water |
| DTI | Deuterium-to-infant method |
| E# | Raw TOBEC reading |
| $E\#_{cor}$ | Net TOBEC reading corrected for the reference phantom (day-to-day variation) |
| $E\#_{net}$ | TOBEC reading, corrected for background noise |
| $EC_{BM}$ | Energy content of breast milk |
| EXP | Exponential function |
| FF | Formula-fed |
| FFM | Fat-free mass |
| FQ | Food quotient |
| GEI-TW | Gross energy intake by test weighing |
| $^2H_2{}^{18}O$ | Deuterium, and 18-oxygen enriched water (ie, doubly-labeled water) |
| ID | Isotope dilution |
| IWL | Insensible water loss |
| $k_H$ | Elimination rate of hydrogen |
| $k_O$ | Elimination rate of oxygen |
| $^{40}K$ | 40-Potassium isotope |
| K-S test | Kolmogorov-Smirnov test |
| $L_c$, $L_{con}$ | Conductive length of the subject |
| LBM | Lean body mass |
| MAA | Midupper-arm area |
| MAFA | Midupper-arm fat area |
| MAFR | Midupper-arm fat ratio |
| MAMA | Midupper-arm muscle area |
| MAMC | Midupper-arm muscle circumference |
| MEI-PRED | Metabolizable energy intake calculated from body composition and energy expenditure |
| MEI-TW | Metabolizable energy intake by test weighing |
| MIC | Michigan, USA |
| MRI | Magnetic resonance imaging |
| $N_H$ | Dilution space of hydrogen |
| $N_O$ | Dilution space of oxygen |
| NDL | Netherlands |
| NMR | Nuclear-magnetic resonance |
| NPN | Non-protein nitrogen |
| $rCO_2$ | Carbon dioxide production |
| RMSE | Root mean squared error |
| RQ | Respiratory quotient |
| SD | Standard deviation |
| SEE | Standard error of the estimate |
| SEM | Standard error of the mean |
| SFT | Skinfold thickness |
| T# | Transformed TOBEC value |
| TBF | Total body fat |
| TOBEC | Total-body electrical conductivity |
| TBW | Total body water |
| TDEE | Total-daily energy expenditure |
| TN | Total nitrogen |
| TW | Test weighing |
| TX | Texas, USA |

*Part 1.*

# Introduction

*Chapter 1*

# Introduction and Study Objectives

## 1.1 Body Composition: History

Not until the start of the 'biochemical era' in the 19th century more detailed descriptions of the body's contents were published. Lawes & Gilbert [1859] documented changes in the amount of body fat and lean body mass when animals were fed different diets. Pfeiffer [1887] discovered the relative constancy of body water when expressed on a fat-free basis, which was the start for the concept of the fat-free mass. Bischoff [1863] analyzed several human adult cadavers for water content. Fehling [1877] and Camerer & Söldner [1900] did the same for human fetuses. Around this time the chemical composition of the fetus as regards water, fat, nitrogen, and major minerals had been established. A full chemical analysis of the human adult was not accomplished until much later [Iob & Swanson, 1938; Widdowson & Dickerson, 1964], as was also true for the description of the changes into 'chemical maturity' of the body during growth [Moulton, 1923]. Analogous to his findings in animals, Moulton assumed that the human body reaches chemical maturity at the age of approximately 3 years which was refuted among others by Widdowson & Dickerson [1964] on their observation that the percentage of extracellular fluid gradually fell until puberty. Until then most of the work on the chemical analysis of the body was by carcass analysis. One of the great break-throughs in body composition techniques was the start of *in vivo* measurement of body composition in living mammals by using (stable) isotopes to measure intra- and extra-cellular body fluids [Von Hevesy & Hofer, 1934; Moore, 1946] and densitometry to measure body fat and fat-free mass using Archimedes' principle [Behnke et al., 1942; Keys & Brozek, 1953]. Fomon and coworkers calculated, based on various direct and indirect estimates of body composition, the average chemical composition of the 'male reference infant' [Fomon, 1967], the 'reference fetus' [Ziegler et al., 1976] and 'the reference child' [Fomon et al, 1982] (see **Appendix 3**). An example of the body composition of the 'reference fetus' and the 'reference child' (as g per 100 g body weight in a female) is given in **Figure 1.1**. Data of the 'reference child' have been extensively used by numerous investigators in the past 15 years but have not yet been compared with more recent body composition techniques. Also, no data or centile standards exist on the physiological scatter

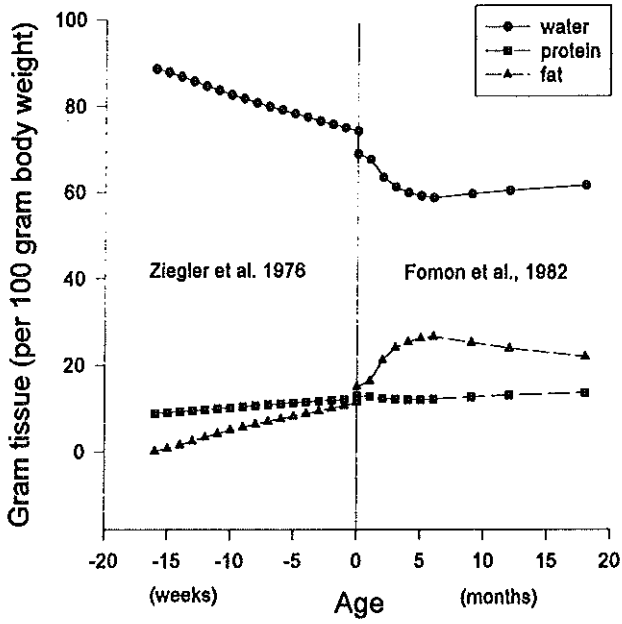


**Figure 1.1.** Body composition of the 'reference fetus' and a female 'reference child' from 24 w gestational age to 18 mo after birth. *Data derived from Ziegler et al [1976] and Fomon et al. [1982].*

in total body fat and fat-free mass.

Keys and Brozek [1953] originally divided the mammalian body into four chemical groups: water, protein, ash (or bone mineral) and fat and used densitometric priciples to measure total body fat (TBF) and fat-free mass (FFM), i.e. water, protein and ash. In the literature several other terms are often used interchangeably with FFM: 'lean body mass' (LBM), which has to be reserved for the water and protein compartment (i.e. muscle mass and intra- and extra-cellular fluid compartments), 'body cell mass' (BCM), which was introduced by Moore et al. [1963] and defined as "the working, energy-metabolizing portion of the human body in relation to its supporting structures". BCM consists of the cellular components of muscle, viscera, blood and brain. The TBF compartment is the most variable one and most sensitive to changes in nutritional status. For this reason TBF has been the prime parameter for disturbances in health, growth and/or nutritional status. From a clinical and nutritional point of view therefore, most emphasis has been laid on the measurement of TBF. Because at present most indirect methods measure FFM, TBF is usually determined as the difference between body weight and FFM.

## 1.2 BODY COMPOSITION: CLINICAL IMPORTANCE

### *1.2.1. Its role in nutritional assessment*

Body composition data give important information on the nutritional status. These data are especially important in the period of rapid growth and cell differentiation as happens during fetal life and infancy. These periods are recognized as so called 'critical periods' for growth and development [Widdowson et al. 1963, 1964]. The timing of these critical periods differs for different tissues.

Adult obesity is a well recognized risk factor for chronic disease. Observations in humans have led to inconsistent conclusions regarding whether infant diet influences child, adolescent and adult body composition. Whether infant obesity is related to obesity in later childhood and adulthood also remains unclear. Few longitudinal studies tracking body composition from infancy to adulthood have been performed. Lewis [1996] recently presented a literature overview of studies on this subject. Several authors find no direct relation [Shapiro et al, 1984; Crisp et al, 1970; Poskitt & Cole, 1977]. Other studies do find a relation between adult obesity and obesity in infancy and childhood [Rolland-Cachera et al, 1984; Charney et al, 1976; Serdula et al, 1993; Dietz, 1994]. Although more longitudinal studies are needed, it is likely that the relation is multifactorial, with social and parental factors [Charney et al, 1976] playing a role, as well as various child-dependent factors concerning education and growth, like for example the timing of the 'obesity rebound' phenomenon in early childhood [Rolland-Cachera et al, 1984]. Also, infant feeding style has been associated with childhood obesity [Agras et al, 1990].

Nutritional deprivation in infancy may result in marked and often irreversible growth retardation and developmental sequelae on short term [Waterlow & Alleyne, 1971; Lechtig, 1991], in later life [Barker & Winter, 1989; Osmond et al, 1993], and even in offspring of food-deprived subjects [Susser & Stein, 1994].

Malnutrition in children is commonly spoken of as protein-energy malnutrition, for deficiencies in energy and to a lesser extend in protein are usually involved in its development. The most obvious evidence of malnutrition in children is abnormally low weight for length, and, in long-standing malnutrition low length for age (stunting). However, early recognition of malnutrition (as well as infant obesity) needs more subtle body composition estimations, with methodologies appropriate for the specific population of interest.

### *1.2.2. Its role in the prediction of energy requirements.*

Energy and nutrient allowances during infancy as recommended by the FAO/WHO/UNU are based on empirical observations of the intake of healthy, well-nourished, thriving infants. Although the breast milk 'gold standard' concept may be challenged with respect to, for instance, vitamin K and D and to a lesser extend to protein in later infancy, the breast-

fed infant is still used as a model for nutrition requirements during the early months of life.

The accuracy of the traditional method for assessment of nutrient intake in breast-fed infants (test-weighing of the whole infants, and manual or mechanical expression of breast milk) has been debated [Lucas et al, 1980; Lucas et al, 1987]. Based on these observations, Prentice recently wrote that the approach to base nutrient and energy requirements in infancy on energy intake determinations *"has a number of disadvantages, which prompted the WHO consultative panel to state that future guidelines should be based, not on estimates of intake, but of measurements of energy expenditure if and when these become available. [FAO/WHO/UNU, 1985]"* [cited in Prentice et al. 1988]. Non-invasive measurement of energy expenditure has become more easily available with the doubly labeled water method [Schoeller et al, 1982; Jones et al, 1987]. Besides estimation of energy expenditure, energy deposition into tissue fat and protein (estimated to be approximately 10-20% of metabolizable energy intake in the first months of life) needs to be estimated to come to proper estimates of energy requirements in the period of rapid growth and tissue accretion in early infancy. Until now no accurate body composition method was available for this purpose. Data on the assessment of energy requirements from energy expenditure and energy deposition by body composition are very scarce, due to the lack of an accurate body composition method and the fact that the doubly-labeled water method is cumbersome and extremely expensive.

## 1.3 BODY COMPOSITION: METHODS FOR USE IN INFANTS

### *1.3.1. Paucity of accurate infant body composition methods.*

Most body composition methods that yield accurate values in adults and children are not applicable to infants. Body composition methods to be used in infants have to be non-invasive, non-radioactive and without the need for active cooperation of the subject. Methods which are either inaccurate or still in an experimental phase for use in infants are: bioelectrical impedance [Mayfield et al, 1991], $^{40}$K-counting [Forbes & Hursh, 1963; Ellis & Shypailo, 1992a], air-displacement [Taylor et al, 1985; Dell et al, 1987], acoustic plethismography [Sheng et al, 1988], inelastic scattering of neutrons [Kehayias et al, 1987; Ellis et al, 1992b], MRI [Fuller et al, 1985] and NMR [Lewis et al, 1986]. Near-infrared interactance yields erroneous values for the thickness of the subcutaneous fat layer [Kabir et al, 1993]. Recently measurements of dual-energy X-ray absorptiometry (DEXA) [Van Loan et al, 1992] were used in infants. Although measurements of FFM and weight were rather accurate, determination of TBF resulted in large systematic errors [Picaud et al, 1996]. In our laboratory absorption and desorption of xenon has been used for direct estimation of TBF in small infants. [Mettau et al, 1977]. At present TBF in infants is measured mainly by means of isotope dilution (using $^{2}$H- or $^{18}$O-enriched water ) [Trowbridge et al, 1984], and various anthropometric methods [Dauncey et al, 1979; Weststrate & Deurenberg, 1989]. Calculation of FFM from isotope dilution data, however, is complicated by the variable

hydration of the FFM (this body compartment rapidly changes in the growing infant, from a FFM hydration of approximately 80% in the neonate to 73.2% in children and adults). Anthropometry is widely used for assessment of nutritional status in adults and children. Measurement of skinfold thickness has been notorious for its inaccuracy in untrained hands and for its interobserver variation [Lohman, 1981]. Recently the non-invasive measurement of total body electrical conductivity (TOBEC) has emerged as a new promising method to estimate body composition in infants [Fiorotto et al, 1987; Fiorotto, 1991].

*1.3.2. Total body electrical conductivity (TOBEC).*

The principle underlying TOBEC is that lean tissue is far more electrically conductive than fat. The body's lean tissue, when introduced in a weak homogeneous electromagnetic (em) field, will 'disturb' the em-field properties. The amount of lean tissue (i.e. the body's fat-free mass) can be deduced from the amount of 'disturbance' of the em-field. A pediatric TOBEC device is commercially available since 1989. The TOBEC technique was introduced in the Sophia Children's Hospital in 1990. The method is not yet widely used, due to the (still) relatively high price of a TOBEC instrument (approximately $ 45,000.-), and the fact that the instrument is large, difficult to move and therefore not suitable for field studies. A TOBEC measurement is rapid, safe, easy to perform and suitable for measurement of large numbers of infants. This makes the technique promising as a screening tool for nutritional assessment, for studies on nutrition and quality of growth and as a reference method.

## 1.4 SUMMARY OF STUDY OBJECTIVES.

The following questions were addressed in the studies described in the subsequent parts of this thesis:

| | |
|---|---|
| In PART 2. | Is TOBEC suitable for reproducible and reliable measurement of body composition throughout the first year of life? |
| In PART 3. | What is the value of anthropometry for nutritional assessment in infants? Can reliable reference centiles be constructed on infant total body fat and fat-free mass? |
| In PART 4. | What is the effect of exclusive breast-feeding or formula-feeding on growth, body composition and energy utilization?<br>Can energy requirements be predicted from the sum of energy deposition (by TOBEC) and energy expenditure?<br>How well do traditional methods assess energy consumption, especially in breast-fed infants? |

## 1.5 STUDIES DESCRIBED IN THIS THESIS

### *1.5.1. TOBEC methodology (PART 2).*

Calibration of the present type of instrument had been performed on one location only (Dr. ML Fiorotto, Children Nutrition Research Center, Houston TX, USA) and instrument variability with respect to strength and homogeneity of the electromagnetic field was not known. Thus we did not know whether the calibration equation derived in Houston could be applied to our instrument. Beside this, only some of the possible pitfalls of the method had been investigated, and several questions were still (partly) unsolved. What is the effect of temperature, body geometry, tissue autolysis, different types of electrolytes and pathological electrolyte shifts on the TOBEC outcome? Can the general physics of the dielectric and conductive properties of tissues be linearly applied to the area of the TOBEC technology? The study on the effects on TOBEC of temperature, physiological and pathological electrolyte changes in non-human mdels and measurements in minipiglets after death is described in **Chapter 2.**

In cooperation with Dr. Marta L. Fiorotto (Children Nutrition Research Center, Houston, USA) and the late Prof. Yves W. Brans (Wayne State University, Detroit, USA) a 'universal' TOBEC calibration equation for use in infants was calculated and instrument specifications as magnetic field homogeneity, long-term and day-to-day variability were determined. For this purpose the TOBEC instrument was also calibrated for use in healthy infants from 0-12 months of age in our own laboratory against carcass analysis data of miniature piglets. These studies are described in **Chapter 3.**

In **Chapter 4** estimates of fat-free mass by TOBEC are compared with fat-free mass estimates derived from total-body water measurements by $D_2O$ and $H_2{}^{18}O$ in 149 healthy, full-term infants. Also, this study describes whether the linearity of the minipig-derived calibration equation is valid for the entire first year of life.

### *1.5.2. Validation of anthropometry for use in infants and calculation of centile standards for TBF and FFM (PART 3).*

To prevent diet-induced short-term and long-term health sequelae sensitive body composition methods for accurate nutritional assessment and nutritional studies in infants and children have to be identified. Anthropometry-based body composition methods are often used in general practice as screening tools for nutritional assessment, also in infants. Because validation studies on these simple and inexpensive body composition methods are very scarce, infant anthropometry needs to be (re-) validated and refined for they are still used in large screening projects, in population surveys and for individual screening purposes to study the quality of growth (malnutrition/obesity). In cooperation with the Rotterdam Home Care Foundation (Stichting Thuiszorg Rotterdam) a cross-sectional study was designed in which known prediction equations on TBF and FFM published for use in infancy (published by Dauncey et al. [1977] and by Weststrate and Deurenberg [1989]) are validated against TOBEC (**Chapter 5**).

In **Chapter 6** traditional anthropometric measurements and indices (originally designed for adults but often applied to infants) are validated against TOBEC. In this chapter also new and more accurate anthropometric prediction equations and simple anthropometric indices are calculated to predict infant body composition.

**Chapter 7** describes the data collection and calculation of reference centiles for body fat and fat-free mass against age, weight and length and the comparison of direct estimates of body fat and fat-free mass by TOBEC with the 'reference child' of Fomon et al. [1982].

*1.5.3. Energy utilization in breast-fed and formula-fed infants and prediction of energy requirements (PART 4).*

In **Chapter 8** the effects of exclusive breast-feeding or formula-feeding on growth, body composition and energy utilization are analyzed in a longitudinal study in 46 healthy full-term infants. From data on energy expenditure and energy deposition (calculated from body composition by TOBEC) predictions on energy requirements can be made and compared with estimations of energy intake by traditional methods (test weighing and expression of breast milk).

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*Part 2.*

# Is the technique of total-body electrical conductivity (TOBEC) measurements suitable to estimate body composition in infants?

*Chapter 2*

# TOBEC Validation with Non-human Models [a)]

## 2.1 SUMMARY

The measurement of total-body electrical conductivity (TOBEC) has become one of the standard methods for the estimation of body composition in infants. We investigated, using non-human models, the effect on the accuracy of TOBEC-derived body composition estimates of alterations in physical and chemical characteristics of the fat-free mass (FFM). The effect of electrolyte type, concentration and volume on TOBEC was determined using 2, 3 and 5 liter solutions of six different chlorides and sodium bicarbonate. Equimolar concentrations yielded TOBEC values in accordance with known ion-conductivities: $H^+ >> Ca^{2+} > Mg^{2+} > K^+ > Na^+ > Li^+$ and $Cl^- > HCO_3^-$. The behaviour of these solutions was described very accurately over a wide range of concentrations (1-200 mM) by a simple exponential law. Dissolved egg-white protein, glycine and L-glutamine elicited no TOBEC signal. *In vitro*, using polyethylene bottles filled with physiologic saline, in the interval of 2 to 45°C a linear relation was observed between temperature and TOBEC. Below the freezing point no TOBEC signal was elicited. The effect of tissue autolysis and body temperature on TOBEC was examined by repeated measurements of TOBEC and temperature in seven fresh infant minipig cadavers. Five minipigs were allowed to cool. Shortly after death TOBEC decreased by 2.5% per °C. Two animals were kept at constant temperature. The TOBEC signal showed a gradual increase of 9% after 7 h due to autolysis. We conclude that *in vivo* TOBEC measurements are affected by ion-concentration (e.g. non-isotonic hydration changes), geometry (e.g. deviations in body shape), temperature (e.g. fever, skin-cooling) and tissue autolysis (measurements after death). Proteins, molecules with strong dipole moments, and ions trapped in crystalline structures do not significantly affect the TOBEC reading.

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[a)] This chapter has been published before as: **De Bruin NC, Luijendijk IHT, Visser HKA, Degenhart HJ.** Effect of alterations in physical and chemical characteristics on TOBEC-derived body composition estimates: validation with non-human models. *Phys. Med. Biol.* 39:1143-1156, 1994

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## 2.2 INTRODUCTION

Total body electrical conductivity (TOBEC) measurements are used for the assessment of body composition (Presta *et al* 1983). The principle underlying TOBEC is that lean tissue is much more electrically conductive than fat due to the greater content of electrolytes dispersed in the fat-free mass (FFM). In essence the TOBEC instrument is a large solenoidal coil driven by a 2.5 MHz oscillating radiofrequency current. When a conductive mass passes through the electromagnetic field, the magnetic component of the field induces weak eddy currents within the conductive mass, producing a small amount of heat. The energy of the eddy currents is dissipated from the magnetic field. The total energy loss is detected as a phase change in coil impedance. This phase change serves as an index of the amount of conductive mass (Harker 1973). The amount of fat is calculated by subtraction of the conductive mass (the FFM) from the body weight. Measurement of TOBEC is rapid, safe and reproducible (Presta *et al* 1983). The first published application, an electronic egg grader, is dated 1947 (Winters 1947). Several industrial and scientific applications for the measurements of lean body content (e.g. of meat hogs, living swine, birds and small animals and recently the measurement of body composition in humans) have been described since (Fiorotto 1991). A pediatric application of the method has only become commercially available since 1989. In spite of the widespread use of this methodology during several decades, the behaviour of electrolytes and other potential FFM components in a TOBEC electromagnetic field have not yet been extensively studied. The method has been validated in adults against reference methods such as hydrodensitometry (Newby *et al* 1990, Van Loan *et al* 1990, Van Loan 1990), which yielded accurate predictions of FFM. For infants no valid reference method for measuring body composition is available. An animal model for the calibration of TOBEC has been described, based on the observation that the physiological changes in conductivity and geometry of the FFM of the maturing piglet approximate very well the FFM changes of growing infants (De Bruin *et al* 1992 Fiorotto *et al* 1987a, Fiorotto 1991). With an instrument-specific calibration equation several centers obtained values for body fat from healthy infants during the first year of life (De Bruin *et al* 1993, Fiorotto *et al* 1987b). These values were in close agreement with the body composition of the "reference infant" (Fomon *et al* 1982).

The TOBEC method is largely empirical. Errors might arise when conductivity and geometry of the subject's FFM deviate from the standard conditions under which the (empirical) calibration equation was measured. These deviations do not necessarily change the absolute amount of FFM or fat, but they could change the conductive properties of the FFM and hence their TOBEC reading. Changes in geometry arise for example with thorax deformations, extremely distended abdomen, hydrocephalus, severe dystrophy, and edema. Changes in conductivity of the FFM might arise by changes in body temperature and by altered fluid, protein or electrolyte status as happens for example in severe malnutrition, non-isotonic dehydration, edema, metabolic or respiratory acidosis and alkalosis, several inborn errors of metabolism, renal, pulmonary and cardiac diseases.

Several authors studied the effect of changes in hydration on TOBEC in animals (Cochran *et al* 1989, Cunningham *et al* 1986, Fiorotto *et al* 1987a), but no detailed studies have been performed which systematically quantify the effect on TOBEC of electrolyte type and concentration, volume, temperature and the behaviour of macromolecules and crystalline structures. Neither has a study been published evaluating the effect on TOBEC of loss of membrane integrity as occurs in tissue autolysis, for example after death.

The aim of this study was to evaluate the effect of the latter parameters on TOBEC using non-human models, which allows better definition and evaluation of the TOBEC responses.

## 2.3 MATERIALS & METHODS

### *2.3.1. TOBEC measurements*

The TOBEC instrument (Body Composition Analyser Model HP-2; EM-Scan Inc., Springfield IL, USA) consists of a large solenoid coil, driven by a 2.5 MHz radio frequency generator, producing a time varying homogeneous electromagnetic field. Electric and magnetic field intensities are less than respectively 0.0002 and 0.004 of the American National Standards Institute limits for continuous human exposure (EM-SCAN 1989).

Harker (1973) presents an expression describing the power ($P$) induced in a homogeneous, cylindrical sample ($P$ is equal to the energy loss, which in turn is represented by the TOBEC number $E\#$):

$$P=(\pi/8)B_0^2\sigma\omega^2R^4L \qquad [1]$$

where $B_0$ is the time-varying induction amplitude, $\omega$ is the angular driving frequency, $\sigma$ is the electrical conductivity of the sample, $R$ is the sample radius and $L$ the sample length; because the sample cross sectional area $A$ is proportional to $R^2$ it can be deduced from equation [1] that $E\# \approx A^2 \cdot L$). Furthermore the volume $V$ is proportional to $A \cdot L$. Hence it is found that $E\# \approx V^2 / L$. This final relation yields the basis for the estimate of the volume of a homogeneous, cylindrical, conductive mass from a TOBEC measurement:

$$V=k_1\sqrt{(E\#)L} \qquad [2]$$

where $k_1$ is an instrument and sample specific constant which incorporates the parameters as described in equation [1]. The term $\sqrt{(E\# \cdot L)}$ has been called the *"transformed TOBEC number"* and is used as the standard term for the calculation of calibration equations for pediatric measurements (Fiorotto *et al* 1987a). The TOBEC instrument does not give an absolute estimation of the conductivity of the sample. However the TOBEC reading is linearly related to conductivity, as can be seen from equation [1].

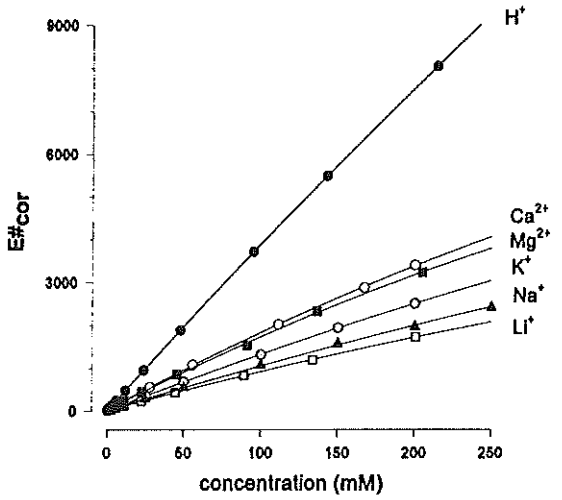


**Figure 2.1.** The effect on TOBEC of different chloride solutions and concentrations. Results are shown for the 3 l phantom.

The TOBEC instrument has two measuring facilities: (i) *fixed mode*, in which the instrument scans the conductor while it is moved slowly through the coil, and (ii) *peak mode,* in which the instrument's on-line computer detects the greatest deflection in coil impedance at the time the object passes through the coil as compared to the impedance of the empty coil. Measurements in *peak mode* are less influenced by head movements and thorax breathing expansions than measurements in *fixed mode*. For non-moving or inanimate objects no difference between *peak mode* and *fixed mode* has been observed (personal observations). All measurements in the present study, including reference phantom and background measurements, were performed in *peak mode*. TOBEC readings were performed by placing the object in the middle of the sledge of the instrument and slowly moving it into the coil. Each TOBEC measurement consisted of a series of ten 5 s readings of the object, from which the average gross TOBEC reading (gross *E#*) was calcu lated. The stability of the instrument was recorded twice a day by measurement of background noise and a reference phantom. Background measurements consisted of a series of ten 5 s TOBEC readings with an empty sledge. Subtraction of this background value from the gross *E#* of the object or reference phantom results in the net *E#* value ($E\#_{net}$). The reference phantom as delivered by the manufacturer consisted of a standard reference cylinder with a standard $E\#_{net}$ value of 1944. All TOBEC measurements have been corrected for background measurement ($E\#_{net}$) and the measurement of the reference phantom ($E\#_{cor}$). Correction for the reference phantom was performed by multiplying $E\#_{net\ (subject)}$ with: $1944/\ E\#_{net\ (phantom)}$.

*2.3.2. Electrolytes: type, concentration and volume*

The behaviour of several types of cations in a TOBEC electromagnetic field was studied. 2, 3 and 5 l solutions of the following cations was measured: $H^+$, $Li^+$, $Na^+$, $K^+$, $Mg^{2+}$ and $Ca^{2+}$. They were measured as chlorides at twelve concentrations (mean values: 200, 150, 100, 50, 25, 10, 5, 2.5, 1, 0.5, 0.25, 0.1 mM). To investigate whether the TOBEC reading of a mixture of electrolytes could be calculated from the known individual TOBEC readings of the ions, 200 mM NaF and KCL solutions were measured separately and as an equimolar mixture. The effect of sodium bicarbonate, the body's second most significant anion (large changes in bicarbonate concentration and thus in the TOBEC outcome may occur), was measured in 3 and 5 l bottles at the same 12 concentrations as mentioned above. All stock solutions were prepared by dissolving the dry salts (weighed on an analytical balance with an accuracy of 0.1 mg) in 5 l of distilled water. Further solutions were prepared by dilution of the stock solution.

We investigated the possible effect on TOBEC of macromolecules possessing a large number of ionized side-chain groups, using a solution of pure egg-white protein. Sixty grams of egg-white protein was dissolved in 1 l of water and dialyzed three times against 25 l of water and three times against equivalent amounts of distilled water. In the final (2 l) solution no $Na^+$, $K^+$ or $Cl^-$ was detectable. By means of freeze drying 20 ml of the solution the concentration of protein was gravimetrically determined to be 15 g $l^{-1}$. Protein was measured at the isoionic pH of the solution (pH = 4.6). Adjustment to the physiologic pH 7.4 would require the addition of e.g. sodium hydroxide, which would undoubtly influence the TOBEC reading: binding of ions to the protein makes the contribution of the individual ions of the added base to the overall TOBEC number virtually impossible to assess.

Aminoacid behaviour in a TOBEC electromagnetic field was investigated by measuring TOBEC of a 2 l solution of 150 mM of glycine and of a 2 l solution of 150 mM of L-glutamine. Both aminoacids have a large electric dipole moment (e.g. glycine has a dipole moment of 16.7 debye whereas water has a dipole moment of 1.83 debye). Glycine has a non-polar side chain (hydrogen), whereas L-glutamine has a polar side chain (an amide group). This allows investigation of the possible interaction of the TOBEC electromagnetic field with dipoles and with polar macromolecules.

For all the above described measurements the same 2, 3 and 5 l bottles were used (circumference 37.6, 43.0 and 50.5 cm respectively, length 26.3, 29.9 and 35.3 cm respectively). All measurements (except those of the temperature curves) were performed with solutions equilibrated at room temperature (22 $\pm$1 °C).

*2.3.3. Temperature*

Two polyethylene bottles with a volume of respectively 2 and 5 l were filled with 159 mM NaCl. The TOBEC was measured at nine different temperatures between -19 °C and 45 °C. The temperature of the solution was measured directly before each TOBEC measurement. Frozen bottles were measured immediately after removal from the freezer. The bottles were equilibrated at all temperatures for at least 24 h.

**Table 1.** Constants of equation (3) for each type and volume of electrolyte.

| | $A_0$ | $A_1$ | $A_2$ | n |
|---|---|---|---|---|
| 5 l Hcl | 0 | 110.7 (2.10) | 0.956 (0.004) | 12 |
| 3 l | 0 | 47.2 (0.67) | 0.959 (0.003) | 12 |
| 2 l | 0.93 (2.9) | 24.0 (0.44) | 0.960 (0.004) | 12 |
| 5 l $CaCl_2$ | 37.6 (18.1) | 62.6 (3.15) | 0.913 (0.009) | 10 |
| 3 l | 19.3 (6.5) | 26.7 (1.12) | 0.915 (0.008) | 10 |
| 2 l | 11.8 (4.7) | 13.4 (0.81) | 0.917 (0.011) | 10 |
| 5 l $MgCl_2$ | 0 | 64.3 (5.80) | 0.899 (0.018) | 10 |
| 3 l | 1.14 (14.4) | 29.7 (2.63) | 0.882 (0.017) | 10 |
| 2 l | 5.73 (8.5) | 14.9 (1.55) | 0.884 (0.019) | 10 |
| 5 l Kcl | 0 | 41.8 (1.15) | 0.930 (0.005) | 9 |
| 3 l | 0 | 18.5 (0.56) | 0.925 (0.006) | 9 |
| 2 l | 0 | 9.74 (0.32) | 0.918 (0.006) | 9 |
| 5 l NaCl | 10.9 (10.2) | 31.8 (1.34) | 0.942 (0.008) | 9 |
| 3 l | 10.2 (3.3) | 14.2 (0.47) | 0.937 (0.006) | 9 |
| 2 l | 6.40 (4.5) | 7.08 (0.51) | 0.939 (0.013) | 9 |
| 5 l LiCl | 0 | 31.4 (0.21) | 0.915 (0.001) | 9 |
| 3 l | 2.61 (2.2) | 13.6 (0.34) | 0.914 (0.005) | 9 |
| 2l | 1.51 (1.6) | 7.15 (0.24) | 0.907 (0.006) | 9 |
| 5l $NaHCO_3$ | 15.3 (27.4) | 22.1 (3.5) | 0.977 (0.029) | 9 |
| 3 l | 7.40 (13.1) | 9.47 (1.65) | 0.979 (0.033) | 9 |

Each row shows the *constants* $A_0$, $A_1$, *and* $A_2$ for a given type and volume of electrolyte, as calculated by nonlinear regression analysis using equation (3). The *SE* of each constant is given between brackets, *n* corresponds to the number of concentrations measured.
The smaller *n* in some experiments is due to the fact that for some electrolyte solutions the TOBEC signal was no longer distinguishable from background. For all regressions: $r^2 > 0.99$.

Seven infant Göttinger minipigs (body weight, 3.04 - 10.1 kg) were used to measure the effect of body temperature and of tissue autolysis, directly from the moment that they were sacrificed by an overdose of barbiturates. The minipigs were positioned in a lateral recumbent position on the sledge of the TOBEC instrument with the head positioned at 45° to the main axis of the body. During the experiment the position of the body on the sledge was not changed. For at least 1 h TOBEC and rectal temperature was repeatedly measured. To investigate the effect of tissue autolysis on TOBEC (with elimination of the effect of temperature) two animals were measured while the body temperature was kept constant by means of an electrical blanket. The animal part of the study was reviewed and approved by the institutional review board on animal research.

**Table 2.2.** Effect on TOBEC of mixing NaF and KCl solutions. Volumes are expressed as liters, all other values are in $E\#_{cor}$ numbers.

| *volumes* | *mixture* [@] | *NaF+KCl*[#] | *(NaCl+NaF)/2 + (KCl+KF)/2* [$] |
|---|---|---|---|
| 3 | 3781 | 4026 | 4025 |
| 5 | 8689 | 9438 | 9427 |

@ = 200 mM KCl and 200 mM NaF mixture.
\# = sum of $E\#_{cor}$ of separate measurements of KCl and NaF.
$ = calculated from the separate measurements of KCl , NaF, NaCl and KF.

*2.3.4. Statistical analysis*

Data are expressed as means, with the standard deviation between brackets (SD). The *Number Cruncher Statistical System* package (Hintze 1989) was used for descriptive statistics and (non-) linear regression analysis.

## 2.4 RESULTS

*2.4.1. Instrument stability and precision of measurements*

Intrameasurement and the day-to-day variability was calculated from the TOBEC measurements of background and of the reference phantoms, which were performed twice a day at the time of the experiments. They were calculated, respectively, as the coefficient of variation (CV) of the ten consecutive 5 s measurements and the CV of the mean *E#* values taken over the total duration of the study (eleven months). The TOBEC readings showed a narrow error range (*E#* numbers). In a typical series of ten measurements the difference between the largest and smallest value did not exceed fifteen *E#* numbers, independent of the magnitude of the signal. Mean daily background values ranged from 28 to 43, with a CV of 11%. Due to (partly) uncontrollable factors, e.g. vibrations, humidity and temperature, the day-to-day variability is considerable. However, the absolute magnitude of the variation is limited as compared to the overall TOBEC signal of the object measurements. The intrameasurement variation of the reference phantom measurements ranged from 0.15% to 0.63%, with a mean of 0.38%. The day-to-day variability of $E\#_{net}$ of the reference phantom was 0.6%.

Because the TOBEC instrument also showed a narrow error range in the study objects, it is obvious that the precision of the measurements increases in larger objects with higher conductivity.

When $E\#_{net}$ values instead of $E\#_{cor}$ values of the electrolyte solutions were entered in the regression model (see equation [3] below), the precision of the prediction equation worsened (mean squared residuals were 13% ($\pm$5.3) larger than in the regressions which used $E\#_{cor}$). This confirmed the need for correction of the $E\#_{net}$ values against the reference phantom measurements (resulting in $E\#_{cor}$).

*2.4.2. Electrolytes: type, concentration and volume*

The effect of various concentrations and volumes of cations on TOBEC is presented in **Figure 2.1**. This figure shows that equimolar concentrations of a given volume of different electrolytes yielded different $E\#_{cor}$ values, always in the following order:

*cations (chlorides):* $H^+ >> Ca^{2+} > Mg^{2+} > K^+ > Na^+ > Li^+$

*anions (sodium salts):* $Cl^- > F^- > HCO_3^-$

A highly significant relation between $E\#_{cor}$ and the concentration of electrolyte was observed ($r^2 > 0.99$ for all equations). The relation between $E\#_{cor}$ and the volume and conductivity of a cylindrical object was stated in the equations [1] and [2]. We used these equations as the basis for a search for the best fitting regression equation, correlating $E\#_{cor}$ and the concentration of electrolytes. For each volume and type of electrolyte all terms of equation[1] can be assumed constant except $\sigma$, which changes with concentration. A power term was added to give the model the freedom to adapt to non-linearities in the relation between concentration and conductivity of an electrolyte solution. Indeed, the behaviour of the cations could be described best for all three volumes over the entire measured range of concentrations by the following equation:

$$(E\#_{cor}) = A_0 + A_1 * (conc)^{A_2} \qquad [3]$$

where $E\#_{cor}$ is the TOBEC value, corrected for background and reference phantom, (conc) is the concentration of a given type of electrolyte. $A_0$, $A_1$ *and* $A_2$ are constants calculated by non-linear regression analysis (see **Table 2.1**). We added the constant $A_0$ to give the model the capability of correction, e.g. for environmental factors influencing the TOBEC measurements. There was a constraint that $A_0$ could not be less than zero (at a concentration of zero, $E\#$ cannot be negative). The constant $A_1$ depended upon volume and type of electrolyte and increased with volume. The magnitude of $A_1$ is explained mainly by the geometry factors and the conductivity of the sample. Harker (1973) stated that the term $R^4 \cdot L$ explained most of the dependence of E# from geometry. We calculated this term for the three phantoms. The $R^4 \cdot L$ term for the 2 l bottle was taken as $Q$; the $R^4 \cdot L$ term for the 3 and 5 l bottles (divided by the value for the 2 liters bottle) was $1.94Q$ and $4.37Q$. With the same procedure, the mean $A_1$ values ($\pm$ 1 SD) of the respective equations were 1.96 (0.04)$Q$ and 4.55 (0.16)$Q$, which is in accordance with the actual $R^4 \cdot L$ values of the 3 phantoms. Also for a given volume the magnitude of $A_1$ was in accordance with known conductivity values for these cations. The exponent $A_2$ is volume and concentration independent and is virtually constant for a given type of electrolyte (see **Table 2.1**).

The magnitude of $E\#_{cor}$ of the three anions, chloride, bicarbonate and fluoride, was in accordance with known ion conductivity values for anions for all three volumes (Moore

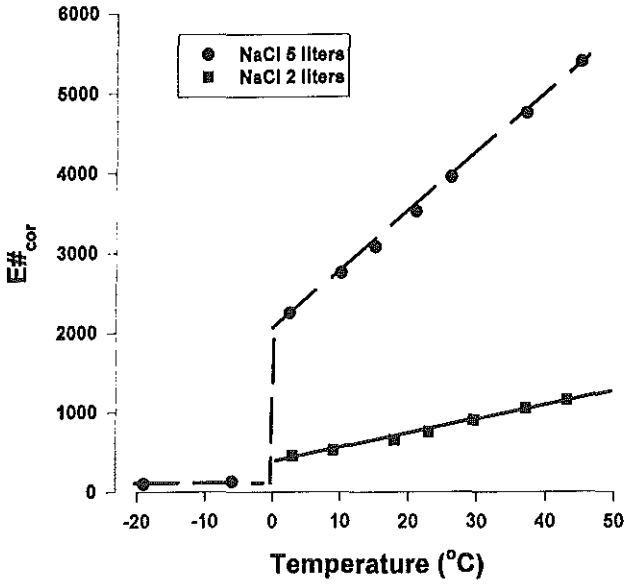


**Figure 2.2.** The effect of temperature on TOBEC of 2 and 5 l solutions of physiological saline (159 mM).

1973). When a solution was made of 200 mM NaF and 200 mM KCl $E\#_{cor}$ values were less than would be expected from a simple addition of the $E\#_{cor}$ values of the separate measurements of KCl and NaF (see **Table 2.2**), thus the effect on TOBEC of mixing of two electrolytes is clearly not additive.

The egg-white protein, glycine and L-glutamine solutions all yielded *E#* values not significantly different from zero.

*2.4.3. Temperature and phase*

With 2 and 5 liter solutions of saline (159 mM) a strictly linear relation between *E#* and temperature was found between 2 °C and 45 °C (see Figure 2). This relation could be described by the following equations:

*For the 2 liter solution:* $E\#_{cor} = 16.16\ (temp) + 448.3 \qquad r = 0.998$
*For the 5 liter solution:* $E\#_{cor} = 74.62\ (temp) + 1990 \qquad r > 0.999$

where $E\#_{cor}$ is the TOBEC value, corrected for background and reference phantom, (*temp*) is temperature in degrees Celsius. A discontinuity of this linearity was observed below the

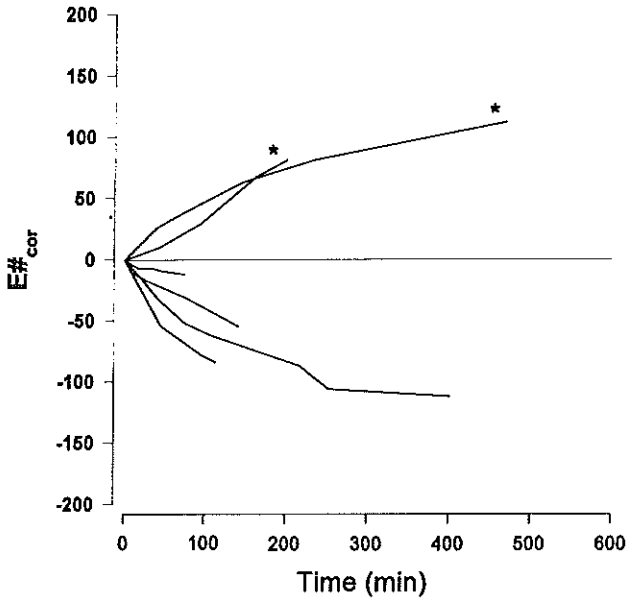


**Figure 2.3.** The effect of temperature in seven minipigs. Two animals were kept at constant body temperature; five animals were allowed to cool. The values plotted are calculated as follows: $E\#_{cor}$ (t=t) - $E\#_{cor}$ (t=0). Curves marked * represent the pigs kept at constant body temperature.

freezing point, where two TOBEC measurements of a 5 l solution of saline yielded *E#* values of virtually zero (see **Figure 2.2**).

*2.4.4. Post mortem measurements*

Data of the minipigs are summarized in **Table 2.3**. Results are presented in **Figure 3** and **Table 2.4**. Body temperature at the time of sacrifice ranged from 34.3 to 40.2°C (n=7). The mean decline (SD) of $E\#_{cor}$ after 1 h was 3.2 (0.8)%. The mean rate of temperature decline after death was 2.3 °C $h^{-1}$ ($n$=5). The change in *E#* per°C was calculated from the data obtained in the first minutes after death (here the effect of autolysis is expected to be minimal), and was approximately 2.5%. However, when in two pigs body temperature was kept constant at, respectively, 39.0 (0.6) and 39.7 (0.6)°C, $E\#_{cor}$ increased by 9.1% and 9.4%, respectively, as calculated from the largest range in $E\#_{cor}$.

## 2.5 DISCUSSION

### *2.5.1 TOBEC*

In spite of the relatively widespread use of the TOBEC methodology in several industrial and scientific applications and recently also in human body composition studies, only a limited number of papers has addressed the question of the effect of physiopathological changes in the subject in question on TOBEC. Fiorotto *et al* (1987a) measured infant minipigs and adult rabbits in the early model M-60 TOBEC instrument. Hydration of the FFM as well as the amount of extracellular fluid per volume FFM (determined by carcass analysis) differed significantly between minipigs and rabbits. They did not find a statistical difference in the slopes of the regression lines of FFM against *E#*, but neither were these lines identical. Cunningham *et al* (1986) used another small early model TOBEC instrument. These authors found no differences between several groups of rats in which altered fluid and electrolyte status was induced. Although not explicitly concluded by the authors an effect on TOBEC by altered ion states is suggested by their results. Cochran *et al* (1989) infused minipigs peritoneally with an isotonic saline solution. An isotonic change in FFM hydration gave a proportional rise of the TOBEC outcome, yielding a proper estimate of body composition. However, all early models of the TOBEC instrument were less accurate and had different electromagnetic field properties as compared to the current second-generation instrumentation.

### *2.5.2. Electrolytes: concentration and volume*

In the present study measurement of solutions of chloride salts of several physiological cations and lithium yielded TOBEC values in close agreement with known values for ion conductivity and mobility in water (Moore 1973). The hydrogen ion was by far the most conductive ion, which is also in agreement with known ion conductivity values. However, under physiological conditions at a plasma pH of 7.4, the plasma $H^+$ concentration is very low and physiological changes in pH are too small to affect the TOBEC outcome.

**Table 2.3.** Data of infant Göttinger miniature pigs (m = male, f = female).

| *Pig No.* | *Gender* | *Age (days)* | *Length (cm)* | *Weight (kg)* |
|---|---|---|---|---|
| 1 | m | 78 | 59 | 7.76 |
| 2 | m | 79 | 56 | 7.29 |
| 3 | m | 40 | 41.5 | 3.04 |
| 4 | m | 41 | 47 | 4.86 |
| 5 | m | 42 | 47.5 | 5.20 |
| 6 | f | 99 | 59 | 10.10 |
| 7 | f | 53 | 53 | 5.48 |

**Table 2.4.** Effect of temperature on TOBEC measurements in 7 minipigs.

| pig# | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | $E\#_{cor}$ | **1274** | **1262** | **1255** | **1253** | **1249** | **1242** | **1233** | **1225** |
| | T | 36.7 | 36.0 | 35.6 | 34.3 | 33.7 | 32.5 | 32.2 | 30.5 |
| | δt | 0 | 14 | 10 | 14 | 26 | 16 | 16 | 35 |
| 2 | $E\#_{cor}$ | **1344** | **1335** | **1312** | **1292** | **1282** | **1257** | **1238** | **1232** |
| | T | 35.5 | 35.1 | 34.0 | 32.5 | 31.0 | 28.5 | 28.2 | 26.1 |
| | δt | 0 | 13 | 29 | 34 | 32 | 109 | 36 | 149 |
| 3 | $E\#_{cor}$ | **363** | **356** | **356** | **354** | **351** | | | |
| | T | 34.3 | 33.8 | 32.8 | 32.4 | 30.7 | | | |
| | δt | 0 | 17 | 18 | 11 | 29 | | | |
| 4 | $E\#_{cor}$ | **776** | **766** | **759** | **756** | **744** | **721** | | |
| | T | 35.5 | 35.0 | 34.2 | 33.8 | 31.3 | 29.8 | | |
| | δt | 0 | 9 | 18 | 11 | 41 | 63 | | |
| 5 * | $E\#_{cor}$ | **929** | **886** | **896** | **915** | **953** | **967** | | |
| | T | 40.2 | 39.0 | 38.4 | 38.6 | 39.1 | 38.8 | | |
| | δt | 0 | 8 | 43 | 51 | 68 | 39 | | |
| 6 | $E\#_{cor}$ | **1502** | **1448** | **1424** | **1418** | | | | |
| | T | 37.9 | 37 | 34.5 | 34.0 | | | | |
| | δt | 0 | 45 | 51 | 18 | | | | |
| 7 * | $E\#_{cor}$ | **620** | **626** | **637** | **663** | **681** | **712** | | |
| | T | 40.0 | 39.1 | 38.9 | 39.9 | 40.2 | 40.2 | | |
| | δt | 0 | 40 | 30 | 76 | 91 | 235 | | |

$E\#_{cor}$ = TOBEC value, corrected for background and reference phantom.
c.v. = coefficient of variation of the 10 consecutive five-second TOBEC readings.
T = temperature (°C).
δt = time interval between TOBEC measurements (minutes).
* = pigs kept at constant temperature with an electrical blanket.

The bicarbonate ion ($HCO_3^-$) is one of the most variable anions in the human body and is present in large amounts. On a molar base its conductivity was slightly less than that of sodium chloride. When the bicarbonate concentration increases (e.g. in metabolic alkalosis and respiratory acidosis), the chloride anion usually decreases. In theory this might have implications for TOBEC measurements. However, changes in electrolyte concentration in acid-base disturbances tend to neutralize each other (Maxwell 1987, Tietz 1987) . Only in patients with diabetic ketoacidosis and renal failure with a large decrease in bicarbonate and with large anion gap values, the TOBEC method might significantly underestimate the volume of conductive (fat-free) mass.

Equation [3] predicts $E\#_{cor}$ very accurately. The factor $A_0$, (which was anticipated to be zero) has to be interpreted as a correction constant of the predictive model for all kinds of exogenous factors such as differences in humidity and temperature. For all electrolytes the constant $A_2$ was less than one, showing a non-linear and electrolyte-specific relation between the concentration of an electrolyte solution and its conductive properties.

Increments in concentration do increase the TOBEC reading but the effect is not additive. This effect is also present when several different types of electrolytes are mixed. The measurement of NaCl and NaF separately yielded values for *E#* that were in accordance with known conductivity values. When solutions of different salt mixtures were studied the resulting *E#* did not equal to the additive effect of the salts measured separately. The mixture was less conductive than the sum of the separate salts, and less than the mean values of the NaCl, NaF, KCl and KF salts. Possibly this effect also occurs when the total number of ions of a heterogeneous electrolyte solution increases. The reason for the non-linearity between conductivity and concentration of an electrolyte solution is due to decreasing ion activity. It is not possible therefore to calculate whole-body TOBEC values from the contributions to the TOBEC signal of the separate body electrolytes.

It is known from literature that the amount of power dissipated from an electromagnetic field by a sample depends on coil factors, sample conductivity and to a large extent the geometry of the object. Equation [1] shows that for a cylinder this dependency is represented by the geometry factor $R^4 \cdot L$ and the conductivity $\sigma$. These two factors contribute to $A_1$. For the same bottle the magnitude of $A_1$ was dependent upon type of electrolyte (i.e. the ion-specific conductive properties): when the constants $A_1$ of the different electrolytes were ranked to magnitude, the order reflected the conductivity values of the given electrolytes. Also, for each type of electrolyte $A_1$ was proportional to $R^4 \cdot L$. Effects of geometry are relatively easy to assess in cylindrical objects but very complex and difficult to extrapolate quantitatively to the human subject. They should be determined empirically, e.g. by calibration procedures. Nevertheless, it is obvious from the above results that a potential source of error lies in the variability in object cross-sectional area. (such as in infants with a distended abdomen or other significant deviations from normal body shape).

### *2.5.3. Protein and aminoacids*

TOBEC measurements of pure distilled water yield *E#* values not significantly different from zero. Also TOBEC measurements of solutions of pure egg-white protein and of the aminoacids glycine and L-glutamine were virtually zero. Both amino acids have strong dipole moments. The electrical dipole moment of water is 1.83 debye, and for example that of glycine is 16.7 debye. The results from this study show that, in addition to non-ionized compounds such as urea (Pethig 1984), local dipoles of water, amino acids and ionized side chains also do not dissipate energy from the electromagnetic field (in accordance with formula [1]), i.e. no eddy currents are elicited by the TOBEC electromagnetic field. When protein and aminoacids are dissolved in pure water (without adding other ions), the pH of the solution reaches its isoionic point (the molecule has a net charge of zero). In most proteins the pH of this point is approximately equal to the pH of the isoelectric point (i.e. the point where a protein does not move on electrophoresis). The isoelectric points of egg-albumin and human albumin are respectively at pH 4.6 and 4.7. This implicates that at pH

4.6 the net charge of the molecule is zero. Hence there will be no conductive effect of the molecule. This is in agreement with the finding of the present study, where a solution of egg-white protein (with egg-albumin as a main constituent) elicited no net TOBEC reading. However, at a physiological pH (of ca. 7.4) the albumin molecule will have a net charge and hence it will possess conductive properties. The relative magnitude of this conductivity can be estimated (e.g. against the conductivity of sodium). At pH 7.4 only the glutamate and aspartate side chains of the albumin molecule will be ionized with a maximum of 97 charged side-chains (the total number of glutamate and aspartate molecules). Hence the net charge will be at most 97 times that of sodium. The friction constant of albumin (a measure of the mobility and thereby a measure of the charge transport function of the molecule in electrophoresis experiments) is roughly twenty times that of sodium (Atkins 1982). Thus on a molar base the net conductivity at pH 7.4 of albumin will be maximally five times that of sodium ions. When the concentration of serum albumin is fixed at 40 g $l^{-1}$ (which is approximately 0.5 mM) its conductivity will not exceed the conductivity of a 2.5 mM sodium solution, which is at the limit of detection of the current pediatric TOBEC device. Any change in serum protein concentration will therefore not influence the TOBEC-derived body composition assessment. The experiment confirms theoretical predictions as to conductivity. Besides this, and more important, the results of this study also implicate that on a local molecular level no energy is dissipated from an electromagnetic field from e.g. rotation or vibration of large macromolecules with charged side chains. As far as the amino acids are concerned, the low concentration of amino acids *in vivo* precludes any significant contribution to conductivity. So it can safely be assumed that under physiological conditions changes in the body's protein and amino acid level will not affect the TOBEC outcome.

*2.5.4. Temperature and phase*

The conductive properties of tissue in an electromagnetic field vary with temperature. Ion mobility decreases with lower temperature, weaker eddy currents arise and less energy is dissipated from the electromagnetic field, resulting in a lower *E#*. We found a strictly linear relation between the temperature and TOBEC readings of a solution of physiological saline. For both volumes the intercept differed significantly from zero, while below the freezing point TOBEC readings dropped virtually to zero (Figure 2.2). Thus in the crystalline structure of ice, where the mobility of ions is small, their conductive properties at this temperature are not detectable in a TOBEC electromagnetic field. Accordingly it is expected that any ion bound in a crystalline structure will not elicit a significant TOBEC signal. This implies that the apatite-water crystals of bone will have no effect on TOBEC either, which has been confirmed by personal observations on freshly excised bovine bone. From the slope of the equations the change in *E#* per°C can be estimated. For a 2 l solution of physiological saline a change was observed of *E#* = 16 per°C, and *E#* = 75 per°C for a 5 l phantom. At body temperature (37 °C) the relative changes in *E#* calculated for a deviation of 1 ° C are 1.5 % and 1.6 %, respectively, for a 2 l and a 5 l solution of physiological

saline, resulting in an equivalent error in the estimate of FFM. A linear relationship between *E#* and temperature, and an equivalent relative change in *E#* per°C of 1.5% was found by Klish *et al* (1984) with the first generation TOBEC device. Because the most dominant effect on TOBEC of a living subject is caused by the ionic content of the FFM, we expected an equivalent change of approximately 1.5% in E# per°C for the minipigs. This change appeared to be approximately 2.5%, as calculated from the change in E# during the first minutes after death (here the TOBEC measurements are least likely to be influenced by the effect of autolysis). This value is higher than that obtained from inanimate objects. Effects occurring at the moment and after the process of dying might contribute to this (redistribution of water and electrolytes, loss of osmotic activity etc.). Also the deviation in *E#* (per°C) of the different animals was much larger than in the measured bottles.

The results of the *in vitro* temperature measurements are in agreement with earlier reports on the measurement of conductivity of small samples in an electromagnetic field. Marchal *et al* (1989) measured gelatine phantoms of 10% - 40 % gelatine at frequencies ranging from 10 to 50 MHz. They found a linear relationship between conductivity of the gelatine phantoms and temperature. With added saline (5 g $l^{-1}$) the relationship became curvilinear above 40 °C, which might be a result of loosening of $Na^+$ binding to the polar protein sidegroups. Schwan (1957) showed at 50 MHz that changes in tissue conductivity per °C were least for fat, brain and kidney tissue (around 1.4%) and highest for muscle tissue, spleen and blood, the major constituents of the FFM (around 2.7%). The calculated changes in *E#* per°C from the phantoms and the minipigs suggest that in living subjects an error in TOBEC measurements of at least 2 - 5 % can be anticipated, thereby clearly overestimating FFM. This fact also complicates the study of TOBEC in neonates and preterm infants who have unstable temperature control mechanisms. In addition the outer layers of the subject probably dissipate more energy from the magnetic field and contribute relatively more to the TOBEC reading than the inner parts of the body. Furthermore when temperature decreases superficial blood vessels constrict, giving rise to a redistribution of the absolute amount of extracellular and intracellular ions in the superficial layer around the body. The effect of superficial eddy current distribution is highly significant at frequencies exceeding around 60 MHz (Harpen 1989), increasing gradually with frequency (Harker 1973). It is not clear whether this effect is also significant at 2.5 MHz. Based on the above-mentioned data one should prevent cooling of the skin before and during a TOBEC measurement.

*2.5.5. Post mortem measurements*

From the measurements on minipigs after death two phenomena can be identified: a clear temperature effect (*E#* decreases with temperature), and a counter-effect (*E#* increases when temperature remains constant). At constant temperature the increase of *E#* continued for the total duration of the experiment. This can be attributed to several causes: (i) autolysis and loss of cell membrane integrity, (ii) disruption of acid-base homeostasis, (iii) breakdown of

macromolecules, which changes the concentration of (bound) electrolytes, and (iv) redistribution of previously specific body-compartment-bound electrolytes throughout the carcass. Many of these phenomena have been observed during studies of conductivity and dielectric properties of biological molecules, or with *in vitro* tissue experiments. The importance of the cell membrane in conductivity experiments was demonstrated by studies where in cell suspensions a large reduction of resistivity was observed when the cells were lysed with digitonin (Fricke and Curtis 1935). Besides the conductance properties of ions as such, the cross sectional area and the length of the total conductive mass also have an effect on the total amount of energy dissipated from the electromagnetic field. Redistribution of ions therefore undoubtedly changes the total mean effect of the FFM in the TOBEC electromagnetic field. A gravitational effect might also occur, which changes the cross sectional area and distribution volume of the electrolytes within the FFM, as happens in cadavers where after some time livid spots develop. The results show that assessment of body composition of cadavers by TOBEC (e.g with use of animal models at calibration or validation studies, and at post-mortem analysis of body composition) is only possible with a minimum of error when performed immediately after death.

### *2.5.6. Conclusions*

Practical consequences of the present study can be summarized as follows: (i) The reproducibility of TOBEC measurements is excellent. Variability of instrument response is only a minor source of error in the estimate of body composition. (ii) Errors arising from changes in temperature will undoubtly disturb TOBEC-derived body composition estimates. (iii) Most physiological changes in FFM electrolyte concentrations will not severely disturb the TOBEC outcome although changes in bicarbonate levels may affect the TOBEC signal in the case of diabetic ketoacidosis and renal failure. (iv) Deviations in plasma levels of protein and amino acids will not affect the TOBEC outcome. (v) Ions bound in a crystalline structure (such as in ice and bone) will not elicit a significant TOBEC signal. (vi) Major deviations from the normal cross sectional area of the measured subjects may have a significant effect on TOBEC. (vii) Due to tissue autolysis and body-temperature decline, TOBEC measurements after death are susceptible to considerable errors.

Finally, the present study shows that electrolyte solutions in a TOBEC electromagnetic field behave in accordance with data on ion conductivity. This is of much importance to the TOBEC methodology: it suggests that much of the knowledge of the behaviour of electrolytes, cells and tissues in an electromagnetic field as has been studied in the past can be transferred to the field of body composition measurements by total body electrical conductivity.

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*Chapter 3*

# TOBEC Instrument Evaluation and Calibration [a)]

## 3.1 SUMMARY

Quantitation of the body's fat and lean masses is an important component of nutritional assessment. Such measurements, however, are difficult to conduct routinely in infants due to the numerous limitations of traditional methods. The application of total body electrical conductivity measurements for quantitating fat-free mass (FFM) overcomes many of these limitations. The instruments required to perform these measurements in pediatric patients (HP-2) have recently become commercially available, but their measurement performance has not been evaluated. In these studies, we compared the precision, day-to-day variability, and magnetic field profile of three HP-2 instruments. We also derived a new calibration equation that relates the FFM to the total body electrical conductivity measurement in piglets, and compared it with an equation (provided currently by the manufacturer) derived on a prototype instrument. The performance of the instruments was generally similar, although a significant difference in the magnetic field of one instrument was identified. The coefficient of variation of inanimate phantom measurements varied from $\pm 0.2$ to $\pm 0.5\%$, and the day-to-day variability was generally similar. Such measurement error is significant ($\pm 0.035$ to $\pm 0.078$ kg FFM) for small subjects. The new calibration equation was similar to the original equation; therefore, all the data were pooled to generate a new equation that is linear at least to 10 kg. Thus, the HP-2 total body electrical conductivity instruments, which can be safely and easily used to measure FFM and fat in infants through 1 y of age, proved to be reliable and precise, and results obtained from different instruments can be confidently compared.

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## 3.2 INTRODUCTION

The quantitation of the body's fat and lean masses are fundamental for the assessment of an individual's nutritional status. The adequacy of fat stores and the FFM traditionally are assessed indirectly from measurements such as skinfold thicknesses and arm muscle area. These latter methods are relatively insensitive, and their accuracy in predicting fat and lean masses is questionable in infants. Although measurements of total body FFM and fat mass are preferable, they are difficult to conduct on a routine basis in infants due to the numerous limitations of traditional methods. The application of TOBEC measurements for quantitating FFM overcomes many of these limitations.

TOBEC measurements have been used to estimate the conductive mass of human subjects and animals *in vivo* (1-4). The conductive mass of the body corresponds to that compartment occupied by total body water and the conductive, fat-free solids of the body throughout which the water is distributed (3, 4). This compartment corresponds to the FFM. The instrument consists essentially of a cylindrical measurement chamber encompassed by a solenoidal coil through which a low frequency oscillating electrical current (2.5 MHz) is passed to generate a magnetic field within the chamber. A conductive object placed in the field dissipates some of the field's energy and in doing so changes coil impedance. The magnitude of this change in impedance is a function of the instrument's magnetic field characteristics, as well as the object's conductivity, and total conductive mass. Because fat is nonconductive, it does not change coil impedance and therefore is not measured; fat mass, however, can be calculated as the difference between body weight and FFM.

The technique is ideally suited for infants because it is safe, noninvasive, requires no active participation by the subject, and can be rapidly performed (in approximately 5 min); hence, it provides immediate estimates of FFM and body fat mass. In addition, the accuracy of the FFM measurements is compromised minimally by isotonic variations in the hydration of the FFM (4). This feature is advantageous for studying populations, such as the pediatric population, in which the hydration of the FFM can be widely divergent even under normal circumstances.

Use of TOBEC in pediatric clinical or research applications has been limited because pediatric instruments were not commercially available until 1989, and the initial evaluations of this technique were conducted exclusively on prototype instruments (models EMME M60 and HP-1) (2, 5). The current, commercially available instrument (5) (model HP-2) differs from the prototypes in the shape, length, and homogeneity of its magnetic field; the units in which the TOBEC values are expressed also differ.

Despite these developments, three concerns still potentially limit the use of the technique in pediatrics. First, each HP-2 instrument is assembled individually. Thus, there is no assurance that all instruments perform similarly. If the instruments differ, then estimates of FFM determined on one instrument would not be directly comparable with those obtained from another. Although the manufacturer standardizes the HP-2 instruments, there has been

no evaluation to establish whether variations in performance regarded acceptable by the manufacturer (because they are within the design tolerances) are acceptable in practice.

Second, there is the concern of calibration (6). Adult TOBEC instruments were calibrated by measuring the FFM of a reference population with an alternative technique (usually hydrodensitometry) and relating this to TOBEC measurements of the same individuals (3). A similar approach could be used for the pediatric TOBEC instrument; however, methods currently available for the estimation of FFM of infants (such as total body water and potassium) do not measure the same body compartment as does TOBEC. A calibration equation based on these methods therefore would provide estimates of FFM that would only be as accurate as body water and potassium in their prediction of FFM. Thus, an alternative approach for calibration was used (7, 13). The conductance of animals (infant miniature pigs) with a chemical composition and size similar to that of human infants was measured, and this was related to the piglets' true FFM measured by chemical analysis (7, 13). These TOBEC measurements were made on prototype instruments, and the calibration equation so derived is currently used to estimate FFM from TOBEC measurements made on the new HP-2 instruments. In view of the changes in magnetic field characteristics, it is essential to compare the original calibration equation with a calibration equation derived directly on an HP-2 instrument.

The final concern relates to the size range of the subjects over which the calibration equation is applicable. The original calibration was confined to piglets weighing less than 5.6 kg; the validity of linear extrapolation for larger subjects has not been tested.

Our studies were designed to address these three concerns. Our first objective was to assess the variability in measurement precision and magnetic field characteristics among three HP-2 instruments. The measurement precision determines the smallest change in FFM that can be discerned with confidence. The evaluation of the magnetic field profiles would allow us to determine whether a universal calibration equation can be used for all instruments to derive FFM or whether each instrument must be separately calibrated. If each instrument must be separately calibrated, it would materially reduce the usefulness and widespread use of the technique in pediatrics.

Our second objective was to compare a calibration equation derived from direct measurements of miniature piglets on one of the three HP-2 instruments with the original equation derived from measurements on an HP-1 instrument. This comparison essentially tests the practical consequences of the modifications associated with the upgrade of the HP-1 to the HP-2 instrument. The use of an animal model that can be subjected to chemical analysis enabled us to circumvent concerns that arise about the accuracy of the reference method for FFM determination.

Finally, our third objective was to determine whether the relationship between FFM and the TOBEC measurement is linear over a wider range of sizes.

**Table 3.1.** Comparison of average day-to-day and within-measurement variability of TOBEC measurements made on three HP-2 TOBEC instruments.

| Laboratory | Reference [2] | *n* | Mean | Variability (SD) [1] | |
|---|---|---|---|---|---|
| | | | | Day-to-day | Within-measurement |
| MIC (0006) [3] | 2012 | 49 | 2007 | 13.8 | 3.6 ± 1.2 |
| NDL (0011) | 1944 | 50 | 1942 | 9.8 | 8.8 ± 2.9 |
| TX (0010) | 2032 | 50 | 2037 | 4.1 | 4.7 ± 1.2 |

[1] All units are E#.
[2] Manufacturer's specified net E# for phantom.
[3] Instrument serial number.

## 3.3 METHODS

### *3.3.1. Comparison of Three HP-2 Instruments*

Our first objective, to compare measurement precision and magnetic field profiles among three (designated as MIC, NDL, and TX) HP-2 instruments (model HP-2, EM-SCAN Inc., Springfield, IL), was accomplished with the use of inanimate standards (phantoms) provided with each instrument. One phantom is a copper hoop with a resistor in series and provides a measurement at a single point in the magnetic field. The other is a 45-cm long cylinder that contains a conductive coil, and provides a measure of the average conductance integrated over 45 cm. Each phantom has an E# determined using standard operating conditions on a reference HP-2 instrument maintained by the manufacturer. Each new instrument is then adjusted (using a normalization constant) so that the phantom E# is the same value as that measured on the manufacturer's reference instrument. This constant therefore is meant to correct for instrument-to-instrument variations in a magnetic field. The correction procedure, however, adjusts only for differences at the center of the measuring range and does not identify discrepancies at the two ends.

Measurement precision (within-measurement variability) was assessed from the average SD of 10 individual, consecutive readings of a phantom measured on numerous separate occasions (**Table 3.1**). Day-to-day variability was assessed from the variation in the average E# of either phantom over a period of 18 to 24 mo All measurements were made with the phantoms placed in the center of the measurement chamber (in the user-determined position, i.e. "fixed" mode). Empty carrier (background) measurements were made concurrently, and this value was subtracted from the gross E# of the phantom to give a net E#.

The magnetic field profiles of the instruments were compared using two procedures. First, the hoop phantom was placed at the distal end of the carrier (furthest from the handle); the carrier was then slowly inserted into the measurement chamber and a reading was taken every 2 cm over the length of the measurement chamber, i.e. 200 cm. This is the method recommended by the manufacturer. In the second procedure, we compared the

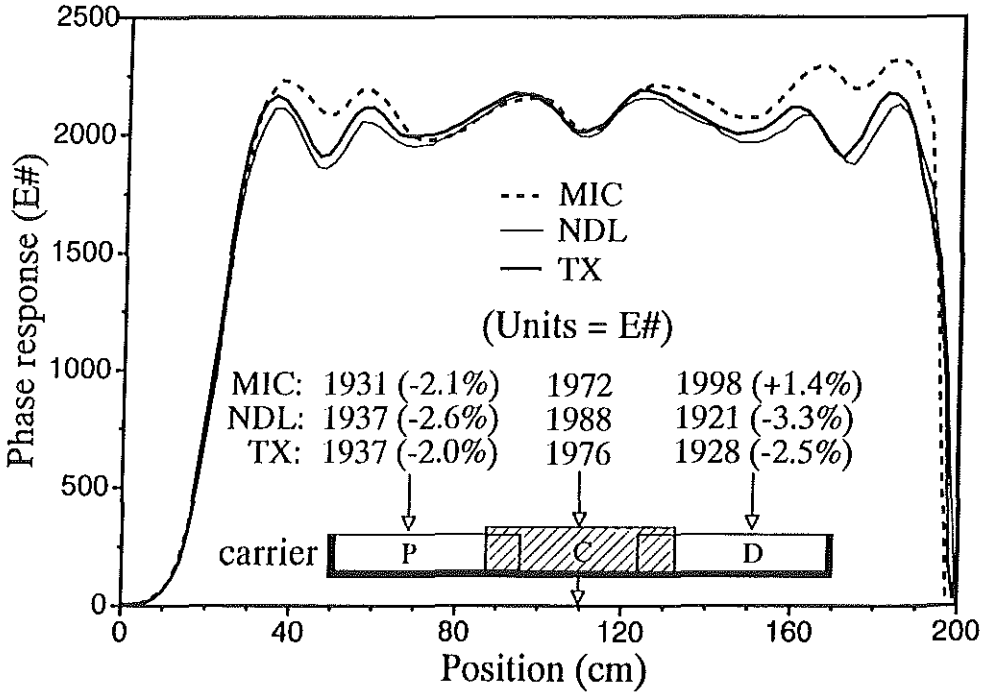


**Figure 3.1.** Magnetic field plots of the MIC, NDL, and TX HP-2 instruments showing the position of the subject carrier when it is centered in the measurement chamber. Boxes on the subject carrier represent the location of the tube phantom in the chamber when measurements were made at the proximal (P), center (C), and distal (D) positions. The values are the average E# of the respective phantoms at these positions for the three laboratories; all measurements were made in the "fixed" mode. The values in parentheses are percent differences between the values of E# in the P or D positions and their respective value in the center position.

average E# obtained for the tube phantom when it was placed at the proximal (closest to the handle) and distal ends of the subject carrier with the E# in the center (reference) position (Fig. 1). In all instances, the subject carrier was positioned in the center of the measurement chamber. This procedure provided a quantitative measure of how the magnetic field of each instrument varied over its length and enabled the magnetic fields of instruments to be compared without the need for the same phantom to be measured on all instruments.

*3.3.2. Relationship between TOBEC Measurements (E#) and FFM*

To address our second objective, we compared the relationship between the chemically

determined FFM and the TOBEC measurement of two groups of piglets: for one group of piglets (TX), the E# was determined on an HP-1 instrument, and for the second (NDL), measurements were made on the NDL HP-2 instrument. The general procedures used have been described previously (7) and essentially involved measuring piglets in the TOBEC instruments and then determining their FFM by chemical analysis. The two laboratories, however, differed in certain details.

*Animals.* The NDL laboratory studied 12 miniature piglets of the Gottingen strain (University of Dusseldorf) ranging in age from 7 to 99 d and weighing from 1.03 to 10.10 kg. The TX laboratory studied 26 miniature piglets of the Hanford strain (Charles River Laboratories, Wilmington, MA) ranging in age from 7 to 33 d and weighing from 1.87 to 5.53 kg. With one exception, all piglets were healthy and had been stabilized after transport to the respective laboratories; during this time piglets had free access to a swine milk replacer. One NDL piglet refused feedings between arrival and measurement time. All piglets were fasted (but provided with water) for at least 6 h before TOBEC measurements were made.

The animal protocols were reviewed and approved by the respective institutional review boards.

*TOBEC measurements.* Measurements were made on anesthetized piglets. NDL piglets were fitted with ear vein catheters before being measured. The piglets were centered on the subject carrier in a lateral recumbent position and the $L_{con}$ was measured as described previously (7). Ten consecutive readings were taken (in "peak" mode) and an average net E# was calculated for each pig after subtracting the background reading of the empty subject carrier. The HP-1 E# units were subsequently converted to HP-2 E# units using an instrument specific conversion equation. A phantom measurement was also made to adjust for short-term variations in instrument performance. For both NDL and TX pigs, the net E# was then corrected by a factor that reflected the extent by which the concurrent phantom reading deviated from its predicted value ($E\#_{cor}$). The square root of the product of $E\#_{cor}$ and $L_{con}$, $\sqrt{[E\#_{cor} \cdot L_{con}]}$, was calculated for each pig; this term was used as the independent variable in the regression analysis against FFM.

*Chemical analysis.* On completion of the TOBEC measurements, the pigs were killed with an overdose of anesthetic and weighed. The analytical procedures used by the two laboratories were similar and have been previously reported (7). Total body water was estimated by desiccation of the whole carcass (at 97°C). Complete desiccation was verified by the absence of weight change with further drying. The fat content was measured by carrying out an initial extraction with methylene chloride (TX laboratory) or hexane (NDL laboratory), followed by a diethyl ether extraction in a Soxhlet apparatus. Both laboratories verified that fat extraction was complete by the absence of further weight change on repeated extraction. The coefficient of variation for the replicate fat analyses were $\pm 1.4\%$ and $\pm 1.1\%$ for the NDL and TX laboratories, respectively. FFM was calculated as the difference between body weight and the analyzed value for total body fat.

**Table 3.2.** Characteristics of piglet body composition and TOBEC measurements.

| | NDL | TX |
|---|---|---|
| *n* | 13 | 26 |
| Length ($L_{con}$) (cm) [1] | 37.8 ± 10.6 [2] (22.0-53.0) | 37.2 ± 4.3 (30.5-47.1) |
| Body weight (kg) | 4.45 ± 3.00 (1.03-10.10) | 3.02 ± 1.00 (1.87-5.54) |
| Fat-free mass (FFM) (kg) | 3.78 ± 2.45 (0.94-7.68) | 2.62 ± 0.84 (1.61-4.73) |
| Fat (% body weight) | 14.2 ± 5.0 (7.0-24.0) | 12.8 ± 3.7 (6.6-19.9) |
| $E\#_{cor}$ [3] | 606 ± 536 (47-1606) | 283 ± 154 (120-733) |
| FFM /$L_{con}$ (g/cm) | 90 ± 40 (41-145) | 67 ± 14 (49-103) |
| Totalwater (% FFM) | 77.3 ± 2.8 [4] (74.4-84.3) | 79.3 ± 1.1 (76.9-81.8) |

1) Conductive length, i.e. rump to lateral canthus of the eye, with the pig lying in a lateral recumbent position on the instrument carrier.
2) Values are mean ± 1 SD; ranges are given in parentheses.
3) A mean of 10 readings (made in the 'peak mode') was obtained per piglet and the empty subject carrier reading subtracted. The resulting value was adjusted by a factor that corrected for the deviation of the net phantom reading obtained on the same day from the manufacturer's specified value.
4) Value for dehydrated piglet, 71.2%, omitted.

### *3.3.3 Statistics*

All statistics were carried out using Minitab statistical software (Minitab Inc., State College, PA). Regression analysis techniques were used to derive calibration equations; dummy variables were used to categorize the equations in the comparison procedures. Only values of $p < 0.05$ were considered statistically significant.

## 3.4 RESULTS

### *3.4.1. HP-2 instrument characteristics.*

The sources of instrument variability that influence the precision and accuracy of TOBEC measurements are shown in **Table 3.1.** The measured mean E# for the phantoms were within 0.3% of the reference value. The day-to-day variability was the SD of mean phantom readings for the 18- to 24-mo period over which data were collected and in all cases was <1% of the mean value. Day-to-day variability (±0.7%) was almost 4-fold

**Table 3.3.** Coefficients for linear regression of FFM versus $\sqrt{[E\#_{cor} \cdot L_{con}]}$ derived from measurements on infant miniature pigs.

| Instrument [1] (laboratory) | *n* | Intercepts (1 SD) (kg) | Slope (1 SD) | SEE (kg) | $r^2$ | Equation |
|---|---|---|---|---|---|---|
| HP-2 (NDL) [2] | 13 | -0.0361 (0.0754) | 0.0269 (0.0005) | 0.143 | 99.7 | 1 |
| HP-2 (NDL) [3] | 12 | -0.0047 (0.0441) | 0.0264 (0.0003) | 0.0082 | 99.9 | 2 |
| HP-1 (TX) [4] | 26 | 0.0261 (0.0506) | 0.0258 (0.0005) | 0.078 | 99.1 | 3 |
| HP-2 [5] + HP-1 | 38 | -0.0213 (0.0274) | 0.0264 (0.0002) | 0.077 | 99.7 | 4 |

[1] Instrument model on which original TOBEC measurements were made.
[2] Regression based on all data points from NDL laboratory.
[3] Regression omitting one point with standard residual of 2.8
[4] Calibration equation currently provided by instrument manufacturer; the units for the HP-1 E# have been converted to HP-2 units.

higher than the within-measurement (+0.18%) variability for the MIC instrument and was more than could be accounted for by within-measurement variability alone. The within-measurement variability was $\pm$0.5% for the NDL instrument and $\pm$0.2% for TX HP-2 instruments. The day-to-day was not different from the within measurement variability for the NDL and TX HP-2 instruments. The average SD for the piglet (NDL) measurements was 7.8 $\pm$ 3.9 E#, a value that was almost identical with that for the phantom measurements and that was not influenced by the absolute value of E#.

The magnetic field profiles for the three instruments (**Figure 3.1**) were generally similar in form, but quantitative differences were discerned at the two ends of the measurement chamber. These differences were largely in portions of the magnetic field that are outside of that part of the coil where subjects are positioned for measurement. A quantitative measure of the between-instrument differences in magnetic field profiles is given by the tube phantom measurements obtained with the phantom placed at the two ends of the subject carrier relative to the reading in the middle (Fig. 1). At the proximal end, the signal generated was similar for all instruments, and on average was 2.2% less than the value obtained in the center. At the distal end, however, the NDL and MIC instruments differed by approximately 5%, which was anticipated in view of the relative difference in magnetic field strength at the distal ends of the measurement chambers.

*3.4.2. Relationship between* $\sqrt{[E\#_{cor} \cdot L_{con}]}$ *and piglet FFM.*

The characteristics of the two sets of piglets analyzed by the NDL and TX laboratories are summarized in **Table 3.2.** The chemical compositions were similar for animals of similar ages. The exception was the piglet that had refused to eat. The total body water (72.2% FFM) of this animal was substantially less than that of a littermate (84.3%), which, in turn, was appropriate for its age (7 d old) (9); this suggests that the fasted piglet was substantially dehydrated. The dehydration, however, did not adversely influence the TOBEC-FFM relationship, and thus the measurements of this piglet have been included in the analyses.

**Table 3.4.** Comparison of FFM of piglets determined by chemical analysis with values predicted from TOBEC measurements.

| Method | FFM (kg) | Δ [1] (kg) | Δ [2] (%) |
|---|---|---|---|
| *NDL piglets (n=12)* | | | |
| Chemical analysis | 3.521 ± 2.362 (0.938-7.675) | | |
| TOBEC [3] | | | |
| Equation 3 | 3.478 ± 2.325 (0.842-7.592) | 0.043 ± 0.086 (-0.064-0.213) | 1.5 ± 3.8 (-5.8-10.2) |
| Equation 4 | 3.504 ± 2.361 (0.828-7.681) | 0.017 ± 0.079 (-0.084-0.158) | 1.4 ± 4.2 (-5.0-11.8) |
| *TX piglets (n=26)* | | | |
| Chemical analysis | 2.619 ± 0.841 (1.609-4.732) | | |
| TOBEC [3] | | | |
| Equation 2 | 2.646 ± 0.850 (1.651-4.832) | -0.027 ± 0.075 (-0.145-0.136) | -1.1 ± 3.4 (-8.2-6.8) |
| Equation 4 | 2.630 ± 0.850 (1.641-4.815) | -0.011 ± 0.075 (-0.129-0.152) | -0.4 ± 3.4 (-7.3-7.7) |

Values are mean ± 1 SD; ranges are shown in parentheses.
[1] Measured FFM - predicted FFM.
[2] [(Measured FFM - predicted FFM) / measured FFM] x 100.
[3] Equations used to predict FFM are described in Table 3.3.

The ratio between FFM and length provides an index of the geometry of the FFM (**Table 3.2**). FFM increased logarithmically with length (NDL: r = 0.99; TX: r = 0.96), and the slope of the relationship did not differ significantly between the two groups of piglets. The apparent difference suggested by the mean values in **Table 3.2** therefore reflected the different range of sizes studied by the two laboratories rather than differences in geometry of the piglets.

The relationship between FFM and $\sqrt{[E\#_{cor} \cdot L_{con}]}$ (**Table 3.3**) was linear for both TX HP-1 and NDL HP-2 instruments. The use of polynomial equations with the inclusion of higher power functions did not improve the fit significantly (significance of higher order power functions, $p > 0.18$; $\Delta$ SEE = 0.001 kg). The SEE of equation 1 was markedly larger than that of equation 3. Closer examination of the NDL data revealed an outlier with a standard residual of 2.8. Omission of the data from this animal reduced the SEE of the NDL equation by 40% (equation 2). The data for this animal were not included subsequently.

Neither the intercepts nor the slopes ($p = 0.285$ and 0.493, respectively) of equations 2 and 3 (**Table 3.3**) were significantly different from each other. There were no differences between equation 2 and 3 in the variability of the residuals about the regression line, nor was there any bias in the distribution of the residuals: the mean values (0.019 $\pm$ 0.079 kg

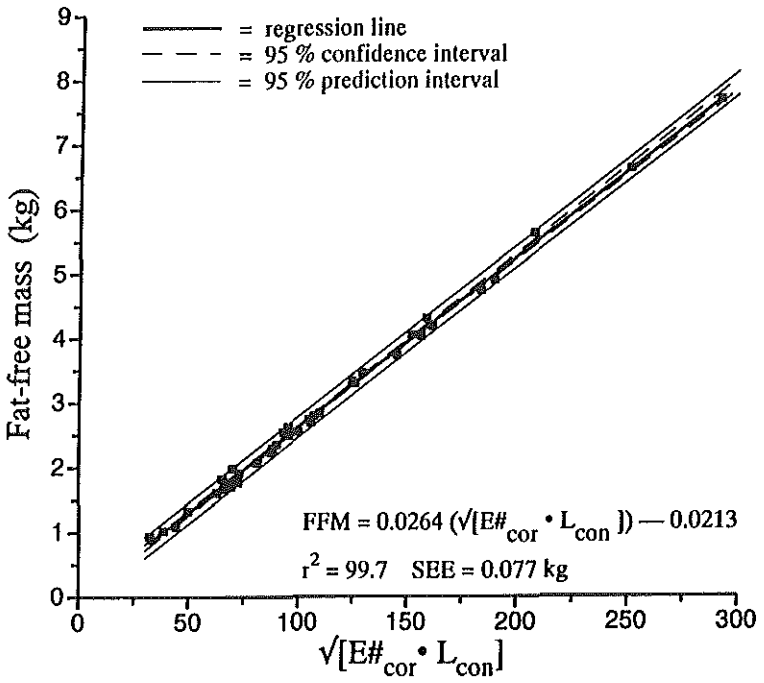


**Figure 3.2.** Regression line (equation 4, Table 3.3) of FFM determined by chemical analysis *versus* the √[E#cor · Lcon], showing the 95% confidence and prediction intervals values for individual piglets.

for equation 2 and -0.009 ± 0.075 kg for equation 3) were not significantly different from each other or from 0. Thus, the data sets were homogeneous. Equation 4 was therefore derived from the pooled data. We also assessed the level of agreement of equations 2 and 3 (Table 3) by using the equation derived on one instrument to predict the FFM from the TOBEC measurements obtained on the other. The difference between predicted and measured estimates of FFM are summarized in **Table 3.4**. Absolute differences between predicted and measured values were as large as 0.213 kg for the NDL piglets and 0.145 kg for the TX piglets. In neither case was the average significantly different from 0. For both sets of piglets, the percent error was randomly and equally distributed around 0; it was greatest for the smallest piglets and decreased nonlinearly as absolute FFM increased. For either group, the prediction using equation 4 (**Table 3.3**) was better than that using the equations derived from the alternate instrument. The difference between measured and predicted values was less than 5% for all values of FFM of more than 2.0 kg. **Figure 3.2** shows the relationship between FFM and √[E#cor · Lcon] for the pooled data set (equation 4; **Table 3.3**), together with the 95% confidence interval for the regression and the 95%

prediction interval for an individual observation. The residuals from the regression of $\sqrt{[E\#_{cor} \cdot L_{con}]}$ against FFM were calculated and then used to determine the extent to which variability in $\sqrt{[E\#_{cor} \cdot L_{con}]}$ was attributable to factors other than FFM; any such factors would increase the uncertainty in the prediction of FFM. The residuals were regressed against those factors which we identified as possibly contributing to variation in the E# on the basis of theoretical considerations, i.e. fat (absolute or as % body weight), body geometry (Lcon, chest circumference, weight/Lcon, chest circumference/ $L_{con}$ weight/$L_{con}2$, chest circumference/$L_{con}2$), or degree of maturity (age, hydration of the FFM). None of these variables made any significant contribution to the variability in the $\sqrt{[E\#_{cor} \cdot L_{con}]}$.

## 3.5 DISCUSSION

### *3.5.1. HP-2 instrument performance.*

Our first objective was to characterize the accuracy and precision of the instruments that are now commercially available for measuring the TOBEC of pediatric subjects. The within-measurement variability (precision) defines the inherent minimum uncertainty for a TOBEC measurement on a given instrument. All three HP-2 instruments were very precise, and the value for the within measurement variability was constant for each instrument. The measurement variability for the NDL instrument, however, was almost 2-fold higher compared with the MIC and TX HP-2 instruments. The greater precision of the MIC and TX HP-2 instruments was likely attributable to instrument and environmental factors. Indeed, we identified retrospectively the presence of an electrical motor in a position coaxial with the NDL HP-2 measuring chamber. Interference caused by the magnetic field generated by the motor would increase instrument noise, *i.e.* the within-measurement variability. The precision of the E# for piglets indicated that the uncertainty associated with the measurement of a live subject was no greater than for the phantom measurements. The practical consequence of the within-measurement variability for the estimation of FFM depends on the size of the subject. For example, for a 1-kg piglet an error of $\pm$9 E# (NDL) *versus* +4 E# (TX HP-2 and MIC) represents an uncertainty of $\pm$0.078 kg FFM *versus* $\pm$0.035 kg FFM, but for a 10-kg pig, an error of 9 E# translates into $\pm$0.022 kg FFM. Thus, control of environmental conditions to minimize the within-measurement variability is important, especially when measuring subjects with a small conductive mass.

The low day-to-day variability in the phantom readings established the excellent degree of constancy of the instruments over time. Such long-term stability ensures that measurements of FFM made at different times can be compared with each other and assumed to be of equal accuracy and not influenced by differences in instrument performance. Under ideal circumstances, the variations in the TOBEC value of an inanimate phantom over time will reflect the within measurement variability, and, indeed, this was found for the TX and NDL HP-2 instruments. The higher value for the MIC HP-2 was atypical, and in retrospect

we noted that a significant increase (50-80 E#) in the phantom readings for several months after relocation of the instrument was responsible. Such long-term drift introduces bias and therefore compromises the accuracy of the FFM prediction. This observation underscores the necessity to document phantom calibrations and to ensure that they remain within a specified range.

*3.5.2. Magnetic field characteristics.*

The TOBEC value of a conductive object is a function of both its conductive mass and the strength of the magnetic field within which it is placed. Thus, two subjects with identical FFM, but measured on different instruments, will have equivalent TOBEC values only if the magnetic fields of the two instruments also are identical. Although instruments are cross-calibrated by the manufacturer, this exercise is only performed in the center of the field. As can be seen from the field plots, this does not ensure that the instruments are equivalent over the whole measuring range. The practical consequence of discrepancies in magnetic field characteristics, such as those observed for the MIC instrument, would be the overestimation of FFM for a subject that extended into the distal end of the measurement chamber. Ideally, when magnetic field characteristics differ from those of the NDL or TX HP-2 instruments, and the user does not have the option of deriving their own calibration, the instrument should be adjusted to bring the magnetic field profile into an acceptable range over the full measuring range. A practical solution is to place subjects within the homogeneous sections of the magnetic field.

*3.5.3. Calibration equation.*

A calibration equation is required to derive FFM from a TOBEC measurement. The primary measurements used to derive this equation were obtained on a prototype HP-1 TOBEC instrument, and its validity for the HP-2 TOBEC instrument previously had not been assessed. Our data show that the equation derived on the HP-2 was very similar to that derived on the TX HP-1 instruments and thereby indicate that the modifications to the design of the prototype HP-1 instrument had no tangible effects on the TOBEC measurements, other than the change in measurement unit. The similarity also gives confidence that differences between laboratories in the chemical analysis procedures, the E# determinations, and the geometry and composition of piglets of different strains were of little practical consequence. This conclusion was strengthened by the analysis of the residuals of the equations, which showed that factors related to geometry, composition, and maturity did not contribute to variation in E# to a greater extent than could be accounted for by FFM alone. The absence of an effect of dehydration on the relationship between FFM and $\sqrt{[E\#_{cor} \cdot L_{con}]}$ extended our previous observations (8) that isotonic variations in the hydration of the FFM do not compromise the accuracy of TOBEC-derived estimates of FFM.

The lack of improvement in the prediction of FFM with the addition of geometry variables contrasted with our previous finding, using an EMME M60 instrument, that the

addition of a term to describe body geometry (weight/$L_{con}^2$) significantly improved the prediction (7). The difference is probably attributable to the improvement in the magnetic field characteristics and the elimination of the electrical field contribution to the measurement. The latter has minimized the contribution of the more geometry-sensitive dielectric component of the measurement (4).

The effect of the difference between equation 3 (**Table 3.3**), the equation currently used for all HP-2 instruments, and the new equation proposed (equation 4; **Table 3.3**) on the estimation of FFM varies according to the size of the subject. The effect is minimal for small subjects, e.g. a subject who weighs 2.8 kg has an $L_{con}$ of 37.9 cm and a net E# of 283, equation 3 yields a FFM of 2.70 kg, whereas equation 4 yields a value of 2.71 kg. Equation 4 gives a slightly higher estimate of FFM for larger subjects: for a 9.5-kg subject with an $L_{con}$ of 59.3 cm and a net E# of 1120, the estimates of FFM are 6.67 kg (equation 3) and 6.78 kg (equation 4). This represents only a 1.6% increase in the estimate of FFM but a 3.9% decrease in the estimate of fat. Great effort was spent to ensure that the TOBEC measuring procedure was similar between the two laboratories. Thus, the proposed equation 4 and the associated errors in estimates of FFM strictly apply to animals that are anesthetized and lying on their sides. E# are obtained in the "peak" mode. As discussed previously (5), if an investigator chooses to use equation 4 to interpret TOBEC measurements made on human infants, a similar measuring procedure should be followed. Infants should be swaddled to ensure that they are motionless, fully extended, and measured on their backs, thereby mimicking the position and geometry of the piglets as placed in the instrument. Additional factors that could influence the accuracy and precision of measurements in human subjects have been addressed previously (5).

*3.5.5. Precision of FFM estimates.*

The uncertainty that should be anticipated in an estimate of FFM is dictated by the SEE. For an individual measurement of FFM, the uncertainty (reflected by the 95% prediction intervals, Fig. 2) will be on average 2 SEE, or 0.154 kg of FFM. This is a fixed value and becomes $\pm 5\%$ or less above approximately 2.80 kg FFM. The magnitude of the uncertainty for individual measurements is one reason to emphasize that for small subjects the technique is more useful for assessing the average body composition of groups of individuals. Even for repeated measurements on the same individual, the uncertainty is dictated by the instrument precision, which, as discussed previously, could be significant for subjects with a small FFM. The uncertainty in the estimate of FFM of a mean value for a group of individuals is measured by the 95% confidence intervals. These varied from $\pm 0.041$ kg FFM ($\pm 4\%$) for a FFM of 1.0 kg to $\pm 0.085$ kg FFM ($\pm 1\%$) for an average FFM of 7.7 kg.

*3.5.6. Application of calibration equation to interpretation of measurements in human infants.*

On the basis of our data set, the proposed calibration equation can be used to interpret

measurements from piglets with FFM at least within the 0.94 to 7.71 kg range. Its usefulness at the lower end of the range is limited for individual predictions by the precision of the instrument. Data on body composition determined from TOBEC measurement of human infants whose body weights range from 2.8 kg and up (10-14) are entirely consistent with body composition determined by chemical analysis (15-17), and reference data (18). Data on human infants from all three laboratories (19) (our unpublished observations), however, have indicated that the equation is inappropriate for infants less than 2.8 kg, in as far as the derived values of FFM were often greater than body weights. The exact cause of the discrepancy between piglets and human infants is not clear, and therefore it is difficult to give a set of parameters with precise limits outside which the calibration equation is no longer valid. Various factors could be responsible for the discrepancy between piglets and the very small human infant, including differences in the shape or density of their FFM and the exact nature of their conductive length (7, 10). Although there is no indication of nonlinearity to preclude extrapolation of the equation beyond 10 kg, there are no published data for infants of this size that would allow us to assess the validity of other assumptions inherent in the use of the proposed calibration equation. Strictly speaking, therefore, the use of equation 4 to interpret TOBEC measurements of human infants should be limited to infants between 2.8 and 10.0 kg. Calibration equation 4 (**Table 3.3**) thus is applicable to TOBEC measurements made in full-term infants from birth to 12 mo of age, at least.

Nevertheless, the HP-2 instruments are sufficiently sensitive to measure groups of smaller infants provided some appropriate, new calibration method can be devised.

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*Chapter 4*

# Measurement of Fat-free Mass by TOBEC and Isotope Dilution [a)]

## 4.1 Summary

Body composition data are important for adequate monitoring of growth and nutritional status in infants. Isotope dilution techniques ($ID_{18\text{-}O}$) are widely used to estimate total body water (TBW) and calculate fat-free mass (FFM). A problem of isotope dilution is an underestimation of TBW by the extrapolation to t=0 approach and an overestimation of TBW by the plateau approach. Using total body electrical conductivity (TOBEC) as the reference technique we validated the extrapolation approach by 149 measurements (boys, n=76; girls, n=73) in 50 healthy infants aged 1-12 months. TOBEC-derived FFM and fat mass were in excellent agreement with Fomon's reference data. Strictly linear relationships with slopes not significantly different from one were found between FFM estimated by TOBEC ($FFM_{TOBEC}$) and FFM estimated by $ID_{18\text{-}O}$ ($FFM_{18\text{-}O}$) (r=0.98 and residual SD=0.29 for boys, r=0.98 and residual SD=0.32 for girls). $FFM_{18\text{-}O}$ was slightly but significantly lower than $FFM_{TOBEC}$, the difference being on average 0.18 (±0.24) kg for girls and 0.08 (±0.21) kg for boys (i.e. respectively 4 (±4.5)% ($p<0.0001$) and 1.5 (±3.9)% ($p=0.004$) of $FFM_{TOBEC}$). We conclude that $ID_{18\text{-}O}$ using the extrapolation to t=0 approach is suitable for TBW and FFM estimations in groups of infants. Due to the considerable measurement error of $ID_{18\text{-}O}$ (estimated at ~6%), individual $TBW_{18\text{-}O}$ and $FFM_{18\text{-}O}$ estimates should be considered with some caution.

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[a)] This chapter has been published before as: **De Bruin NC, Westerterp KR, Degenhart HJ, Visser HKA.** Measurement of fat-free mass in infants. *Pediatr Res* 1995; 38:411-417. The manuscript was dedicated by the authors to Prof. Dr. H.K.A. Visser in honor of his retirement.

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## 4.2 INTRODUCTION

Body composition data are important for adequate monitoring of nutritional status and quality of growth, especially for preterm and young infants. However, for infants no "gold standard" body composition method exists and a limited number of data on infant body composition has been published. Traditional body composition methods used in infancy are anthropometry [1-4] and isotope dilution [5, 6]. These methods are easy to perform and suitable for bedside and field studies. Although they have been extensively validated in adults and older children, the lack of a good reference method prohibited accurate validation in infants.

Recently measurement of total body electrical conductivity (TOBEC) has emerged as an accurate, precise and reproducible method for the estimation of FFM and total body fat (TBF) in infants [4, 7-11]. Calibration against carcass analysis data of minipigs as well as assessment of precision of TOBEC has been performed [12]. The validity of the minipig calibration equation for use in human infants has been proven in two ways. First, all reported TOBEC data for TBF of full-term infants throughout the first year of life [4, 7, 13] are in excellent agreement with reference data on TBF, which had been calculated from the combination of total body water (TBW), total body potassium and skinfold thickness measurements [14]. Second, Fiorotto [8] showed that when the changes in the actual amount of TBF present in intrauterine life (measured by carcass analysis of human fetuses) and extrauterine life (measured by TOBEC during the first 4 months of life) are plotted against age, the lines of the extra- and intrauterine period nicely coincide (with equal slopes) around the time of birth. Physiologic changes in hydration of the FFM during the process of FFM maturation in early life are accounted for by the calibration procedure [15]. The method has been found to be resistant to changes in extracellular fluid volume [16], so physiological changes in FFM hydration (*i.e.* water content of the FFM) will not seriously affect TOBEC outcome [10]. A TOBEC measurement is rapid, safe and easy to perform, and suitable for measurement of large numbers of infants. The instrument is commercially available since 1989. At present TOBEC is a one of the most reliable methods to estimate infant body composition, but is not widely used, due to the relatively high price of a TOBEC instrument ($ 45,000.-), and the fact that the instrument is large, difficult to move and therefore not suitable for field studies. However, its good reproducibility, precision and accuracy justifies the use of TOBEC as a reference method for *e.g.* cross-validation studies against anthropometry and isotope dilution. Based on this fact we recently described a cross-validation against TOBEC of two known anthropometric methods for TBF and FFM estimation in infants [4].

It is known that isotope dilution either underestimates "true" TBW when calculated by the extrapolation to $t=0$ or overestimates TBW when calculated by the plateau approach [17]. This is due, respectively, to the fact that the assumption of instantaneous mixing of the label is not valid (extrapolation approach) and that only urinary loss of label in the

equilibration phase can be accounted for (plateau approach). Because the exact magnitude of these errors is unknown, we cross-validated the extrapolation approach (based on two urine samples) against the TOBEC technique. The extrapolation approach is convenient for body composition studies and is often used in combination with energy expenditure studies using doubly labeled water. We assessed whether TOBEC and isotope dilution were strictly linearly related throughout the entire first year of life. Accuracy and precision, compared with TOBEC, of the isotope dilution-derived TBW and FFM estimates were determined, and gender- and age-related differences between methods were explored.

## 4.3 METHODS

### *4.3.1. Subjects.*

The present study was part of a prospective study on growth, body composition and energy metabolism of breast-fed and formula-fed infants. Fifty infants were enrolled after written informed consent was obtained from their parents. All were healthy full-term Caucasian infants from healthy mothers and vaginally born without complications. Measurements were performed at the age of 1, 2, 4, 8, and 12 months. Isotope dilution measurements (n=175) were performed as part of an (doubly-labeled water) energy expenditure experiment. The study protocol was approved by the ethical review board of the Medical Faculty and University Hospital of the Erasmus University Rotterdam.

### *4.3.2. Anthropometry.*

Infants were weighed naked on an electronic baby scale (Instru Vaaka Oy, Finland) to the nearest 1 g (0-3 kg body weight), 2 g (3-6 kg) or 5 g (6-10 kg) at the time of the TOBEC measurement and at the end of the isotope dilution period (d 9). Recumbent length and head circumference were measured according to Lohman *et al.* [18].

### *4.3.3. TOBEC.*

Body temperature affects TOBEC outcome [10], therefore no infants with apparent or anamnestic fever were measured. Infants were not fed for at least 2 h preceding the measurement. To prevent cooling and to ensure geometric homogeneity between infants with respect to the introduction of the conductive mass into the electromagnetic field, infants were undressed and carefully swaddled in a large blanket, while care was taken that limbs were not flexed and did not touch each other or the trunk. Infants were placed on their back on the sled of the instrument. A pacifier was allowed when necessary. One TOBEC reading took approximately 10 s. A complete TOBEC measurement consisted of 10 reliable 10-s readings which were averaged for calculation of $FFM_{TOBEC}$. When the infant had urinated, it was swaddled again in a dry blanket and remeasured. Movement or crying during a reading was also a reason for remeasuring the infant.

Instrument specification and safety, measurement procedure and FFM calculation

from raw TOBEC data have been described earlier [4, 10, 19]. TBF and FFM were calculated from raw TOBEC data using a (theoretically deduced) transformed TOBEC value (*T#*) [15]. The calibration equation relating $FFM_{TOBEC}$ to *T#*, derived from minipig data and described in detail before [12], was: $FFM_{TOBEC} = 0.0264 \cdot T\# - 0.0213$. Precision for an individual measurement was 0.154 kg of FFM (*i.e.* the 95% prediction interval of the minipig-calibration curve), which is consistent with an uncertainty in the FFM estimate of less than 5% in infants with an above ~3-kg FFM [12]. Intra-measurement variation was < 0.5% and long-term instrument drift, measured over a 2-y period, was 0.5% as measured with a cylindrical reference phantom with known conductivity index and supplied by the manufacturer [12].

*4.3.4. Isotope dilution.*

Directly after the TOBEC measurement, TBW was determined by standard isotope dilution techniques using 2 ml/kg body weight of water enriched with 5% $^2H$ and 10% $^{18}O$. After collecting a baseline urine sample with a disposable adhesive collection bag, the $^2H_2{}^{18}O$ solution was administered orally by means of a bottle with a known amount of formula or dextrose added to the mixture. In a few infants some fluid was spoiled, which was collected, weighed and subtracted from the dose. Bottles were rinsed with ~20 mL of formula or dextrose, which was also consumed by the infant. A postdose urine sample was collected after at least 5 h and two urine voids. A second urine sample was collected at d 9. Samples were collected with new disposable collection bags at home by the mother. Urine samples were transferred to glass jars immediately after collection and stored at -25°C. Exact times of the urine collections were noted by the mother. No further intake or excretion of water/label between time of dosing and first postdose urine sampling was recorded. Infants were not weighed at the time of the postdose urine sampling. The time zero intercept approach, based on more than one postdose data point per individual, allows for continuous intake and excretion of water. Because this model assumes instant mixing of label in the body water pool, which is obviously untrue, the only problem is the fluid intake during the process of mixing of label with the body water pool. Based on plasma-isotope-data from Trowbridge *et al.* [5] and Whyte *et al.* [20], we assumed for infants this would not exceed 1 h. The normal feeding pattern of the infants therefore was allowed to be continued 1 h after dosing. Stabilizing of urinary tracer output has been shown to lag behind plasma equilibration for at least 2-3 hours [5], so start of postdose urine sampling was kept at >5 h postdose.

Isotope analyses of the initial $^2H_2{}^{18}O$ solutions and urine samples were performed in duplicate using an isotope ratio mass spectrometer (Aqua-SIRA, VG Isogas, Cheshire, UK) as described earlier [21]. Briefly, 5-$\mu$l urine samples were introduced into the heated inlet system of the mass spectrometer with an autoinjector. After evaporation the water vapor flows directly to one analyzer for $^{18}O$ measurement and through a uranium furnace into a second analyzer for $^2H$ measurement after conversion to $^2H_2$ and $H_2$. The analytical precision was 0.2 ppm for $^2H$ and 0.4 ppm for $^{18}O$.

**Table 4.1.** Subject characteristics.

| Age (mo) | n | Age (d) | Body Weight | | Length (cm) | Head Circumference (cm) |
|---|---|---|---|---|---|---|
| | | | d 1 (kg) | d 8 (kg) | | |
| **Boys** | | | | | | |
| 1 | 20 | 36±3 | 4.56±0.46 [b] | 4.89±0.52 [b] | 55.9±2.3 | 38.1±1.0 [a] |
| 2 | 19 | 66±5 | 5.49±0.57 [a] | 5.96±1.25 [a] | 60.0±2.3 [a] | 39.7±1.1 [a] |
| 4 | 18 | 124±4 | 6.66±0.83 | 6.80±0.80 | 65.5±2.0 [b] | 41.7±1.1 [b] |
| 8 | 14 | 244±9 | 8.46±0.36 | 8.47±0.80 | 71.7±2.4 | 44.6±1.1 |
| 12 | 8 | 371±7 | 10.5±0.63 [a] | 10.57±0.61 [a] | 78.9±2.0 [a] | 46.7±1.3 |
| **Girls** | | | | | | |
| 1 | 18 | 33±4 | 4.19±0.42 | 4.42±0.42 | 55.1±2.3 | 37.1±1.2 |
| 2 | 16 | 65±5 | 4.85±0.48 | 5.13±0.50 | 58.0±1.7 | 38.6±1.0 |
| 4 | 18 | 123±7 | 6.13±0.66 | 6.30±0.67 | 63.2±2.3 | 40.9±1.1 |
| 8 | 13 | 248±10 | 8.19±0.74 | 8.35±0.74 | 70.8±2.6 | 44.1±1.1 |
| 12 | 9 | 369±11 | 9.49±0.60 | 9.47±0.65 | 75.6±2.0 | 45.9±1.0 |

Mean ± SD
Difference between sexes (Mann-Whitney *U* Test): [a] $p < 0.01$ [b] $0.01 < p < 0.05$

Urinary tracer concentrations were corrected for additional isotope dilution caused by change of the body water compartment during the 8 d of the experiment, as well as for the timing error of each urine sample caused by mixing of urine with decreasing concentrations of label in the bladder between two subsequent voids. The study protocol did not account for timing of the previous void (*i.e.* the void before the actual urine collection), therefore only a first order correction could be applied for this phenomenon. Because an average voiding interval of 2 h as observed in neonates is reasonable as a maximal frequency throughout the first year of life, 1 h was subtracted from the time of collection of each urine sample.

$^{2}H$ pool size ($N_H$) and $^{18}O$ pool size ($N_O$) were calculated by extrapolation to t=0 [22]. As both isotopes were administered concomitantly, the ratio $N_H$ / $N_O$ is very narrowly defined and was used as a measure for the reliability of the urine sample. Data were excluded when the $N_H/N_O$ ratio was beyond 3 SD from the mean $N_H/N_O$. This ratio is normally distributed (results not shown), resulting in a loss of $<1\%$ of normal data that will be rejected. Nineteen data points were excluded on this ground. An additional seven measurements were excluded on the basis of the fact that not all spoiled tracer could be collected (six cases) and of unclear notation of urine collection times (1 case). $TBW_{18\text{-}O}$ was calculated as $N_O$ / 1.01, where 1.01 is a correction for rapidly exchangeable nonaqueous oxygen [23].

**Table 4.2** TOBEC and isotope dilution results.

| Age (mo) | *n* | $TBF_{TOBEC}$ (kg) | $TBF_{TOBEC}$ (%) | $FFM_{TOBEC}$ (kg) | $TBW_{18\text{-}O}$ (%) | $TBW_{18\text{-}O}$ (kg) | $FFM_{18\text{-}O}$ (kg) | $N_H/N_O$ ratio |
|---|---|---|---|---|---|---|---|---|
| **Boys** | | | | | | | | |
| 1 | 20 | 0.69±0.22 | 15.0±3.9 | 3.87±0.35 | 66.7±3.4 | 3.04±0.30 [c] | 3.78±0.38 | 1.025±0.005 |
| 2 | 19 | 1.09±0.23 | 19.7±3.6 | 4.40±0.38 [b] | 62.9±3.0 | 3.43±0.31 [a] | 4.32±0.39 [a] * | 1.028±0.007 |
| 4 | 18 | 1.65±0.43 | 22.4±4.0 | 5.01±0.44 [b] | 59.7±3.9 | 3.99±0.28 [b] | 4.97±0.36 [b] | 1.030±0.006 |
| 8 | 14 | 2.25±0.26 | 26.6±2.4 | 6.21±0.26 | 58.3±2.3 [b] | 4.93±0.26 [b] | 6.19±0.33 [b] | 1.028±0.006 |
| 12 | 8 | 2.56±0.44 | 24.3±3.1 | 7.96±0.36 [b] | 58.7±4.0 | 6.15±0.19 [b] | 7.80±0.23 [b] | 1.030±0.008 |
| Mean±SD | 76 | 1.47±0.73 | 21.2±5.5 | 5.08±1.32 [c] | 61.9±4.6 [b] | 3.99±1.02 [b] | 5.00±1.31 [b] | 1.028±0.006 |
| **Girls** | | | | | | | | |
| 1 | 18 | 0.59±0.12 | 14.1±2.3 | 3.60±0.36 | 66.4±3.1 | 2.78±0.30 | 3.46±0.37 ** | 1.028±0.004 |
| 2 | 16 | 0.97±0.22 | 19.8±3.5 | 3.88±0.33 | 60.5±3.3 | 2.93±0.26 | 3.68±0.33 *** | 1.028±0.004 |
| 4 | 18 | 1.58±0.32 | 25.6±3.5 | 4.55±0.44 | 58.1±4.5 | 3.55±0.42 | 4.44±0.51 * | 1.029±0.007 |
| 8 | 13 | 2.22±0.31 | 27.1±2.9 | 5.97±0.56 | 55.3±2.7 | 4.50±0.32 | 5.64±0.41 ** | 1.028±0.007 |
| 12 | 9 | 2.56±0.30 | 27.0±2.8 | 6.93±0.52 | 56.2±3.2 | 5.34±0.56 | 6.77±0.71 | 1.030±0.003 |
| Mean±SD | 73 | 1.43±0.75 | 21.9±6.0 | 4.70±1.24 | 59.9±5.4 | 3.60±0.95 | 4.52±1.22 | 1.028±0.006 |
| **All data:** Mean±SD | 149 | 1.45±0.75 | 21.5±5.8 | 4.89±1.29 | 60.9±5.1 | 3.80±1.01 | 4.76±1.28 | 1.028±0.006 |

Mean±SD.
Difference between sexes (Mann-Whitney-U test): [a] $p<0.001$, [b] $0.001<p<0.01$, [c] $0.01<p<0.05$.
Differences between $FFM_{TOBEC}$ and $FFM_{18\text{-}O}$ (Wilcoxon matched-pairs signed-ranks test): ***$p<0.001$, **$0.001<p<0.01$, *$0.01<p<0.05$.

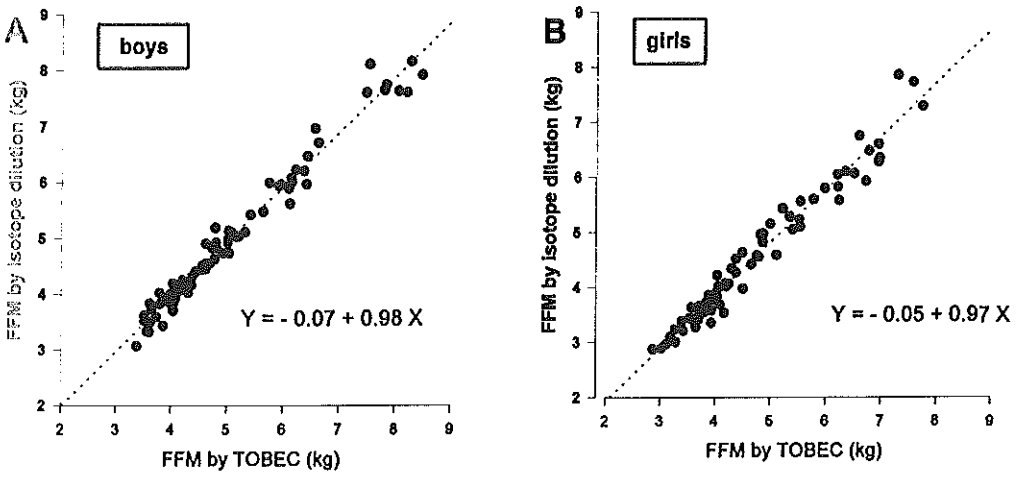


**Figure 4.1.** $FFM_{TOBEC}$ (kg) against $FFM_{18\text{-}O}$ (kg) by gender. Dotted line represents the calculated regression equation. ***A.*** Boys. ***B.*** Girls

In early life the hydration of the FFM compartment rapidly changes with age. To convert TBW into FFM, we used data on changes in FFM hydration in infants by gender published by Fomon *et al.* [14].

*4.3.5. Statistical analysis.*

Determination of linearity and correlation between methods was calculated using the statistical technique to assess linear regression from combined longitudinal data as described by Draper and Smith [24]. Comparison of $FFM_{TOBEC}$ and $FFM_{18\text{-}O}$ was performed by a paired t-test. Estimation of agreement was performed using the method as proposed by Bland and Altman [25]. An effect was assumed to be significant at $p < 0.05$. Unless stated differently data are expressed as mean ($\pm$SD).

## 4.4. RESULTS

*4.4.1. Subject characteristics and body composition.*

Subject characteristics are shown by age and gender in **Table 4.1**. Weight, length and head circumference were in accordance with Dutch reference data [26]. Significant differences between sexes were observed for body weight, length and head circumference.

Body composition estimations by TOBEC are summarized by age and gender in **Table 4.2**. Mean TOBEC background reading was 38 (±4) TOBEC units. Room temperature and relative room humidity at the time of the TOBEC measurements were respectively 22.7 (±0.9) °C and 41.6 (±6.6) %. Intra-measurement uncertainty (coefficient of variation of the consecutive 10-s TOBEC readings) averaged 1.3 % (range 0.1 - 4.4%); only two cases showed a coefficient of variation > 3%, which shows the excellent reproducibility of TOBEC measurements. A significant difference between sexes was observed for $FFM_{TOBEC}$.

Isotope dilution data are summarized in **Table 4.2**. Mean $N_H/N_O$ was 1.028 (SD=0.006; SEM=0.001). TBW calculated from $^{18}O$ dilution ($TBW_{18\text{-}O}$) was on average 5 (±25) mL higher than TBW calculated from $^{2}H$ dilution (not significant) . Significant differences between sexes were observed (see **Table 4.2**) for $FFM_{18\text{-}O}$, $TBW_{18\text{-}O}$ (kg) and $TBW_{18\text{-}O}$ (%).

*4.4.2. Comparison of TOBEC and isotope dilution.*
Regression [24] of $FFM_{TOBEC}$ (Y) against $FFM_{18\text{-}O}$ (X) revealed:

*all data:* $Y = -0.07\ (\pm 0.12) + 0.98\ (\pm 0.02)\ X$
$r = 0.98$; *residual SD* $= 0.29$ *1a*

*boys:* $Y = -0.08\ (\pm 0.15) + 0.99\ (\pm 0.03)\ X$
$r = 0.99$; *residual SD* $= 0.27$ *1b*

*girls:* $Y = -0.05\ (\pm 0.18) + 0.97\ (\pm 0.04)\ X$
$r = 0.98$; *residual SD* $= 0.32$ *1c*

Slopes were not significantly different from one and intercepts were not significantly different from zero.

**Figure 4.1** shows a graphical representation of the relation between $FFM_{TOBEC}$ and $FFM_{18\text{-}O}$ by gender. After correction for the covariable age no significant correlation was found between absolute residual errors of the regression of $FFM_{TOBEC}$ and $FFM_{18\text{-}O}$ and potential confounding parameters as *e.g.* weight, length and $N_H$ / $N_O$ ratio.

In **Figure 4.2** the difference of the values obtained using both methods is plotted against their averaged value. The limits of agreement or 95% confidence limits for an individual estimate [25] is 8-9% for both sexes and not significantly different with age. Although the regression slopes 1a through 1c were not significantly different from one and intercepts not significantly different from zero, paired *t* test showed that TOBEC resulted in significantly higher values of FFM than isotope dilution, both in boys ($p$=0. 0004) and girls ($p < 0.0001$). Calculated separately by age and gender, $FFM_{TOBEC}$ was significantly lower than $FFM_{18\text{-}O}$ in girls for all age groups except at 12 months of age, whereas in boys

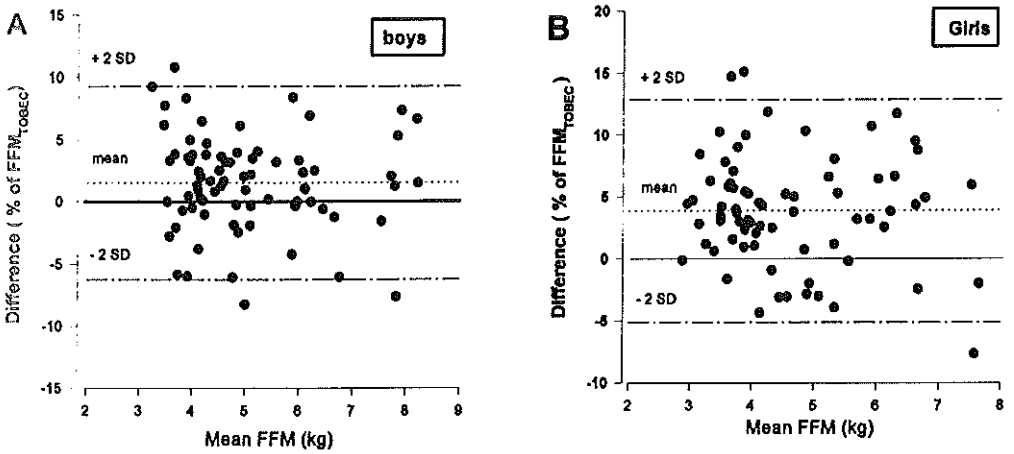


**Figure 4.2.** Difference against mean for $FFM_{TOBEC}$ (kg) and $FFM_{18\text{-}O}$ (kg) by gender. Differences are expressed as % of $FFM_{TOBEC}$. *A. BOYS:* mean difference=1.5%. Limits of agreement (± 2 SD)=-6.3% en 9.3%. SE of limits of agreement=0.77% [24]. *B. GIRLS:* mean difference=3.9%. Limits of agreement (± 2 SD)=-5.1% en 12.9%. SE of limits of agreement=0.91% [24].

only at 2 months of age a significant difference between isotope dilution and TOBEC was found ($p=0.016$) (see also **Table 4.2**). **Table 4.3** shows the differences between $FFM_{TOBEC}$ and $FFM_{18\text{-}O}$ by age and gender (in kg and as percentage of $FFM_{TOBEC}$). On average the difference in FFM between both methods was 0.13 (±0.23) kg (*i.e.* 2.7% of $FFM_{TOBEC}$). Values for the difference between TOBEC and isotope dilution differed significantly by gender ($p=0.004$): on average 0.08 (±0.21) kg for boys and 0.18 (±0.24) kg for girls (which is consistent with respectively 1.5% and 4% of $FFM_{TOBEC}$). For TBW the difference between $TBW_{TOBEC}$ and $TBW_{18\text{-}O}$ averaged 0.06 kg and 0.14 kg (which is also consistent with 1.5% and 4% of $TBW_{TOBEC}$). All differences between isotope dilution and TOBEC as calculated by gender were not significantly related to age.

## 4.5 DISCUSSION

The majority of data on TBW in infants have been derived in the past from $^2H$ dilution by the traditional plateau approach, as formerly used in adults (short equilibration time) and with plasma or urinary $^2H$ concentrations measured by the falling drop method or infrared spectroscopy. More detailed data on the isotope dilution methodology in infants, as *e.g.*

Table 4.3 Differences between $FFM_{TOBEC}$ and $FFM_{18\text{-}O}$ by age and gender.

| ($FFM_{TOBEC}$ - FFM18-O ) | boys | girls |
|---|---|---|
| Difference (kg): | | |
| 1 mo | 0.09±0.18 | 0.13±0.12 |
| 2 mo | 0.08±0.13 | 0.21±0.19 |
| 4 mo | 0.06±0.20 | 0.11±0.22 |
| 8 mo | 0.02±0.21 | 0.32±0.29 |
| 12 mo | 0.16±0.39 | 0.17±0.38 |
| mean (SD) | 0.08±0.21 | 0.18±0.24 |
| Difference (% of $FFM_{TOBEC}$): | | |
| 1 mo | 2.2±4.8 | 3.7±3.3 |
| 2 mo | 1.8±2.9 | 5.2±4.7 |
| 4 mo | 1.1±3.8 | 2.6±4.8 |
| 8 mo | 0.4±3.3 | 5.2±4.5 |
| 12 mo | 1.8±4.9 | 2.5±5.4 |
| mean (SD) | 1.5±3.9 | 3.9±4.5 |

Mean±SD.
Results did not differ significantly between age groups.

plasma and urine equilibrium time in infants, have been published more recently [5, 20]. Until now very few authors reported data on infant TBW using $^{18}O$ dilution, especially for older infants. More TBW and FFM data from isotope dilution will certainly become available in the near future, as the doubly labeled water method used for estimation of energy expenditure has recently been validated for use in infants and TBW and FFM are among the outcome parameters [27-29].

Because body weight , $FM_{TOBEC}$ and $FFM_{TOBEC}$ of the present study were in accordance[b] with reference data [14], our data suggest that $H_2{}^{18}O$ dilution underestimates FFM, at least when using on the extrapolation approach. Underestimation is to be expected, as one of the basic assumptions of the extrapolation approach is instantaneous mixing of the label with the body water pool after dose administration. Mean time of urine sampling in

[b] These authors only presented the mean values per age group, without any indication on biological and instrumental scatter. We assumed a parameter to be "in accordance" when the reference data point was within 1 SD of the present study parameter.

our study was 10.4 (±6.3) h after dosing. Average water loss between time of dosing and first urine sample can be estimated from the elimination rate of $^2H$. Mean elimination rate of $^2H$ ($k_H$) was 0.225 (±0.045), which equals a water loss ~35 mL/h. It takes approximately 5 h before label has completely mixed with the body water pool [5]. During this time water intake, water output and loss of label with water output are not yet in equilibrium, each of which factors can result in errors. During this time on average 175 mL of water are lost with an unknown amount of tracer. This observation shows that water and tracer loss in the early equilibrium phase on its own will account for most if not all of the underestimation of TBW by $^{18}O$ dilution.

As Coward already pointed out [17] the plateau as well as the extrapolation approach rest upon basic assumptions which cause respectively an overestimation and an underestimation of TBW. Our $TBW_{18\text{-}O}$ data were comparable with those of Davies *et al.* [3], who also used the extrapolation approach for isotope dilution. Fjeld *et al.* [6] measured infants between 3 and 30 mo (on average 13 mo) using the plateau approach. They reported mean TBW values for the study groups which were, when back-extrapolated to 12 mo of age, slightly higher then our data. We found one report supplying raw data on both the extrapolation and the plateau approach [1]. The authors measured TBW in 15 infants aged 0-3 mo. Although a small number of infants was measured, a gender-related difference between plateau and extrapolation can be calculated from the raw data, averaging respectively 0.05 (±0.26) kg and 0.14 (±0.15) kg for boys and girls. This difference between plateau and extrapolation almost fully accounts for the discrepancy between TOBEC and isotope dilution found in the present study.

It should be noted that TOBEC measures FFM, which is converted into TBW, while isotope dilution measures TBW, which is converted into FFM. In the present study the same FFM hydration constants as published by Fomon *et al.* [14] have been used for both calculations. The dependence upon gender in the difference between TOBEC and isotope dilution (respectively 1.5% and 4% FFM) is not attributable to these FFM hydration factors, for it was also observed in the raw $TBW_{18\text{-}O}$ data. A possible explanation for this difference between boys and girls could have been a difference in water turnover, however, $k_H$ values were not significantly different between boys and girls. Another explanation for the difference might be a lesser miction frequency in girls. More time between subsequent voids will result in an increased error in the "real" sample time related to the concentration of label in a certain sample. It is not likely that urine collection itself, which is more difficult to perform in girls than in boys, will be the source of the difference between boys and girls, for this would most likely also have affected the $N_H$ / $N_O$ ratio. It is also not likely that gender differences in FFM density and hence in conductivity would have affected the TOBEC signal and so produced an artificial difference in FFM between boys and girls, for it has been reported that small changes in FFM hydration and density do no affect TOBEC outcome [8, 10], whereas we showed that TOBEC body composition data were in excellent agreement with former reports and published reference data. A final explanation might be a difference in feeding mode between both sexes. The study group

existed of infants which were exclusively breast-fed or formula-fed for at least 4 months. It has been postulated formula feeding results in a more pronounced growth of the FFM compartment and formula has a higher renal solute load, which both may result in a difference in labeled water clearance. However, although a significant difference in feeding mode was present (boys: 35% breast-fed and 65% formula-fed; and girls: 56% breast-fed and 44% formula-fed, $p=0.01$ by $\chi^2$ test) the difference between $FFM_{TOBEC}$ and $FFM_{18\text{-}O}$ (as shown in **Figure 4.2a** and **4.2b** and expressed as a percentage of $FFM_{TOBEC}$) was not significantly correlated to feeding mode and not significantly different between feeding mode ($p=0.6$, by analysis of variance), neither for girls nor for boys. The present data therefore do not support the idea that mode of feeding has an effect on the rate of excretion of labeled water.

The present study shows that, besides a significant underestimation of 4% in TBW and FFM in girls and 1.5% in TBW and FFM in boys, isotope dilution results are linearly related to TOBEC and are on average not significantly different from unity. Therefore isotope dilution is suitable for TBW estimations and subsequent calculation of FFM in groups of infants. However, one should exert caution in using isotope dilution data for individual estimations of TBW, due to the relatively large measurement error (maximally 8-9 % for an individual estimate). Also, isotope dilution has no ability to account for biological scatter in FFM hydration, as fixed values for FFM hydration are used to convert TBW into FFM. Differences in FFM hydration will be averaged out when groups of infants are described, but will significantly affect an individual estimate of FFM. TOBEC calibration studies using minipig carcass analysis data show a precision of an individual measurement of ~0.15 kg [12]. The residual SD of the regression of $FFM_{TOBEC}$ and $FFM_{18\text{-}O}$ in the present study on average was 0.23 kg, which suggests that isotope dilution is less precise than TOBEC. One could argue however that the precision of carcass studies cannot be extrapolated to infants. Precision of anthropometry-derived FFM ($FFM_{anthro}$) has been reported to be approximately 0.35 kg [4]. Because anthropometry data were also available in the present study, $FFM_{18\text{-}O}$ was regressed [23] against $FFM_{anthro}$, which revealed a correlation of $r=0.98$ and residual SD=0.40 kg for boys and $r=0.97$ and residual SD =0.43 kg for girls. The increase in residual SD compared with regression against $FFM_{TOBEC}$ confirms the superior precision of TOBEC compared with anthropometry and isotope dilution. Moreover, **Figure 4.2** shows that, although the average difference between methods is small (respectively 1.5% and 4% for boys and girls) the limits of agreement (*i.e.* 2 SD of the difference between methods) are considerable, being approximately 8-9% for both sexes. This error results from both the TOBEC as well as the isotope dilution technique. An approximation of the amount of error arising from the isotope dilution technique can be derived as follows. The limits of agreement (i.e. 2SD) of the difference between $FFM_{TOBEC}$ and $FFM_{18\text{-}O}$ were ~400 g. Assuming that the measurement error of anaesthetized living minipigs approximates that of carefully swaddled infants, the TOBEC technique exerts an error (expressed here as 2SD) of ~150 g. This shows that the isotope dilution technique must exert an additional error in the order of 250

g, which is almost twice as much as the TOBEC technique and will approximate 6%. Beside this, one should be even more cautious when $FFM_{18\text{-}O}$ is used for calculation of fat mass (FM). In a child with e.g. 4 kg FFM and 1 kg FM, an error of ~ 6% in FFM results in an error of ~ 25% in FM.

A potential source of error in the estimation of $^2H$ and $^{18}O$ pool sizes, which to our knowledge has never been accounted for, is the mixing of urine with decreasing concentrations of label in the bladder between two subsequent voids. Inherent to the bladder's function to store *e.g.* hypertonic fluid, urine water is not in direct equilibrium with TBW and thus the bladder cannot be a direct part of the TBW pool. Assuming that urine was collected at $t_2$ (with the previous void at $t_1$), the concentration of tracer in this sample in any calculation of TBW or $CO_2$ production should not be related to $t_2$ but to $\frac{1}{2}(t_1 + t_2)$. To roughly estimate the error due to this phenomenon, we recalculated $TBW_{18\text{-}O}$ while subtracting a fixed time value from the time of collecting the first and the second postdose sample. A subtraction of a minimum of 1 h was based on an assumed average miction frequency of approximately 2 h as found in newborns. This value might well be a valid upper limit of voiding frequency, for all infants were not yet tidy at this time. Subtraction of 1 h through 2.5 h from the second postdose sample at d 9 had no significant effect on $TBW_{18\text{-}O}$ (on average < 2 mL) while the average effect on the first sample was ~45 mL for a 1 h subtraction, 65 mL for 1.5 h , 85 mL for 2 h and 110 mL when subtracting 2.5 h (*i.e.* 5 h between voids) from the sample time. It was not possible to individually correct our data for this phenomenon for no accurate data on time of previous urine voids were available.

Although the literature is not consistent about the value for FFM hydration at birth (values ranging from 80-84% TBW have been reported [30-32]), and hydration factors during the first year of life have only once been estimated indirectly from deuterium dilution by the (obsolete) "falling drop method" and by whole body $^{40}K$ counting [14], these uncertainties do not account for the observed discrepancy between $FFM_{TOBEC}$ and $FFM_{18\text{-}O}$. On average the difference between TOBEC and isotope dilution was 0.13 kg FFM. To account for this difference the FFM hydration at birth should become ~75%, while carcass data show FFM hydration factors of 80-84% [30-32]. This example shows that, although true FFM hydration in infants is not well known, the discrepancy between $FFM_{TOBEC}$ and $FFM_{18\text{-}O}$ cannot be caused by FFM hydration factors.

A significant difference between $FFM_{TOBEC}$ and $FFM_{18\text{-}O}$ was observed, although the regression slopes of these parameters showed no significant difference from unity. This paradox can be explained by the magnitude of the (relatively small) difference (~ 0.13 ($\pm$0.23) kg) between both methods as compared to the large absolute FFM values (~5 kg). A paired t-test compares differences between methods *versus* zero and is sensitive to small changes, while a regression is based upon the values themselves and therefore more robust to small changes. A large scatter therefore causes an initially significant but relatively small difference between methods, as found in the present study, to disappear when the values of these parameters are regressed. This phenomenon also shows that an important feature of

the present study is the disclosure of the random error in the isotope dilution technique and a relatively small systematic difference between methods.

Summarizing, it can be concluded that FFM estimations by TOBEC and isotope dilution, although based on widely divergent principles, are strictly linearly related over the entire first year of life. A small but significant underestimation of $TBW_{TOBEC}$ and $FFM_{TOBEC}$ by isotope dilution was found which averaged 1.5% ($\pm$3.9) for boys and 4% ($\pm$4.5) for girls. Energy expenditure studies using doubly labeled water, as a "byproduct" also allow for TBW and FFM estimations. Because of its moderate precision but relatively good accuracy , the isotope dilution technique is suitable for TBW and FFM estimations in groups but one should exert caution in individual estimates. Isotope dilution is suitable for body composition measurements in *e.g.* bedside and field studies.

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# *Part 3.*

## Are anthropometric methods suitable for assessment of body composition in infants: comparison with total-body electrical conductivity (TOBEC)?

## Construction of reference centiles for body fat and fat-free mass, as measured by total-body electrical conductivity (TOBEC).

*Chapter 5*

# Quantitative Assessment Of Infant Body Fat [a)]

## 5.1 SUMMARY

Measurement of total-body electrical conductivity (TOBEC) has emerged as a rapid, safe and reproducible method for estimation of infant total body fat (TBF). Agreement of two anthropometric methods [by Dauncey et al (1977) and Weststrate et al (1989)] with TOBEC-TBF was assessed in 435 healthy infants aged 21-365 d. Dauncey-TBF correlated with TOBEC-TBF by $r^2 = 0.61$ and exceeded TOBEC TBF by $0.14 \pm 0.25$ kg in infants $< 4$ mo of age. Thereafter, TOBEC-TBF exceeded Dauncey-TBF by $0.20 \pm 0.47$ kg . We modified Dauncey's method, which significantly improved the correlation to $r^2 = 0.75$. Weststrate-TBF correlated with TOBEC-TBF by $r^2 = 0.87$, but exceeded TOBEC-TBF by 0.5 kg. Both methods showed poor agreement with TOBEC-TBF. We conclude that both methods, although suitable for comparison of TBF between groups, cannot be used to accurately assess TBF in an individual infant.

## 5.2 INTRODUCTION

The two-compartment model (which assumes the body to consist of fat and fat-free mass) is still the most widely used model for the study of body composition in infants. It is a suitable model for the clinician, it yields quantitative data on total body fat (TBF) and fat-free mass (FFM), and gives important information on the quality of growth in infants, the nutritional adequacy of their diets, the severity of malnutrition, and the progress of recovery from malnutrition.

Most body composition methods that yield accurate values in adults and children are not applicable to infants. Body composition methods used in infants have to be non-invasive and

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[a)] This chapter has been published before as: **De Bruin NC, Van Velthoven CAM, Stijnen T, Juttmann RE, Degenhart HJ, Visser HKA.** Quantitative assessment of infant body fat by anthropometry and total-body electrical conductivity. *Am J Clin Nutr* 1995; 61:279-86.

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non-radioactive and must not require the active cooperation of the subject. Methods such as bioelectrical impedance (1), dual energy X-ray absorptiometry (2, 3), absorption and desorption of xenon (4), and nuclear magnetic resonance spectroscopy (5), are either inaccurate or still in an experimental phase for use in infants. Near-infrared interactance yields erroneous values for the thickness of the subcutaneous fat layer (6). At present, TBF in infants is measured mainly by means of isotope dilution (with $^{2}H$- or $^{18}O$-enriched water ) and various anthropometric methods. Calculation of FFM from isotope dilution data, however, is complicated by the variable hydration of the FFM (this body compartment rapidly changes in growing infants, from a hydration of ≈ 80% in neonates to 73.2% in children and adults). Recently, measurements of total-body electrical conductivity (TOBEC) have emerged as reproducible, safe and precise estimates of TBF in full-term infants (7-9).

Anthropometry-based methods are widely used to quantify the amount of TBF and FFM. The widespread use of anthropometry is mainly attributable to the fact that it is inexpensive, easy to do, and very convenient for field studies and bedside measurements. Various calculations for the quantitation of TBF from skinfold-thickness (SFT) measurements in adults have been proposed and validated, and summarized by Lohman (10). It is known that quantitative assessment of infant TBF from raw skinfold-thickness data is not possible because of the poor correlation between TBF and raw SFT values (subscapular and triceps) measured with calipers (11). Two methods for the quantitation of TBF in infants directly from various anthropometric measurements use a more realistic approach (12, 13). The first and best known is the method proposed by Dauncey et al (12), who assumed the body to be a sphere (the head) and various hollow cylinders of fat (the trunk and the limbs). The estimate of TBF was calculated from the volume of the "fat cylinders" by using triceps and subscapular SFT data, body length, and various body circumferences. The second method is by Weststrate and Deurenberg (13). They calculated a series of prediction equations for body-fat percentage on the basis of total body density and/or sum of four skinfold-thickness measurements for children from 0 to 18 years of age. The equations are constructed on the basis of published data on changes in the density of the FFM with age in children. Both Dauncey's and Weststrate's methods, however, have never been compared with more recently established methods.

The first objective of the present study was to determine the agreement in infants of TBF estimations by TOBEC with the anthropometric body-composition methods based on skinfold-thickness measurements published by Dauncey et al (12) and Weststrate and Deurenberg (13). Possible age and sex effects on the agreement between TOBEC and anthropometry were also investigated.

## 5.3 SUBJECTS & METHODS

This study was part of a larger study on growth and body composition in healthy infants. A random sample of 2000 infants (living in the Rotterdam, Netherlands, metropolitan area

and aged 0-12 months) was drawn from the database of the local child health clinics of the Rotterdam Home Care Foundation. The parents were sent a letter with detailed information, in which they were invited to participate in the study. After written informed consent was obtained from the parents, 435 infants were enrolled in the present study. All measurements were performed by one observer at the Sophia Children's Hospital, Rotterdam. The study protocol was approved by the ethical review boards of the University Hospital Rotterdam and the Rotterdam Home Care Foundation.

*5.3.1. TOBEC measurements*

The principle underlying TOBEC is that lean tissue is far more electrically conductive than fat, due to the much greater content of water and electrolytes dispersed in the fat-free mass (FFM). In essence the TOBEC instrument (Body Composition Analyser Model HP-2; EM-SCAN Inc., Springfield IL, USA) is a large solenoidal coil driven by a 2.5-MHz oscillating radio frequency current. When a conductive mass passes through the electromagnetic field, the magnetic component of the field induces small eddy currents within the conductive mass, producing a small amount of heat. The energy of the eddy currents is dissipated from the magnetic field. The total energy loss is detected as a phase change in coil impedance. This phase change serves as an index of the amount of conductive mass (14). The amount of fat is calculated by subtraction of the conductive mass (FFM) from body weight. Electric and magnetic field intensities are $< 0.02\%$ and $0.4\%$, respectively, of the American National Standards Institute limits (in $mW/cm^2$) for continuous human exposure (15).

The TOBEC instrument has two measuring facilities: 1) fixed mode, in which the instrument scans the conductor, while it is moved slowly through the coil, and (2) peak mode, in which the instrument's on-line computer detects the greatest deflection in coil impedance at the time the object passes through the coil as compared to the impedance of the empty coil. Measurements in peak mode are less influenced by head movements and thorax breathing expansions than measurements in fixed mode. Before each TOBEC measurement a cylindrical reference phantom is measured (obtained from EM-SCAN and measured on the manufacturer's reference instrument, which yielded a reference value of 1944 for our instrument's reference phantom). Also, a background reading was taken (with empty sledge). All measurements of the present study, including reference phantom and background measurements, were performed in peak mode.

All infants were fasted for $\geq$ 2 h before the measurement. A routine physical examination was performed. Infants were undressed and carefully swaddled in a fully extended position in a large blanket. Care was taken, for reasons of possible changes in the conductive properties of the subject, that the limbs did not touch each other or the trunk. Infants were placed in a recumbent position on their back on the sledge of the instrument. A pacifier was allowed to calm the infants if necessary. For one TOBEC reading, the sledge was slowly moved into the coil, which took $\approx$10 s. A complete TOBEC measurement consisted of 5-10 readings, depending on the age and the emotional condition of the infant (older infants need $<$ 10 readings, because the CV of the readings decreases with

**Table 5.1.** Physical characteristics of the infants and body-composition results derived by total-body electrical conductivity (TOBEC), and the methods of Dauncey et al (12) and Weststrate and Deurenberg (13)

| | Boys ($n$=225) | Girls ($n$=210) | Significance of difference[1] |
|---|---|---|---|
| ***Physical characteristics:*** | | | |
| age (d) | 168 (81) | 166 (78) | NS |
| parents white/nonwhite[2] | 193 / 32 | 177 / 33 | |
| total length (cm) | 67.5 (5.9) | 65.8 (5.6) | $p < 0.001$ |
| weight (kg) | 7.49 (1.61) | 6.91 (1.54) | $p < 0.001$ |
| head circumference (cm) | 43.1 (2.7) | 42.1 (2.4) | $p < 0.001$ |
| Quetelet 's index ($kg/m^2$) | 16.2 (1.3) | 15.7 (1.4) | $p < 0.001$ |
| biceps SFT (mm) | 6.71 (1.81) | 6.93 (2.05) | NS |
| triceps SFT (mm) | 9.99 (2.43) | 10.19 (2.32) | NS |
| subscapular SFT (mm) | 7.07 (1.81) | 7.18 (1.43) | NS |
| suprailiac SFT (mm) | 5.87 (1.86) | 6.15 (1.79) | NS |
| quadriceps SFT (mm) | 15.90 (3.76) | 16.26 (3.85) | NS |
| ***TOBEC method:*** | | | |
| TBF (kg) | 1.84 (0.65) | 1.79 (0.64) | NS |
| FFM (kg) | 5.65 (1.08) | 5.13 (0.96) | $p < 0.001$ |
| TBF (%) | 24.0 (4.9) | 25.1 (5.0) | $p = 0.004$ |
| ***Dauncey's method:*** | | | |
| $TBF_{Dauncey}$ (kg) | 1.77 (0.59) | 1.73 (0.57) | NS |
| $FFM_{Dauncey}$ (kg) | 5.71 (1.26) | 5.18 (1.18) | $p < 0.001$ |
| $TBF\%_{Dauncey}$ (%) | 23.5 (5.5) | 24.8 (5.6) | $p = 0.014$ |
| ***Weststrate's method:*** | | | |
| density (kg/L) | 1.0083 (0.0059) | 1.0075 (0.0060) | NS |
| $TBF_{Weststrate}$ (kg) | 2.36 (0.63) | 2.21 (0.63) | $p = 0.001$ |
| $TBF_{Weststrate}$ (%) | 31.1 (3.4) | 31.6 (3.5) | NS |

Mean (SD) unless otherwise noted. SFT, skinfold thickness; TBF, total body fat; FFM, fat-free mass.

1 Significant difference between boys and girls, by analysis of covariance with age as covariable (NS = not significant).

2 Number of infants with two white parents/infants with one or two non-white parent(s).

increasing weight). The mean of the TOBEC readings was taken for further body-composition calculations. When the infant urinated, it was swaddled again in a dry blanket and remeasured. Unacceptable movement or crying of the infant was also a reason for remeasuring the infant.

TOBEC measures FFM and has a maximal technical error for an individual measurement (ie, width of the 95% prediction interval based on the animal calibration curve) of 0.154 kg FFM, consistent with an uncertainty in the FFM estimate of <5% in infants >3 kg FFM (16). In the present study mean background reading was 40.1 ± 2.86 ($\bar{x}$ ± SD) TOBEC units. Room temperature and relative room humidity at the time of the TOBEC measurement were, respectively, 22.6 ± 0.87 °C and 38.5 ± 4.13 %. On average the CV of the consecutive 10-s readings was 1.23 %. One percent of the measurements had a CV >3%, with only 1 case with a CV >4% (6.2%).

*5.3.2. Anthropometric measurements*

Infants were weighed naked on an electronic baby scale (Instru Vaaka Oy, Finland) to the nearest 1 g (<3 kg body weight), 2 g (3-6 kg) or 5 g (>6 kg). Recumbent crown-heel length and crown-rump length were measured to the nearest 1 mm on a length board. Body circumferences and limb lengths were measured with a standard plastic measuring tape (1-cm wide), circumferences to the nearest 1 mm, and limb lengths to the nearest 0.5 cm; circumferences measured were the head (fronto-occipital), chest (at the level of the nipples), abdomen (recumbent, at the level of the largest cross-sectional area), midupper arm, midthigh, and calf; and limb lengths measured were upper arm (inferior border of acromion to tip of olecranon) and lower arm (tip of olecranon to tip of the lateral styloid). All SFT measurements were performed with Harpenden calipers. Calibration of the calipers before and after the study revealed no difference. SFT was assessed in duplicate at the following 5 sites: biceps, triceps, subscapular, suprailiac and quadriceps femoris. All anthropometric measurements were performed on the left side of the body, by one observer, according to the technique of Lohman (17).

CVs of the individual duplicate skinfold measurements were all <3%, except for the suprailiac SFTs, for which the CV was 5-6 % at the start of the study. A decrease in CV for all SFT values to 1-2 % at the end of the study was observed, independent from the average age or amount of body fat of the measured subjects. This might be explained as a learning effect of the observer.

*5.3.3. Body composition calculations*

***TOBEC.*** Raw TOBEC numbers (*E#*) were corrected for background (by subtraction of the background value, yielding $E\#_{net\text{-}subject}$) and for the reference phantom (by multiplying $E\#_{net\text{-}subject}$ by the factor: 1944 / $E\#_{net\text{-}phantom}$, yielding $E\#_{cor}$). TBF and FFM from TOBEC measurements were calculated using a transformed TOBEC value, T# (18):

$$T\# = \sqrt{(E\#_{cor} \cdot L_c)}$$

where $L_c$ is the conductive length of the subject, derived from:

$$L_c = \{ (\text{crown-heel length}) - (\text{head circumference} / \pi) \}.$$

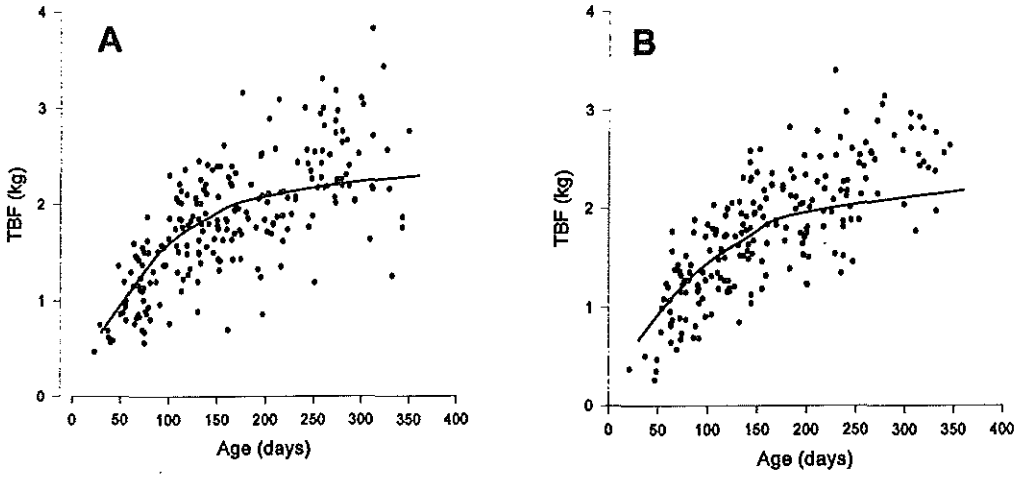


**Figure 5.1.** Total-body fat (TBF) by total-body electrical conductivity plotted against age. The solid line refers to the mean reference values (in kg) derived from Fomon et al (21) **A.** boys. **B.** girls.

The calibration equation used to relate T# with FFM was derived from an animal model using minipigs, and based on the observation that the physiological changes in conductivity and geometry of the FFM of maturing piglets approximate very well the FFM changes of growing infants (8). Infant minipigs were measured by TOBEC, and TBF and FFM was determined by carcass analysis (9). The resulting calibration equation was compared with a calibration equation by Fiorotto et al (16), which showed no significant difference between the curves. Magnetic field characteristics of both instruments were also comparable; therefore, we considered it to be justified to pool the data from both centers. We calculated the following calibration curve based on the pooled data : FFM (kg) = - 0.0213 + 0.0264 ·T# ($r^2$ = 0.997; residual SD = 0.077 kg). This equation was used in the present study to calculate FFM and TBF from TOBEC data.

***Dauncey's method.*** Dauncey et al (12) assumed that the distribution of infant's body-fat volume could be described as three sets of hollow cylinders: the trunk and the two pairs of limbs. Originally, the thickness of the subscapular skinfold was used to calculate the volume of the subcutaneous trunk fat layer, whereas the triceps skinfold thickness was used for the calculation of the fat of both arms and legs.

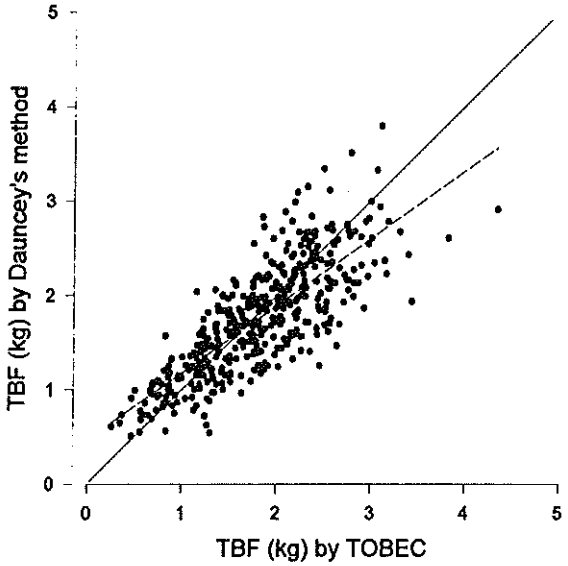


**Figure 5.2.** Total body fat (TBF) by total-body electrical conductivity plotted against TBF by the original Dauncey equation: Y=0.49+0.69X, $r^2$=0.61, ( ----- ), and the line of identity ( —— ).

$$V_{trunk} = (L_{CR} - C_{head} / \pi) \cdot (C_{chest}) \cdot (SFT_{subsc} - 0.2),$$
$$V_{lower\ limb} = (L_{CH} - L_{CR}) \cdot 0.5 \cdot (C_{thigh} + C_{calf}) \cdot (SFT_{tric} - 0.2),$$
$$V_{upper\ limb} = (L_{UA} + L_{LA}) \cdot (C_{upperarm}) \cdot (SFT_{tric} - 0.2)$$
$$TBF = 0.9\ (V_{trunk} + 2V_{lower\ limb} + 2V_{upper\ limb}),$$

where: V is volume, $L_{CR}$ is crown-rump length (cm), $L_{CH}$ is crown-heel length (cm), $L_{UA}$ is upper arm length (cm), $L_{LA}$ is lower arm length (cm), $C_{head}$ is head circumference (cm), $C_{chest}$ is chest circumference (cm), $C_{thigh}$ is thigh circumference (cm), $C_{calf}$ is calf circumference (cm), $C_{upperarm}$ is upper arm circumference (cm), $SFT_{subsc}$ is subscapular skinfold thickness (cm), $SFT_{tric}$ is tricipital skinfold thickness (cm). An assumed value of 2 x 0.1 cm is subtracted from all skinfolds to correct for the thickness of the dermis. The three sets of cylinder volumes are added and multiplied by 0.9, which Dauncey et al (12) assumed to be the density of body fat.

***Weststrate's method.*** Weststrate and Deurenberg (13) related the sum of biceps, triceps, subscapular and suprailiacal SFT to total body density. From total body density total

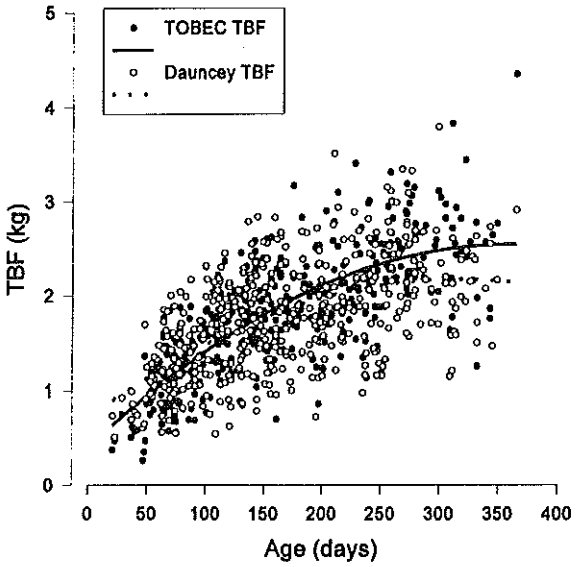


**Figure 5.3.** Total body fat (TBF) plotted against age. Regression equation for total-body electrical conductivity (TOBEC): $TBF_{TOBEC} = 0.384 + 1.19 \cdot 10^{-3}(age) - 1.64 \cdot 10^{-5}(age)^2$, $r^2 = 0.56$, residual SD = 0.428 kg. Regression equation for the original method of Dauncey: $TBF_{Dauncey} = 0.710 + 9.2 \cdot 10^{-4}(age) - 1.46 \cdot 10^{-5}(age)^2$, $r^2 = 0.32$, residual SD = 0.480 kg.

body fat percentage was calculated by use of age-specific prediction equations. For the age group of 0-1.99 years of age the authors published the following equations:

$$\text{TBF percentage} = [(585 - 4.7\ [\text{age (month)}]^{0.5}) / \text{density}] - (550 - 5.1\ [\text{age (month)}]^{0.5})$$

$$\text{Total body density} = [1.1235 + (0.0016\ [\text{age (month)}]^{0.5})] - 0.0719\ \log(\text{skinfold thickness}).$$

*5.3.4. Statistical analysis*

An effect was assumed to be significant if $P < 0.05$. Unless stated differently, data are expressed as mean (SD). For assessment of agreement between methods the statistical approach as proposed by Bland and Altman (19) was used. Limits of agreement were calculated as 2 SD above and below the mean of the difference between the two methods being compared. For the description of the relationships between TBF and age a quadratic-regression model was used.

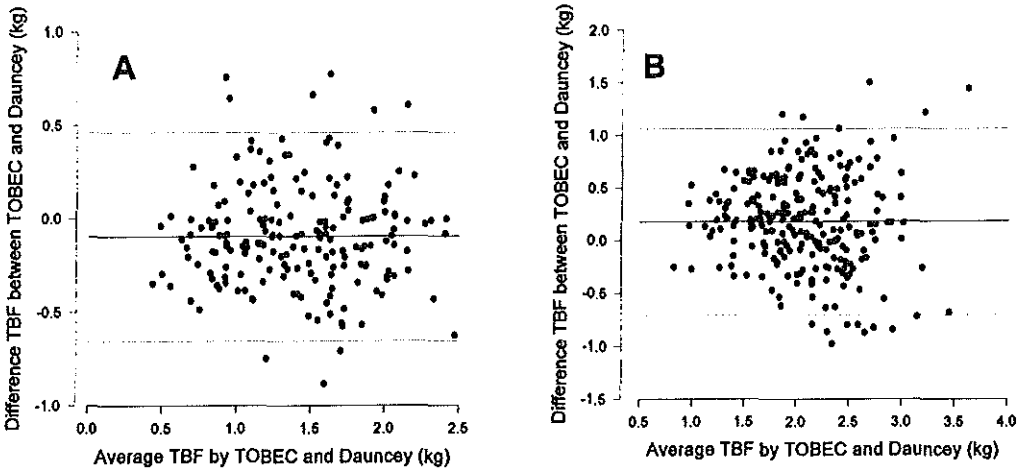


**Figure 5.4.** Difference between total-body fat by total-body electrical conductivity ($TBF_{TOBEC}$) and $TBF_{Dauncey}$ plotted against their mean. **A.** Age < 140 days. Mean difference (d) = 0.099 , $SD_d$ = 0.280, $SE_d$ = 0.020), limits of agreement (calculated as d ± 2 SD) are -0.658 and 0.460 ($SE_{limits}$ = 0.035), *see* reference 17). **B.** Age > 140 days. Mean difference (d) = -0.183 , $SD_d$ = 0.453, $SE_d$ = 0.029), limits of agreement (calculated as d ± 2 SD) are -0.723 and 1.089 ($SE_{limits}$ = 0.050), *see* reference 17).

## 5.4 RESULTS

### *5.4.1. TOBEC body composition and growth.*

Physical characteristics by sex of the study infants are described in **Table 5.1**. A significant difference between boys and girls was observed for length, weight, head circumference and Quetelet's index. Head circumference, body weight and total length for both sexes were distributed between the 3rd and 97th percentile of the 1980 Dutch national growth survey (20). Although length and weight were in accordance with recent Dutch reference values, Quetelet's indexes of the study population were lower than Quetelet's indexes calculated from Fomon et al's reference infants (21). This is mainly due to increased lengths and equal weights of the infants in the present study as compared with Fomon et al's reference infants (21). As shown in **Figure 5.1 A** and **B**, $TBF_{TOBEC}$ (kg) data were in accordance with mean values reported by Fomon et al (21), except for girls in the first 2 mo of life ($TBF_{TOBEC}$ was lower than Fomon's values) and for girls aged 11 and 12 mo ($TBF_{TOBEC}$ was higher than Fomon's values). A significant difference between sexes was observed for $FFM_{TOBEC}$ and $TBF\%_{TOBEC}$. Triceps SFT values were in accordance with reference data of Tanner

and Whitehouse (22) and subscapular SFT values, on average, were slightly lower. We were not able to find reference data for infants on biceps, quadriceps and suprailiacal SFT. Calculated arm circumference percentiles of the present study group were in accordance with (extrapolated) reference percentiles of 1-74-y-old subjects of Frisancho (23). Means and SDs of the various SFT measurements are summarized in **Table 5.1**.

*5.4.3. TOBEC vs Dauncey's method*

In **Figure 5.2**, $TBF_{TOBEC}$ is plotted against TBF derived from Dauncey's method ($TBF_{Dauncey}$; 12). The figure shows that in infants with approximately the same $TBF_{TOBEC}$ there was a large variability in $TBF_{Dauncey}$. No difference in variability between sexes was found. In **Figure 5.3**, $TBF_{TOBEC}$ and $TBF_{Dauncey}$ are plotted vs age, and shown with their quadratic regression curves. Inclusion of age squared in the regression equation significantly improved the correlation coefficient. For infants aged <4 mo, Dauncey's method overestimated $TBF_{TOBEC}$ on average by 0.135 kg (SD = 0.254, SEM = 0.021; paired t-test: $P < 0.0001$), whereas later in infancy (> 4 mo of age) $TBF_{TOBEC}$ was underestimated on average by 0.202 kg (SD = 0.471, SEM = 0.033; paired t-test: $P < 0.0001$).

The agreement of the Dauncey method with the TOBEC method was assessed. We calculated the limits of agreement for two age periods: before and after the point of intersection (140 days) shown in Figure 5.3. **Figure 5.4, A** and **B,** shows plots of the difference between the two methods ($TBF_{TOBEC}$ - $TBF_{Dauncey}$), plotted against their mean. In the age group < 140 days (Fig 6.4, A), in which the mean difference between both methods was the smallest, the limits of agreement were ≈40 % above and below the averaged mean values of the two methods.

*5.4.4. Modifications of Dauncey's method*

We tried several modifications of the original Dauncey method to increase the agreement and correlation with TOBEC. It has been reported that the density of the subcutaneous fat layer is <0.9 and varies considerably between subjects (6, 24). For this reason we chose to avoid this factor and entered the three Dauncey values for fat of trunk, upper and lower extremities separately into a multiple-linear-regression model with $TBF_{TOBEC}$ as dependent variable. This procedure only slightly improved the prediction of $TBF_{TOBEC}$. Also three modifications of the standard, original Dauncey equation were tested: 1) values of all skinfold thicknesses were halved to adapt for the bilayer nature of the original skinfold grasp, 2) the quadriceps skinfold thickness was used for the calculation of the lower limbs fat masses (ie. the second term of Dauncey's equation), and 3) the combination of the two above modifications was tried. In the linear regression model the combined modification

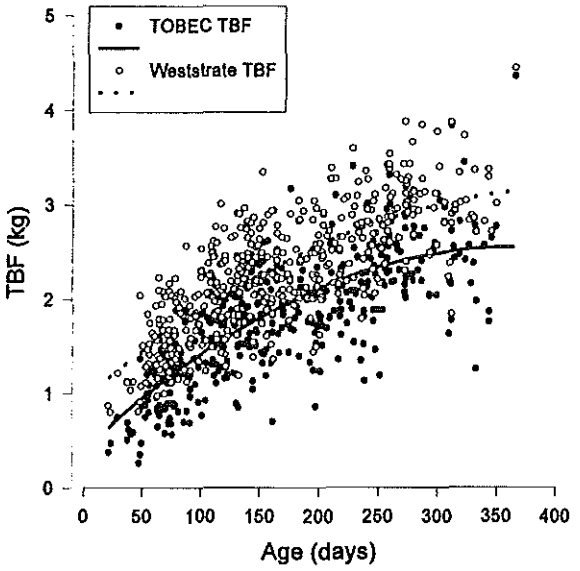


**Figure 5.6.** Total body fat (TBF) plotted against age. Regression equation for total-body electrical conductivity (TOBEC): $TBF_{TOBEC}$ = 0.384 + $1.19 \cdot 10^{-3}$(age) - $1.64 \cdot 10^{-5}$(age)$^2$, $r^2$ = 0.56, residual SD = 0.428 kg. Regression equation for $TBF_{Weststrate}$: $TBF_{Weststrate}$ = 0.937 + $1.07 \cdot 10^{-3}$(age) - $1.29 \cdot 10^{-5}$(age)$^2$, $r^2$ = 0.59, residual SD = 0.406 kg.

$TBF_{Dauncey\text{-}modified}$ yielded the best correlation with $TBF_{TOBEC}$ ($TBF_{Dauncey\text{-}modified}$ = 0.19 + 0.51 $TBF_{TOBEC}$). This improved the $r^2$ from 0.61 for the original Dauncey equation to $r^2$ = 0.75.

*5.4.5. TOBEC vs Weststrate's method*

A significant difference between sexes was observed for $TBF_{Weststrate}$ (Table 5.1; $P$ = 0.001, analysis of covariance with age as covariable). In **Figure 5.5** $TBF_{TOBEC}$ is plotted against $TBF_{Weststrate}$, showing a large systematic difference . Residual variation around the regression line was less than found with Dauncey's method. $TBF_{Weststrate}$ correlated better with $TBF_{TOBEC}$ than did $TBF_{Dauncey\text{-}modified}$ ($r^2$=0.85 vs $r^2$=0.75 for boys, and $r^2$=0.90 vs $r^2$=0.75 for girls, respectively). **Figure 5.6** shows $TBF_{TOBEC}$ and $TBF_{Weststrate}$ plotted vs age. Weststrate's method significantly overestimated $TBF_{TOBEC}$ ($P < 0.0001$, paired t-test) on average 0.516 (0.256) kg for boys and 0.422 (0.208) kg for girls. This average overestimation was independent from age: when the difference between $TBF_{TOBEC}$ and $TBF_{Weststrate}$ was regressed against age, the slope was not significantly different from zero. A graphical representation of the limits of agreement between the two methods for boys and

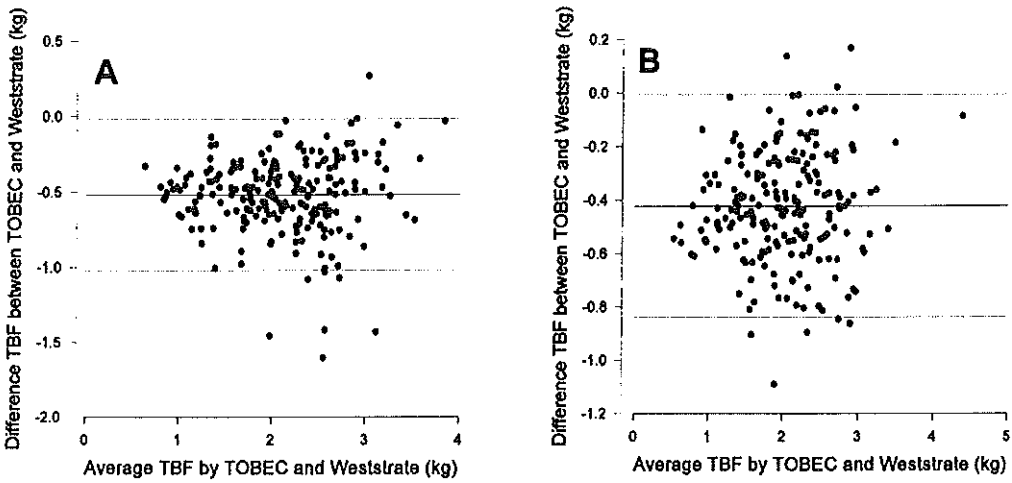


**Figure 5.7.** Difference between total body fat by total-body electrical conductivity ($TBF_{TOBEC}$) and $TBF_{Weststrate}$ plotted against their mean. **A.** boys. Mean difference (d) = -0.516 ($SD_d$ = 0.256, $SE_d$ = 0.017), limits of agreement (calculated as d ± 2 SD) are -1.028 and -0.005 ($SE_{limits}$ = 0.030), *see* reference 17). **B.** girls. Mean difference (d) = -0.422 ($SD_d$ = 0.208, $SE_d$ = 0.014), limits of agreement (calculated as d ± 2 SD) are -0.838 and -0.006 ($SE_{limits}$ = 0.025), *see* reference 17).

girls [ie, ± 2 SD of the difference ($TBF_{TOBEC}$ *minus* $TBF_{Weststrate}$) plotted against their mean values] is given in **Figure 5.7, A** and **B**.

## 5.5 DISCUSSION

### *5.5.1 TOBEC chosen as a reference method*

Measurement of TOBEC is a recently developed, safe, and reproducible method for the estimation of infant TBF and FFM. Because at present an alternative method for estimating fat and FFM in infants with comparable ease and precision does not exist, TOBEC was chosen as a reference method for the validation of anthropometry-based body-composition methods. Two things should be taken into account when looking at the validity of the TOBEC method for being a reference method. First, does TOBEC measure what it has been claimed to measure, namely the FFM compartment? And second, how precise is the estimate of FFM? In two reviews on the TOBEC methodology, Fiorotto et al (7, 25) demonstrate that TOBEC does not measure total body water, but explicitly measures FFM.

Although the lack of a valid reference method in infants compromises a true validation of the TOBEC technique, there is indirect evidence for the accuracy of the method. Mean values for TBF and FFM of healthy full-term infants aged 0-4 mo of age (7) were in excellent agreement with Fomon et al's reference data (21). $TBF_{TOBEC}$ data from the present study were also in agreement with Fomon et al's reference data (see Fig 5.1, A and B). Also, and this is important indirect evidence too, the pattern and amount of fat accretion in the first weeks of life very closely follow the pattern anticipated from known intrauterine fat accretion rates (8). Moreover, we have shown by 131 body composition measurements in healthy full-term infants between 1 and 12 mo of age that FFM estimations by TOBEC are in excellent agreement over the entire range of data with FFM values estimated from total body water measurements by dilution of $^2H_2{}^{18}O$ (24). Total body water was calculated from the average of the corrected dilution spaces of $^2H$ and $^{18}O$. Hydration of FFM was calculated by using Fomon et al's reference data (21). Although a small systematic difference between the $^2H_2{}^{18}O$ and the minipig-derived calibration equation was present ($\approx$120 g), this will not affect the correlation and the limits of agreement in the comparison of methods described in the present study. Minipigs which have a hydration of the FFM and geometry of the FFM (ratio of abdominal circumference to FFM length) comparable to infants of the same FFM weight (8, 25, and personal observation) were used for the animal-derived calibration equation.

We realize the limitations of this approach. We suggest that at least for healthy infants the present calibration equation will yield accurate estimates of FFM. Assuming that the measurement error in FFM by TOBEC in anaesthetized minipigs and carefully swaddled human infants is approximately equivalent, the maximum measurement error for an individual, reflected by the 95% prediction interval, is 154 g. This results in a relative error of $<5\%$ for individuals with FFM $>3$ kg.

*5.5.2. Dauncey's method*

We expected that the original equation of Dauncey et al (12) would yield TBF values lower than $TBF_{TOBEC}$ (the latter being an estimate of *total* body fat). This holds true for infants older than $\approx$5 months of age. In early infancy, however, the method of Dauncey et al (12) overestimates $TBF_{TOBEC}$. This agrees with a recent report by Kabir and Forsum (6), who also found Dauncey's method to overestimate TBF. Three possible explanations for this age-related discrepancy are as follows:

1) Changes in hydration of the adipose tissue, which result in different compressibility of skinfolds. The age-related discrepancy cannot be attributed to errors in the caliper measurement procedure, for calipers were applied until the measurements had been stabilized and SFT measurements agreed with known reference values (22, 23). However, the possible influence of changes in hydration of the adipose tissue might still be valid, because it is unknown whether the caliper measurement compresses all interstitial/extracellular water from the tissue (in both this study and the reference data studies). Aside from this, it is also known that intracellular water within the fat cells is increased in

early infancy.

2) Dauncey's method uses measurements of body dimensions in combination with skinfold thickness. Changes in the shape and thickness of skinfolds during infancy might also contribute to the discrepancy between $TBF_{TOBEC}$ and $TBF_{Dauncey}$ over time. In the original equation, the triceps skinfold is used to calculate the fat of the legs. The ratios (triceps SFT) / (quadriceps SFT) decreases with age, so the use of the triceps SFT for the leg fat calculation is not justified.

3) Finally, a change in internal (non-subcutaneous) body fat over time might add to the difference between $TBF_{TOBEC}$ and $TBF_{Dauncey}$.

*5.5.3. Modification of Dauncey's method*

We have tried several modifications of Dauncey's equation to improve the agreement and correlation with TOBEC. The rationale for the first modification (to halve the SFT values) was the assumption that the skinfold grasp takes a bilayer of dermis and subcutaneous body fat. No reports have been published that unfold the exact nature of the skinfold grasp at different locations (eg, by concomitant ultrasonography at the time of the actual calipers application). Kabir and Forsum (6) showed for infants $< 3$ mo of age that on average the thickness of the fat layer measured with ultrasonography agreed best with SFT's divided by two. The second modification was the use of the quadriceps SFT instead of the triceps SFT for the assessment of the amount of fat in the legs. Kabir and Forsum (6) also showed by ultrasonography that the thickness of the calf fat layer was approximated more closely by the quadriceps SFT than by the triceps SFT, whereas the thickness of the quadriceps and triceps fat layer significantly differed, both with ultrasonography as well as when measured by calipers. This holds true also for the present study. Therefore, it is not surprising that the best modification was found by combining both the above described approaches.

*5.5.4. Weststrate's method*

Compared with TOBEC the predictive value of the SFT-based prediction equations of Weststrate and Deurenberg (13) was better than Dauncey's method, and not influenced by age. However, a large systematic error of $\approx 0.5$ kg TBF was observed, which was slightly (but significantly) higher for boys than for girls. It is uncertain to what the discrepancy between TOBEC and Weststrate's TBF can be attributed. We were not able to find any reference data on biceps, quadriceps and suprailiac SFT. Although triceps and subscapular SFT values agreed with known reference data, it might well be that differences in measurement techniques (a well-known phenomenon with calipers measurements) of the biceps, quadriceps or suprailiac skinfold thicknesses between Weststrate and Deurenberg's (13) and our laboratory might contribute to the systematic difference. This also shows the limited applicability of the extrapolation of anthropometry-based prediction equations to different populations and between different centers or observers. Although the correlation and thus the predictive value between $TBF_{TOBEC}$ and Weststrate's TBF was better than for our modification on Dauncey's method, the large limits of agreement prohibit the use of this

method for individual estimates of body composition in infants.

*5.5.5. Conclusions*

This study shows the limited applicability of anthropometry-based methods to assess absolute amounts of body fat in infants. For the assessment of internal body fat from $TBF_{TOBEC}$ and $TBF_{Dauncey\text{-}modified}$ the inaccuracy even increases, because measurement errors of both methods are additive in this case. This study does not implicate that SFT measurements and other anthropometric measurements or combinations of anthropometric measurements have lost their value in body-composition studies. Mean values of anthropometry-based TBF indexes might still be of value, provided that sufficient numbers of infants are incorporated in the study groups to be compared. In this respect, both Weststrate's method as well as our modification of Dauncey's method can be used. Also, with use of the recent generation of safe and more precise body-composition methods (eg, TOBEC) a systematic search might reveal new and better combinations of anthropometric parameters. However, as this study shows, the large errors associated with anthropometric measurements prohibit their use for accurate estimations of TBF in individual infants.

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*Chapter 6*

# Traditional and New Anthropometric Indexes Validated against TOBEC [a)]

## 6.1 Summary

Anthropometry is frequently used for nutritional assessment. Little is known in infants about the validity of anthropometric measurements in relation to whole-body fat (TBF) and fat-free mass (FFM) composition. We compared TBF and FFM estimations by total-body electrical conductivity (TOBEC) with anthropometry in 435 healthy infants ages 21-365 d. TBF was best correlated with weight-for-length and calf circumference ($r^2=0.84$, $r^2=0.83$). FFM was best correlated with body weight ($r^2=0.93$). Upper-arm anthropometry, skinfold thickness, and Quetelet's and Ponderal indexes were poorly correlated with TBF and FFM ($r^2<0.65$). New anthropometry-based prediction equations were calculated ($r^2=0.90$ for TBF and $r^2=0.95$ for FFM). New simple indexes (analogous to Quetelet's index) were calculated for TBF (weight · calf circumference / length, $r^2=0.87$) and for FFM ($\sqrt{}$(weight · length), $r^2=0.95$). Prediction equations and indexes were cross-validated in a second population by a second observer. Interobserver variation was largest for equations with skinfold thicknesses included. We conclude that anthropometry can be used for rough estimations of body composition, although indexes different than those used in children and adults are preferred.

## 6.2 Introduction

The sophistication of nutritional support in infants nowadays makes it necessary to identify sensitive body composition methods for accurate nutritional assessment in infancy. For this goal several body composition techniques have been used in the past, some of which are inexpensive and easy to use (eg, anthropometry), others are more precise but time-

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a) This chapter has been published before as: De Bruin NC, Van Velthoven CAM, Stijnen T, Juttmann RE, Degenhart HJ, Visser HKA. Body fat and fat-free mass in infants: new and classic anthropometric parameters and prediction equations compared with total- body electrical conductivity. *Am J Clin Nutr* 1995; 61:1195-205

We thank Mr Theo Brugman and Ms. Maria de Ridder for their assistance in the study. We extend our appreciation to the parents and infants who volunteered their time to take part in this study.

consuming or very expensive (eg, isotope dilution with $^{18}O$-labeled water).

Recently, measurement of total-body electrical conductivity (TOBEC) has emerged as an accurate, precise and reproducible method for the estimation of fat-free mass (FFM) and total body fat (TBF) in infants [1-4]. Calibration against carcass analysis data of minipigs as well as assessment of precision of TOBEC has been performed [5]. The validity of the minipig calibration equation for use in human infants has been proven in two ways. First, all reported TOBEC data for TBF of full-term infants throughout the first year of life [1-4] are in excellent agreement with reference data [6]. Second, the observed accretion of body fat in the fetus (measured by carcass analysis) and in the first 4 months of extrauterine life (measured by TOBEC) fit very well [2]. Small changes in FFM hydration, as found between individuals of different ages because of maturation of the FFM compartment in early life and as found between individuals of the same age because of normal biological scatter in FFM hydration, will not seriously affect TOBEC outcome [5, 7-9]. A TOBEC measurement is rapid, safe and easy to perform, and suitable for measurement of large numbers of infants. The instrument has been commercially available since 1989. At present TOBEC is one of the most reliable methods to estimate infant body composition, but is not widely used because of the relatively high price of a TOBEC instrument (≈ $ 45,000.00), and because it is large and difficult to move and therefore not suitable for field studies. However, its good reproducibility, precision, and accuracy justify the use of TOBEC as a reference method.

Anthropometry is widely used for assessment of nutritional status in adults and children. The method has been notorious for its inaccuracy in untrained hands and for its inter-observer variation [10]. However, because the method is inexpensive, simple to use, and portable, its use is very common and widespread. Only during the last decade have accurate body composition techniques become generally available that are suitable for use in infants [1, 11, 12]. As a result of this, a limited number of studies on the validation of only a few anthropometric measurements in relation to infant whole-body composition (TBF and FFM) have been published [1, 13-16]. These studies deal with body composition during the first weeks of life. We recently evaluated two well-known anthropometric body-composition prediction equations [17, 18] explicitly designed for quantitative estimation of TBF in infants [4]. Although well correlated with TBF, the agreement of these methods with TOBEC was not good enough to use them as an alternative for accurate quantitative estimations of body composition in individual patients. To our knowledge no further studies have been published that systematically compare anthropometric indexes with more precise body-composition techniques over the entire range of the first year of life. Beside this, no studies are known to us that describe a systematic search for new combinations of anthropometric indexes to predict TBF and FFM from anthropometric measurements.

The first objective of the present study was to correlate single anthropometric measurements and classic combinations of anthropometric variables (eg, upper-arm anthropometry, Quetelet index, weight by length, etc) with TOBEC-derived body composition in healthy full-term infants. The second objective was to calculate in a multiple-

linear-regression model the most optimal combinations of all measured anthropometric variables and a subsequent search for simple indexes to predict TBF and FFM. The third objective was to cross-validate the newly derived prediction equations and indexes in a second population by a second observer.

## 6.3 SUBJECTS & METHODS

### *6.3.1. Study population*

This study was part of a larger study on growth and body composition in infants. In cooperation with the local child health clinics of the Rotterdam Home Care Foundation a random sample of 2000 infants (living in the Rotterdam metropolitan area and aged between 0 and 12 months) was drawn from their database. A total number of 601 parent-infant couples responded. All infants measured by the main observer ($n$ = 435) were enrolled in the present study. The second sample used in this study and measured by a second observer ($n$ = 110) was also drawn from this population. Written informed consent was obtained from the parents. The study protocol was approved by the ethical review boards of the Erasmus University / University Hospital Rotterdam and the Rotterdam Home Care Foundation.

### *6.3.2. TOBEC measurements*

The principle underlying the TOBEC technique is that lean tissue (FFM) is far more electrically conductive than fat, because of its much greater content of electrolytes [7]. When a conductive mass passes through the electromagnetic field, the magnetic component of the field induces small eddy currents within the conductive mass, producing a small amount of heat. The energy of the eddy currents is dissipated from the magnetic field. The total energy loss is detected as a phase change in coil impedance. This phase change serves as an index of the amount of conductive mass, ie, the infant's FFM [19]. TBF is calculated by subtracting FFM from body weight. Changes in hydration of the FFM during maturation have been incorporated in the calibration procedure [8]. The method has been claimed to be robust under changes in extracellular fluid volume [9].

In essence the TOBEC instrument (Body Composition Analyzer Model HP-2; EM-SCAN Inc., Springfield, IL) is a large solenoidal coil driven by a 2.5-MHz oscillating radio frequency current. Electric and magnetic field intensities are minimal, $< 0.02\%$ and $0.4\%$, respectively, of the limits (in $mW/cm^2$) set by the American National Standards Institute for continuous human exposure [20].

Before each measurement, background noise (with empty sledge) and a cylindrical reference phantom were measured. All measurements of the present study, including reference phantom and background measurements, were performed with the TOBEC instrument set in the peak mode [7].

All infants were not fed for $\geq 2$ h before the measurement. A routine physical

examination was performed. Infants were undressed and carefully swaddled in a fully extended position in a large blanket. Care was taken that the limbs did not touch each other or the trunk. Infants were placed on their back on the sledge of the instrument. A pacifier was allowed to calm the infants when necessary. One TOBEC reading took ≈10 s. A complete TOBEC measurement consisted of 5 to 10 of these 10-s readings. The mean of the 5 to 10 10-s readings was taken for further body-composition calculations. When the infant urinated, it was swaddled again in a dry blanket and remeasured. Movement or crying of the infant was also a reason for remeasuring the infant.

*6.3.3. Anthropometric measurements*

Infants were weighed naked on an electronic baby scale (Instru Vaaka Oy, Finland) to the nearest 1 g (0-3 kg body wt), 2 g (3-6 kg) or 5 g (6-10 kg). Recumbent crown-heel length and crown-rump length were measured to the nearest millimeter on a length board. Body circumferences and limb lengths were measured with a standard plastic measuring tape (1 cm wide), circumferences to the nearest millimeter, and limb lengths to the nearest 0.5 cm. Circumferences of the head (fronto-occipital), chest (at the level of the nipples), abdomen (recumbent, at the level of the largest cross-sectional area), midupper arm, midthigh, and calf were measured. Upper-arm (inferior border of acromion to tip of olecranon) and lower-arm (tip of olecranon to tip of the lateral styloid) were also measured. All skinfold-thickness measurements were performed in duplicate with one Harpenden caliper at the following 5 sites: biceps, triceps, subscapular, suprailiacal and quadriceps femoris. The caliper was applied while keeping the skinfold between thumb and forefinger. Special care was taken that the caliper remained applied until the measurement had been fully stabilized. All anthropometric measurements were performed by one observer on the left side of the body according to Lohman et al [21]. CVs calculated from (duplicate) skinfold-thickness measurements were $<3\%$ [4].

*6.3.4. Body composition calculations*

*TOBEC.* Raw TOBEC numbers (*E#*) were corrected for background by subtraction of the background value ($=E\#_{net}$) and for the reference phantom by multiplying $E\#_{net\text{-}subject}$ with the factor 1944 /$E\#_{net\text{-}phantom}$ ($=E\#_{cor}$) [7]. A transformed TOBEC value (T#) was calculated as:

$$T\# = \sqrt{(E\#_{cor} \cdot L_c)},$$

with $L_c$ as the conductive length of the subject [2,8], derived from

$$L_c = \{(\text{crown-heel length}) - (\text{head circumference}/\pi)\}.$$

The calibration equation used to relate T# with FFM was FFM = 0.0264 T# - 0.0213. The calibration procedure as well as the theoretical basis for the transformed TOBEC value has

been described in detail elsewhere [5, 7]. Briefly, the calibration was derived from an animal model using carcass analysis data of minipigs, based on the observation that the physiological changes in conductivity and geometry of the FFM of the maturing piglet very well approximate the changes in FFM of growing infants [8,22].

Intra-measurement variability (CV of 10 consecutive readings) was 0.5% for the cylindrical reference phantom and 1.2% for the infants in the present study. Day-to-day variability of the cylindrical reference phantom measured over a 2-y period was 0.5%. In the present study mean (± SD) background reading was 40.1 ± 2.86 TOBEC units. Room temperature and relative room humidity at the time of TOBEC measurement were respectively 22.6 ± 0.87 °C and 38.5 ± 4.13%.

Accuracy of the TOBEC method has been calculated from the residual SD of the calibration equation . Accuracy of an individual FFM (as well as TBF) measurement was 0.154 kg (ie, twice the residual SD), which is consistent with an uncertainty of < 5% in infants > 3 kg FFM. Accuracy of an individual TBF estimate becomes ≈30% in a neonate with 3 kg FFM and 0.5 kg TBF, and 7% in a 1-yr-old infant with 7.5 kg FFM and 2.2 kg TBF.

*Classic anthropometry.* Regional anthropometric measurements at the site of the upper arm have been widely used as a screening tool for malnutrition in adults and children. According to Sann et al [23], we calculated from midupper-arm circumference (mm) and triceps skinfold thickness (mm) midupper-arm area (MAA), midupper-arm muscle circumference (MAMC), midupper-arm muscle area (MAMA) and midupper-arm fat area (MAFA), midupper-arm muscle ratio (MAMR) and midupper-arm fat ratio (MAFR). Arm-head ratio was calculated according to Eregie [24]. The sum of three skinfolds thicknesses was calculated from the sum of the values of the triceps, subscapular and quadriceps skinfolds thickness; for the sum of five skinfolds thicknesses, suprailiacal and biceps values were added. Weight by length was calculated as weight (kg) divided by length (m), Quetelet's index was calculated as weight by length squared, Ponderal index as weight by length cubed.

*6.3.5. Statistical analysis*

Because the variance of the dependent variables TBF and FFM increased with TBF and FFM, respectively, a logtransformation was applied on TBF and FFM. For TBF (kg) the independent (predictor) variables needed to be log-transformed also to assure a linear relation of the independent variable to the log-transformed dependent variable. All transformations resulted in linearity compared with the dependent variable and in a

**Table 6.1.** Physical characteristics of the main study population and body-composition results derived by total-body electrical conductivity (TOBEC).

| | boys ($n$ = 225) | | girls ($n$ = 210) | |
|---|---|---|---|---|
| | mean±SD | range | mean±SD | range |
| age (d) | 168±81 | 23 - 349 | 166 ± 78 | 21 - 365 |
| white / nonwhite | 193/32 | | 177/33 | |
| weight (kg) | 7.49 ±1.61 | 3.77 - 11.9 | 6.91 ± 1.54 [1)] | 3.47 - 12.5 |
| Lengths (cm) | | | | |
| total | 67.5 ± 5.9 | 54.0 - 80.5 | 65.8 ± 5.7 [1)] | 51.0 - 79.0 |
| crown-rump | 45.5 ± 3.6 | 36.5 - 59.0 | 44.3 ± 3.5 [1)] | 34.0 - 54.1 |
| upper arm | 12.3 ± 1.3 | 9.0 - 16.0 | 12.0 ± 1.2 [1)] | 8.5 - 16.0 |
| lower arm | 10.2 ± 1.2 | 7.5 - 14.0 | 9.8 ± 1.0 [1)] | 7.0 - 12.5 |
| Circumferences (cm) | | | | |
| head | 43.1 ± 2.7 | 36.0 - 49.0 | 42.1 ± 2.4 [1)] | 36.5 - 49.0 |
| chest | 43.3 ± 3.2 | 34.0 - 54.0 | 42.2 ± 3.1 [1)] | 35.0 - 49.0 |
| mid-thigh | 22.5 ± 2.7 | 16.0 - 32.0 | 22.0 ± 2.7 [2)] | 15.0 - 31.0 |
| calf | 17.3 ± 2.0 | 12.0 - 22.5 | 16.9 ± 1.9 [2)] | 11.0 - 22.0 |
| midupper-arm | 15.0 ± 1.5 | 11.0 - 20.5 | 14.7 ± 1.5 [1)] | 10.5 - 18.0 |
| Skinfolds (mm) | | | | |
| biceps | 6.7 ± 1.8 | 3.4 - 12.8 | 6.9 ± 2.1 | 3.3 - 13.7 |
| triceps | 10.0 ± 2.4 | 4.2 - 16.8 | 10.2 ± 2.3 | 5.1 - 16.2 |
| subscapular | 7.1 ± 1.5 | 3.9 - 11.2 | 7.2 ± 1.4 | 4.2 - 11.6 |
| suprailiac | 5.9 ± 1.9 | 3.0 - 13.8 | 6.2 ± 1.8 | 2.7 - 12.2 |
| quadriceps | 15.9 ± 3.8 | 6.2 - 24.8 | 16.3 ± 3.9 | 6.5 - 25.9 |
| Quetelet's index | 16.2 ± 1.3 | 12.9 - 19.3 | 15.7 ± 1.4 [1)] | 12.6 - 20.0 |
| Sum of 3 skinfolds | 33.0 ± 6.0 | 16.5 - 47.9 | 33.6 ± 6.2 | 16.1 - 47.8 |
| Sum of 5 skinfolds | 45.5 ± 8.7 | 24 - 67.2 | 46.7 ± 8.9 | 23.9 - 64.9 |
| MAA ($mm^2$) | 1817 ± 364 | 963 - 3344 | 1725 ± 341 | 877 - 2578 |
| MAMC (cm) | 11.9 ± 1.4 | 8.5 - 17.8 | 11.5 ± 1.3 [1)] | 8.2 - 15.4 |
| MAMA ($mm^2$) | 1142 ± 279 | 573 - 2530 | 1057 ± 246 | 540 - 1893 |
| MAFA ($mm^2$) | 675 ± 184 | 287 - 1168 | 669 ± 178 | 298 - 1171 |
| body composition (TOBEC) | | | | |
| TBF (kg) | 1.84 ± 0.65 | 0.47 - 4.05 | 1.79 ± 0.64 | 0.26 - 4.34 |
| TBF (%) | 24.0 ± 4.9 | 10.8 - 36.5 | 25.1 ± 5.0 [1)] | 7.5 - 35.2 |
| FFM (kg) | 5.65 ± 1.08 | 3.31 - 8.90 | 5.12 ± 0.96 [2)] | 3.13 - 8.16 |

MAA = midupper-arm area, MAMC = midupper-arm muscle circumference, MAMA = midupper-arm muscle area, MAFA = midupper-arm fat area., TOBEC = total-body electrical conductivity, TBF = body fat, FFM = fat-free mass.
Significantly different from boys (ANOVA with sex as factor and age as covariable): [1)] $P < 0.001$, [2)] $P < 0.010$

stabilization of the variance. TOBEC was used as reference method and correlated with classic anthropometric variables (the latter entered as independent variables in a linear-regression model).

The "All Possible Subsets Regression" module of the BMDP Statistical Software package (1990; Los Angeles) was used to calculate the best combinations of variables to predict TOBEC-derived TBF, percentage body fat (TBF%) and FFM. As predictor variables a total of 24 variables were investigated: age; gender; weight total length; crown-rump length; length of upper arm and lower arm; head, upper arm, lower arm, chest, thigh, and calf circumferences; Quetelet's index; all skinfolds thicknesses separately; sum of three skinfolds thicknesses; sum of five skinfolds thicknesses; MAA; MAMC; MAMA; and MAFA. For each subset (containing one to five independent variables) the best through 10th-best combination was calculated by the program. First, the best through 10th-best single predictor variable ($k = 1$) of these 24 variables was found by the program. Next, the best through 10th-best combinations of two predictor variables ($k = 2$) was determined. The same was done for $k = 3$, $k = 4$ and $k = 5$. In all cases TOBEC-derived body-composition estimates were entered as the dependent variables.

With the best-scoring variables from the all possible subsets regressions new anthropometric indexes were calculated using a heuristic approach. An index was defined as a simple combination of anthropometric variables (ie, analogous to the Ponderal or Quetelet's index, for example). The optimal exponent of each variable in an index was calculated by nonlinear regression. For example when by nonlinear regression $[(\text{weight})^{0.43} \times (\text{length})^{0.47}]$ was found, this was rounded to the nearest 0.5 to yield $\sqrt{[(\text{weight})(\text{length})]}$.

The best-scoring newly derived prediction equations and indexes were cross-validated in an independent second population by a second observer. To obtain untransformed TBF and FFM values, the equations were back-transformed as follows (here , as an example, TBF is taken):

$$TBF_{EQUATION}\ (kg) = EXP(\text{prediction equation})$$

where $TBF_{EQUATION}$ is TBF calculated by using the anthropometric prediction equations with log-transformed dependent variables. First, the magnitude of interobserver variation was assessed. No measurements were available that were performed by both observers on one subject. Therefore only indirect evidence for interobserver difference could be assessed. We used analysis of covariance (ANCOVA), with "observer" as factor, and with as many covariables as possible without interobserver variation, this to account for biological dissimilarities in fatness, age and growth between the infants of the two populations. In the past we found no interobserver variation for TOBEC measurements (personal observation). We assumed absence of interobserver variation for weight measurements. Therefore we used $TBF_{TOBEC}$, weight, age and gender as covariables in the ANCOVA. The adjusted means (adjusted for the covariables by ANCOVA) were used to calculate the magnitude of the interobserver difference, which was hence expressed for clarity as the percentage

**Table 6.2.** Numbers of boys and girls, by age of the main study population.

| age (mo) [1)] | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| boys | 6 | 27 | 21 | 38 | 30 | 21 | 20 | 21 | 21 | 11 | 6 | 222 |
| girls | 5 | 25 | 28 | 32 | 31 | 21 | 21 | 24 | 9 | 10 | 6 | 212 |

[1)] n = 435, 1 mo = 15 - 45 d, 2 mo = 46 - 75 d , etc.

difference between the two adjusted means. These mean differences only give an impression of the *average* deviation (accuracy) of a series of measurements from the "standard measurement" (which here is TOBEC) . Because mean differences can average out positive and negative errors, and thereby cover up larger individual differences, the uncertainty (precision)of anthropometric prediction equations was calculated using the root mean squared error (RMSE)[b]. For reasons of comparison, we also calculated the relevant RMSE's of the main population ($n$=435).

In general, percentage explained variability [ie, the square of the correlation coefficient ($r^2$) of the linear regression] was used as a measure for the predictive value. An effect was assumed to be statistically significant if $P < 0.05$. Unless stated differently data are expressed as mean $\pm$ SD.

## 6.4 RESULTS

### *6.4.1. Study population*

Physical characteristics of the study population are described by gender sex in **Table 6.1**. In **Table 6.2** numbers of boys and girls entered in the study are given per age group. A significant difference between boys and girls was observed for length, weight, body circumferences, MAMC, Quetelet's index, and TOBEC-derived fat-free mass (FFM) and percentage body fat (TBF%). Head circumference, body weight, and total length, distributed against age, were in accordance for both sexes to the 1980 Dutch national growth-survey percentiles [25]. Calculated upper-arm circumference percentiles of the present study group were in accordance with (extrapolated) reference percentiles of 1-74-y-old subjects of Frisancho [26]. Triceps skinfold-thickness values agreed with the reference data of Tanner and Whitehouse [27], subscapular skinfold-thickness values were slightly lower on average. We were not able to find any reference data for infants on biceps, quadriceps and suprailiacal skinfold-thicknesses. MAA and MAMC values agreed with reference values by Sann et al [23]. Because the triceps values were slightly higher in the present study than values reported by Sann et al. [23], MAMA values in the present study

b Here: root mean squared error = $\sqrt{\{\sum(\text{TOBEC} - \text{anthropometry})^2 / n\}}$

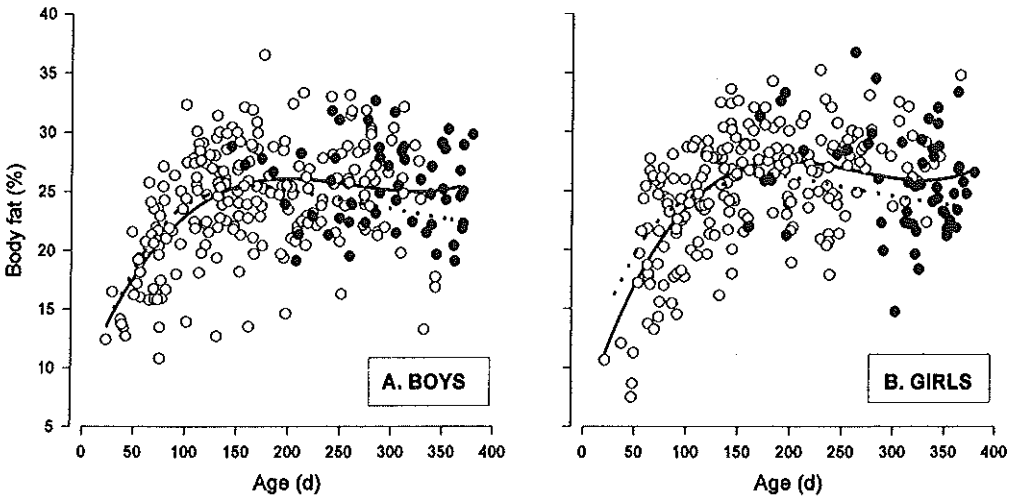


**Figure 6.1.** Percentage body fat by total-body electrical conductivity (TOBEC) for boys and girls plotted against age for the first study group (○) and the second study group (●). Solid line represents a third-degree polynomial fit on the pooled data of both study groups, the dotted line refers to Fomon et al 's reference data [6] for total-body fat.

were slightly lower than reported by Sann et al. Mean TOBEC body fat (TBF) values agreed with published mean values of Fomon's reference child [6]. **Figure 6.1** shows the distribution against age of TBF% for both study populations, as well as the only published reference values for infant TBF, reported as mean values by Fomon et al [6].

*6.4.2. Classic anthropometry*

First, traditional anthropometric indexes for body composition and single anthropometric measurements were correlated with TOBEC-derived body-composition estimates. Only minor differences in $r^2$ were observed between sexes (data not shown). **Table 6.3** shows that most classic anthropometric indexes are at best only moderately correlated with composition as derived by TOBEC measurements. The ratio of weight to length as well as the calf circumference were well correlated with TBF. Weight and length were well correlated with FFM. The relevant linear regression equations are as follows:

$$\begin{aligned} \ln TBF &= -8.6928 + 3.2527 \ [\ln \{\text{calf circumference (cm)}\}] \\ r^2 &= 0.83 \\ SE &= 0.173 \ \ln(kg) \end{aligned} \quad (1)$$

$$\ln TBF = -5.5460 + 2.5719\ [\ln(\text{weight / length})\ (\text{kg/m})] \quad (2)$$
$$r^2 = 0.84$$
$$SE = 0.164\ \ln(\text{kg})$$

$$\ln FFM = 0.8205 + 0.1174\ [\text{weight (kg)}] \quad (3)$$
$$r^2 = 0.93$$
$$SE = 0.050\ \ln(\text{kg})$$

$$\ln FFM = -0.4286 + 0.0314\ [\text{length (cm)}] \quad (4)$$
$$r^2 = 0.89$$
$$SE = 0.063\ \ln(\text{kg})$$

As **Table 6.3** shows, TBF% was poorly predicted by all tested anthropometric variables. Dividing anthropometric variables by body weight did not improve the correlation.

*6.4.3. Best multiple-linear-regression equations predicting TBF and FFM*

With use of all possible subset regression a systematic search was performed for the most optimal combinations of anthropometric variables in a multiple-linear-regression model. Log-transformed TBF (lnTBF), TBF%, and log-transformed FFM (lnFFM) were used as dependent variables.

In **Appendix 1** the adjusted $r^2$ values, residual SDs and regression coefficients of the best subset regressions are summarized. Inclusion of more than three variables in the subset regression analysis resulted in only a minor improvement of the residual SD, although the increase in $r^2$ was still statistically significant. The improvement was $<15$ g in the prediction of $TBF_{TOBEC}$ and $FFM_{TOBEC}$, and $<0.1\%$ body fat in the prediction of $TBF\%_{TOBEC}$.

Ln(calf circumference), being the best single predictor of lnTBF ($r^2 = 0.83$, Table 6.3), was always present in subsequent subsets. The combination of three variables best predicting lnTBF was: calf circumference, weight and sum of three or five skinfold thicknesses ($r^2 = 0.90$). For the prediction of lnFFM weight and length were the best single predictors (Table 6.3). There was a minor improvement in $r^2$ over that of weight alone, when the computer was allowed to search for two optimal independent variables, and no improvement when a subset of three variables was allowed ($r^2 = 0.95$). No combination of anthropometric variables predicted TBF% more accurately than $r^2 = 0.67$.

**Table 6.3.** Relationship between TOBEC-derived body-composition estimates and traditional and single anthropometric variables derived from the main study population.

| | $r^2$ | | |
|---|---|---|---|
| Independent variable: | TBF [1] | TBF% [2] | FFM [3] |
| Weight (kg) : length (m) | **0.84** [4] | 0.43 | **0.85** [4] |
| Quetelet's index (kg/m$^2$) | 0.59 | 0.44 | 0.38 |
| Ponderal index (kg/m$^3$) | 0.10 | 0.03 | 0.11 |
| Weight | **0.81** [4] | 0.33 | **0.93** [4] |
| Age | 0.60 | 0.19 | 0.73 |
| Upper arm anthropometry | | | |
| area | 0.65 | 0.37 | 0.47 |
| muscle circumference | 0.40 | 0.16 | 0.46 |
| muscle area | 0.40 | 0.15 | 0.35 |
| fat area | 0.45 | 0.38 | 0.20 |
| muscle ratio | 0.02 | 0.02 | 0.12 |
| fat ratio | 0.09 | 0.08 | 0.03 |
| ratio of arm/head circumference | 0.25 | 0.26 | 0.07 |
| Skinfold thickness | | | |
| biceps | 0.30 | 0.26 | 0.17 |
| triceps | 0.21 | 0.25 | 0.05 |
| subscapular | 0.15 | 0.20 | 0.05 |
| suprailiac | 0.13 | 0.24 | 0.08 |
| quadriceps | 0.57 | 0.49 | 0.22 |
| sum of 3 skinfolds | 0.52 | 0.52 | 0.16 |
| sum of 5 skinfolds | 0.51 | 0.53 | 0.13 |
| Circumferences | | | |
| head | 0.58 | 0.18 | **0.84** [4] |
| chest | 0.65 | 0.25 | **0.85** [4] |
| midthigh | 0.75 | 0.44 | 0.64 |
| calf | **0.83** [4] | 0.52 | 0.66 |
| midupper arm | 0.62 | 0.36 | 0.53 |
| Lengths | | | |
| total | 0.63 | 0.22 | **0.89** [4] |
| crown-rump | 0.63 | 0.24 | **0.81** [4] |
| upper arm | 0.50 | 0.20 | 0.64 |
| lower arm | 0.50 | 0.19 | 0.66 |

TBF, total body fat; FFM, fat-free mass.
[1] TBF and independent variables both log-transformed.
[2] TBF% and independent variables not transformed.
[3] FFM log-transformed, independent variables not transformed.
[4] $r^2 > 0.80$.

**Table 6.4.** Physical characteristics of the second population and body-composition results derived by total-body electrical conductivity (TOBEC).

| | boys (n = 54) | | girls (n = 56) | |
|---|---|---|---|---|
| | mean ± SD | range | mean ± SD | range |
| Age (d) | 229 ± 62 | 144-379 | 304 ± 62 | 159-379 |
| White:nonwhite | 48:6 | | 53:3 | |
| Weight (kg) | 9.35 ± 1.41 | 6.25-12.4 | 8.64 ± 1.28 [1)] | 5.48-10.8 |
| Lengths (cm) | | | | |
| total | 74.8 ± 4.2 | 63.8-83.0 | 73.5 ± 4.6 [1)] | 62.3-82.4 |
| crown-rump | 49.1 ± 2.9 | 42.8-56.4 | 47.8 ± 2.6 [1)] | 39.9-52.7 |
| upper arm | 14.4 ± 1.1 | 11.8-17.1 | 13.8 ± 1.0 [1)] | 11.7-15.5 |
| lower arm | 12.3 ± 1.0 | 10.0-15.0 | 11.9 ± 1.1 [2)] | 9.8-15.7 |
| Circumferences (cm) | | | | |
| head | 46.4 ± 1.9 | 42.1-51.0 | 45.0 ± 1.8 [1)] | 40.0-48.0 |
| chest | 47.5 ± 2.8 | 42.1-53.2 | 45.9 ± 2.7 [1)] | 38.8-51.3 |
| mid-thigh | 24.0 ± 2.1 | 19.5-29.0 | 23.6 ± 2.5 | 18.5-30.6 |
| calf | 18.4 ± 1.5 | 15.0-21.2 | 17.9 ± 1.4 [3)] | 14.0-20.3 |
| midupper-arm | 15.7 ± 1.3 | 13.2-18.7 | 15.1 ± 1.1 [2)] | 12.4-18.1 |
| Skinfolds (mm) | | | | |
| biceps | 8.7 ± 2.1 | 5.2-13.9 | 8.7 ± 2.1 | 4.5-12.6 |
| triceps | 13.1 ± 2.8 | 7.3-20.9 | 13.3 ± 3.3 | 5.9-19.1 |
| subscapular | 7.6 ± 1.5 | 5.0-11.2 | 7.8 ± 1.9 | 4.3-12.6 |
| suprailiac | 5.9 ± 1.7 | 3.3-11.0 | 5.8 ± 1.9 | 3.0-12.5 |
| quadriceps | 18.7 ± 3.4 | 10.0-26.6 | 19.6 ± 3.1 | 12.7-26.8 |
| Quetelet's index | 16.6 ± 1.3 | 14.1-19.4 | 15.9 ± 1.3 [2)] | 13.1-18.4 |
| Sum of 3 skinfolds | 39.4 ± 5.5 | 28.3-54.0 | 40.7 ± 7.1 | 27.2-58.3 |
| Sum of 5 skinfolds | 54.0 ± 8.1 | 38.7-75.2 | 55.2 ± 9.7 | 37.1-79.4 |
| MAA ($mm^2$) | 1984 ± 323 | 1386-2783 | 1831 ± 273 [2)] | 1223-2607 |
| MAMC (cm) | 11.6 ± 1.2 | 8.0-14.0 | 10.9 ± 1.1 [2)] | 8.7-13.9 |
| MAMA ($mm^2$) | 1086 ± 219 | 519-1564 | 962 ± 195 [2)] | 601-1530 |
| MAFA ($mm^2$) | 898 ± 210 | 469-1436 | 869 ± 228 | 338-1312 |
| Body composition (TOBEC): | | | | |
| TBF (kg) | 2.40 ± 0.58 | 1.47-3.72 | 2.25 ± 0.58 | 0.97-3.57 |
| TBF (%) | 25.5 ± 3.6 | 19.1-32.6 | 25.8 ± 4.4 | 14.8-36.7 |
| FFM (kg) | 6.95 ± 0.98 | 4.75-8.85 | 6.39 ± 0.89 [1)] | 4.28-8.02 |

MAA = midupper-arm area, MAMC = midupper-arm muscle circumference, MAMA = midupper-arm muscle area, MAFA = midupper-arm fat area., TOBEC = total-body electrical conductivity, TBF = body fat, FFM = fat-free mass
[1 - 3)] Significantly different from boys (ANOVA with gender as factor and age as covariable): [1)] $P < 0.001$, [2)] $0.001 < P < 0.010$, [3)] $0.010 < P < 0.050$

*6.4.4. New simple anthropometric indexes*

The best correlation with TOBEC-derived lnTBF was found for the (log-transformed) product of calf circumference and weight by length ($r^2 = 0.87$), which was only slightly improved by inclusion of the square root of the sum of skinfolds ($r^2 = 0.89$). No difference was found in $r^2$, neither when three or five summed skinfolds were entered. Best correlation with lnFFM was found for the square root of the product of weight and length ($r^2 = 0.95$). The relevant linear regression equations are

$$\begin{aligned} \ln TBF &= -0.358 + 1.499\,[\ln(\text{weight} \cdot \text{calf circumference/length})] \\ r^2 &= 0.87 \\ SE &= 0.148 \end{aligned} \tag{5}$$

$$\begin{aligned} \ln TBF &= -2.219 + 1.176\,[\ln(\text{weight} \cdot \text{calf circumference} \sqrt{\{\text{sum 3 skinfolds}\}/\text{length}})] \\ r^2 &= 0.89 \\ SE &= 0.138 \end{aligned} \tag{6}$$

$$\begin{aligned} \ln FFM &= 0.433 + 0.056 \sqrt{(\text{weight} \cdot \text{length})} \\ r^2 &= 0.95 \\ SE &= 0.044 \end{aligned} \tag{7}$$

All other tested combinations yielded poorer relations with TBF and FFM. No good index was found for TBF%.

*6.4.5. Validation of anthropometric body-composition predictors in a second population of infants*

We validated the best-scoring anthropometric parameters and the new prediction equations in a second population of 110 healthy full-term infants (54 boys and 56 girls, aged between 144 and 379 d). Measurements were performed by a well-trained second observer. See **Table 6.4** for physical characteristics of the infants.

***Interobserver variation.*** Skinfold thickness measurements (on all five locations) were overestimated on average by ≈10% by the second observer as compared to the first ($P<0.001$). Upper- and lower-arm length were overestimated by ≈6% by the second observer ($P < 0.001$) and crown-rump length by ≈1% ($P < 0.001$). On average calf, thigh, chest and upper-arm circumferences differed ≈2.5% ($P< 0.001$). No significant interobserver difference for head circumference and total length was found.

***Body-composition calculation with new prediction equations.*** The multiple-linear-regression prediction equations from **Appendix 1** as well as the prediction equations given for the well-correlating classic and new anthropometric variables and indexes (Eq 1, 2, 3, 5, 6, and 7) were used to calculate $TBF_{EQUATION}$ and $FFM_{EQUATION}$.

**Table 6.5**. Validation of anthropometric prediction equations against total-body electrical conductivity (TOBEC) in a second population.

| | Regression against TOBEC | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Body fat (kg) | | | | | Fat-free mass (kg) | | | | |
| Prediction equations | $r^2$ | Residual SD | Mean difference (kg) [1] | RMSE (kg) | RMSE of main population (kg) ($n$ = 435) | $r^2$ | Resid-ual SD | Mean difference (kg) [1] | RMSE (kg) | RMSE of main population (kg) ($n$ = 435) |
| From Appendix 1 | | | | | | | | | | |
| Eq 2a | 0.77 | 0.282 | -0.040 | 0.168 | 0.276 | 0.92 | 0.279 | -0.016 | 0.292 | 0.255 |
| Eq 3a | 0.85 | 0.230 | -0.202 | 0.332 | 0.234 | 0.92 | 0.271 | -0.007 | 0.282 | 0.239 |
| From Results | | | | | | | | | | |
| Eq 1 | 0.73 | 0.302 | 0.186 | 0.358 | 0.315 | - | - | - | - | - |
| Eq 2 | 0.78 | 0.272 | -0.105 | 0.328 | 0.276 | - | - | - | - | - |
| Eq 3 | - | - | - | - | - | 0.86 | 0.361 | 0.055 | 0.400 | 0.295 |
| Eq 5 | 0.81 | 0.253 | -0.001 | 0.273 | 0.265 | - | - | - | - | - |
| Eq 6 | 0.86 | 0.220 | -0.127 | 0.288 | 0.248 | - | - | - | - | - |
| Eq 7 | - | - | - | - | - | 0.92 | 0.278 | -0.105 | 0.286 | 0.257 |

[1] $FFM_{TOBEC} - FFM_{prediction\ equation}$

Table 6.5 shows the resulting $r^2$ and residual SDs when $TBF_{EQUATION}$ and $FFM_{EQUATION}$ from the second population were regressed against $TBF_{TOBEC}$ and $FFM_{TOBEC}$. Correlations were only slightly lower than in the main population. Table 6.5 also shows the mean absolute differences (kg) between TOBEC- and anthropometry-derived TBF and FFM, as calculated from the corresponding adjusted means. $TBF_{APPENDIX\text{-}1\ 3A}$ (with skinfold thicknesses incorporated) had a larger mean difference with $TBF_{TOBEC}$ than $TBF_{APPENDIX\text{-}1\ 2A}$. FFM equations (all without skinfold thicknesses incorporated) exhibited smaller differences with TOBEC body composition than with the TBF equations, which agrees with the presently found interobserver differences. RMSEs did not differ much between prediction equations, being somewhat larger in the second population than in the main population (Table 6.5).

## 6.5. DISCUSSION

### *6.5.1. Classic anthropometry*

A basic assumption for using body-composition data for the assessment of nutritional status is that these measurements have to be a direct estimation, or a good reflection, of energy deposition (ie, total body fat storage as reflected by the adipose tissue, or TBF, compartment) and/or total-body protein storage (as reflected by the lean body mass, or FFM, compartment). The present study shows that, based on this basic assumption, nutritional assessment in infants is not accurate when using most of the classic anthropometric indexes.

A poor correlation with whole-body TBF and FFM has been described for Quetelet's index and skinfold thickness in early infancy [13, 14], and for upper-arm anthropometry in adults [28]. We found that these traditional anthropometric indexes as well as sum of skinfold thicknesses and Ponderal index (weight by length cubed) did not correlate well in the present population of infants with body-composition estimations by TOBEC. Although upper-arm anthropometry has been described, for example, for children and adults as an inexpensive and simple screening tool for gross protein-energy malnutrition in developing countries [29-31], it does not reflect the composition of the infants' whole body and probably is of limited value for nutritional assessment in infants $\leq 1$ y. In contrast with Ponderal and Quetelet's index the ratio of weight by length was well correlated with $TBF_{TOBEC}$. This might be due to the slightly less-accurate supine length measurements in infants as compared with height measurements in children and adults. The contribution of these less-accurate length measurements in the indexes with length squared and cubed will therefore be more exaggerated.

We were surprised to find calf circumference amongst the major predictors of TBF. It was the best single predictor of TBF, also in the second population of infants. Calf circumference independently contributes to TBF ($r^2 = 0.83$, $P < 0.0001$), also when length is introduced into the multiple-linear-regression equation (partial $r^2 = 0.76$ for calf circumference, $P < 0.0001$), and when length and weight are introduced (partial $r^2 = 0.44$

for calf circumference, $P < 0.0001$). However, the introduction of weight will result in an underestimation of the relation between calf circumference and TBF, because TBF is also directly correlated with weight (infants with equal lengths and increasing amounts of TBF will show an increase in both calf circumference and weight).

This relatively good correlation between calf circumference and TBF is no artifact caused by the TOBEC methodology. It is known that the cross sectional area of a conductor in an electromagnetic field (like that inside the TOBEC instrument) largely contributes to the output signal [2, 19]. However, when this phenomenon is the underlying cause of the good correlation between $TBF_{TOBEC}$ and calf circumference, then chest, thigh, and abdominal circumference should have been correlated even more with TOBEC-derived body fat because of their larger cross sectional areas. Also, because the TOBEC signal is proportional to the subject's FFM and not directly to $TBF_{TOBEC}$, in case of an artifact, one would expect a relationship of calf circumference with FFM rather than with TBF. Kabir and Forsum [15] showed by ultrasonography in healthy infants $<3$ mo of age that the thickness of the fat layer over the calf muscle was almost as much as the fat layer thickness over the quadriceps muscle. The calf is a relative stiff part of the limbs and can be more accurately measured to the nearest millimeter than other limb circumferences. Therefore we suggest that calf circumference is a good and inexpensive alternative to measure body fatness in infants and suitable as a screening tool for infant nutritional assessment in, for example, developing countries. Correlation of calf circumference with TBF and FFM was better than for traditional screening tools for malnutrition used in older children and adults. We have provided the linear prediction equations for conversion of calf circumference data to TBF(kg). An inter-observer variation should be taken into account , which was, however, much smaller than for the skinfold thickness measurements (3% as compared to 10% for skinfolds in the present study ). The equations have not been proven to be valid in ethnic groups other than whites. The results of the present study should be used with caution in premature infants. The same holds true for infants and toddlers which have learned to walk, because this ability might very well change the local composition of the cross sectional area of the calf (e.g. by increasing the contribution of the soleus muscle).

*6.5.2. New prediction equations*

We used "All Possible Subsets Regression" software to find the best linear combinations of anthropometric measurements in a multiple-linear-regression model to predict TOBEC-derived TBF, TBF% and FFM. Inclusion of more than three input variables in the subset still resulted in a significant improvement of the $r^2$; however the net effect on the residual SE was too small to be of any clinical importance. For this reason we here presented multiple-linear-regression equations with at most three anthropometric variables. Only a minority of the 24 anthropometric input variables were found in the results of the all possible subsets regression (see **Appendix 1**)., which shows that, as stated earlier, most measurements of body dimensions are not closely related to the composition of the body in terms of total TBF and FFM.

It should be said that, apart from the fact that all possible combinations (in a multiple-regression model and as searched for by a heuristic approach using nonlinear regression) of anthropometric indexes to predict TBF and FFM have now been fully explored, no attempt has been made to develop more complex algorithms, like Weststrate and Deurenberg's [17] and Dauncey et al's equations [18]. These two classic prediction equations used for the quantitative estimation of TBF in infants were recently evaluated by us against TOBEC [4]. The original Dauncey equation was modified by us, which improved the correlation with TOBEC-derived body composition (skinfold thickness values were halved to adapt for the bilayer nature of the skinfold grasp, and the quadriceps skinfold was used for calculation of lower-extremities fat instead of the triceps-skinfold thickness). Weststrate's equation overestimated $TBF_{TOBEC}$ by ≈0.5 kg. In that study we used untransformed TBF values. When log-transformations were applied as described in the present study the following $r^2$ values for the regression against $TBF_{TOBEC}$ and $FFM_{TOBEC}$, respectively, are found: $r^2$ = 0.80 and $r^2$ = 0.94 for the modified Dauncey equation and $r^2$ = 0.88 and $r^2$ = 0.95 for Weststrate's equation.

For the prediction of TBF, prediction equation 3a (see **Appendix 1**) is slightly better than Weststrate's equation. For the prediction of FFM, comparable results are found for both Weststrate's and the all-possible-subsets-regression" equations. The same holds true when Dauncey's and Weststrate's TBF and FFM were calculated for the second population ($r^2$ = 0.73 and $r^2$ = 0.92 for modified Dauncey TBF and FFM, and $r^2$ = 0.76 and $r^2$ = 0.92 for Weststrate's TBF and FFM, respectively).

*6.5.3. New simple indexes*

Indexes might be preferred in many (clinical) circumstances to complex prediction equations if they correlate well with composition. Therefore we also extensively searched for new, simple, anthropometric body-composition indexes. The new indexes we calculated were highly correlated with TOBEC-derived composition in both populations. Their relation with $TBF_{TOBEC}$ and $FFM_{TOBEC}$ was almost as good as the multiple-linear-regression prediction equations. The best anthropometric indexes we found were as follows: 1) for TBF (kg): (weight · calf circumference/length) and (weight · calf circumference · √(sum of skinfolds)/length), 2) for FFM (kg): √(weight · length). These indexes were also highly correlated with $TBF_{TOBEC}$ and $FFM_{TOBEC}$ in a second population of healthy, full-term infants aged between 5 and 12 mo, measured by a second observer.

Several of the best anthropometric indexes and multiple-linear-regression equations contain length, which is also used in the calculation of the transformed TOBEC value. One might argue that these good correlations are therefore inherent artifacts. Anthropometric indexes are always implicitly related (and not necessarily in a linear way) to length, especially in a population of growing subjects. However, as can be seen from Appendix 1, the best anthropometric prediction equations either did not contain length (*see* TBF), or substitution of length by sum of skinfold thicknesses (for example) in the multiple-linear-regression did not decrease the correlation (*see* FFM). It is therefore not likely that

incorporation of length in the simple indexes will have a major (artificial) effect on the correlation with FFM or TBF by TOBEC.

Mean differences from TOBEC-derived body composition were smallest for anthropometric indexes without skinfold thicknesses incorporated (Table 6.5). RMSE, however, was not related to whether or not skinfolds were included in the equation. For all equations the RMSE was ≈0.3-0.4 kg. This indicates that the accuracy is more affected than is the precision of the body-composition measurement when anthropometric prediction equations are used. This is in line with the large interobserver variation found for skinfold thicknesses between the two observers of the present study and the relatively small CV of the duplicate skinfold measurements (CV < 3%).

The second population mainly consisted of infants aged between 6 and 12 mo whereas the first population covered almost the entire first year. In later infancy the new FFM and TBF indexes correlated as good as or even better than the multiple-linear-regression prediction equations and Weststrate's method. This indicates that these indexes are robust predictors of body composition in the entire first year of life and thus a simple alternative for nutritional assessment in infants in the absence of a precise, modern body-composition technique.

Beside their use as indexes, we have provided their linear-regression equations for conversion of the indexes to absolute amounts of TBF and FFM. Also here it must be stressed that the equations have not been proven to be valid in ethnic groups other than whites.

*6.5.4. Accuracy and precision.*

As we found earlier for two anthropometry-based algorithms to calculate TBF in infants [4], the precision of anthropometric measurements based on skinfold thickness is not good enough to use these variables and their derivatives for individual estimates of TBF and FFM. This is corroborated by the large residual SD found in the present study between TOBEC-derived body composition estimates and the various anthropometric derivatives. In general the precision of an individual prediction derived from a prediction equation is roughly twice its residual SD, which for the present study is ≈0.6 kg. This results for an individual estimate in a relative error of 16% of FFM and 90% of TBF in 1-mo-old reference child, and 7.6% of FFM and 26% of TBF in a 1-y-old reference child [6]. Only a small part of the residual error of ≈0.30 kg is due to TOBEC, for it has been found that the residual SD of TOBEC versus carcass analysis is only 0.077 kg [5]. Accuracy of anthropometric skinfold-based estimates is known to be affected by interobserver variation. Except for use in intra individual comparison of body-composition changes and comparison of body composition between study groups and measured by one observer, prediction equations based on these measurements should be used with great caution, performed by people well-trained in anthropometric measurements, and not be misused for individual estimates of infant body composition. This study was performed in healthy, term infants. It is not known whether the accuracy and precision of the derived prediction equations can be

extrapolated to sick infants. Especially large deviations from normal body proportions may exhibit changes in the outcome of combined anthropometric variables.

The present study was a cross-sectional study and hence the validity of both the TOBEC and the anthropometric prediction equations to assess changes associated with growth during the first year of life on a longitudinal basis remains to be determined. The minimum detectable change in body composition as measured by TOBEC can be roughly estimated from the residual SD of the calibration procedure. The 95% confidence limits of the difference between two measurements (ie, the minimal significant detectable increase in TBF and FFM) can be calculated as $\sqrt{[(error_1)^2 + (error_2)^2]}$ , which is a general expression for the error observed in the difference of two independent observations, where $error_1$ and $error_2$ are the 95% confidence limits (ie, 2SD) of the subsequent observations. The residual SD of the anthropometric prediction equations and indexes was ln(0.15), which is consistent with a 95% confidence limit range in TBF of 0.37 - 0.67 kg for a child with 0.5 kg of TBF and with a range in TBF and FFM of 2.22-4.05 kg TBF range in a child with 3 kg of TBF.

As the present study was performed in normal, healthy subjects, the validity of the newly derived prediction equations and indexes for use in sick infants remains to be determined. For the TOBEC technique, as has been studied earlier by us using nonhuman phantoms [7], only major changes in body electrolytes (mainly bicarbonate shifts) and large deviations from normal body shape will disturb TOBEC outcome.

*6.5.5. Conclusions*

We conclude from this study that for infants between 1 and 12 mo of age:

1) Upper arm anthropometry, skinfold-thickness measurements and Ponderal and Quetelet's index are poorly correlated with TOBEC-derived body composition estimates.

2) Calf circumference is a very convenient and simple anthropometric measurement and corresponds well with whole-body fat estimations in infants. Interobserver variation is present but much less than for skinfold thickness measurements (3% in the present study). It might be worth while to further explore the specificity and sensitivity of calf circumference measurements for the prediction of malnutrition in infants in developing countries, for example.

3) Several prediction equations are provided with their corresponding $r^2$ and residual SDs to derive TBF and FFM from various anthropometric indexes. In field studies and nutritional screening programmes in developing countries weight, length, and/or skinfold thickness measurements are not always available. Depending upon the available anthropometric measurements the appropriate equation relating anthropometric indexes to infant body composition can be chosen from Appendix 1 [c].

4) However, as was true for Dauncey et al's [18] and Weststrate and Deurenberg's [17] prediction equations, the large residual SD prohibits accurate body-composition estimations by these equations in individual patients (this is especially true for TBF). Measurement precision

[c] Details of additional equations are available from the authors.

did not differ much between the various prediction equations. However, because of the notorious effect of interobserver variation in skinfold measurements (≈10% as found in the present study), users should bear in mind possible interobserver differences when using skinfold-related prediction equations. We therefore recommend the use of prediction equations and anthropometric indexes without incorporation of skinfold thickness.

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*Chapter 7*

# Standards for body fat and fat-free mass in infants [a)]

## 7.1 SUMMARY

Data on body composition in conjunction with reference centiles are helpful in identifying the severity of growth and nutritional disorders in infancy and for evaluating the adequacy of treatment given during this important period of rapid growth. Total body fat (TBF) and fat-free mass (FFM) were estimated from total-body electrical conductivity (TOBEC) measurements in 423 healthy term Caucasian infants, aged 14 -379 days. Cross-sectional age, weight, and length related centile standards are presented for TBF and FFM. Centiles were calculated using Altman's method based on polynomial regression and modeling of the residual variation. The TBF percentage steeply increased during the first half year of life, and slowly declined beyond this age. Various simple, TOBEC derived anthropometric prediction equations for TBF and FFM are available to be used in conjunction with these standards. Regression equations for the P50 and the residual SD, depending on age, weight or length, are provided for constructing centile charts and calculating standard deviation scores.

## 7.2 INTRODUCTION

Assessment of body composition provides important data on nutritional status and quality of growth of children. This is especially true for the period of rapid growth, as happens during infancy. Malnutrition in intrauterine and early extrauterine life has been associated with altered growth [1,2], adult morbidity [3,4] and decreased birth weight of their offspring [5]. The style of infant feeding may be relevant to the development of childhood

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obesity [6]. Infant and childhood obesity have been related to adult obesity [7-9]. With these observations in mind the need for reliable tools to monitor nutritional status in infancy and early childhood becomes more urgent. The availability of body composition standards will greatly enhance the usefulness of body composition data in the treatment of nutritional disorders and in evaluating the adequacy of treatment interventions. However, centile standards on total body fat (TBF) and fat-free mass (FFM) in infants have not yet been published.

Limited data are available on the body composition of human infants and the changes that occur during the first year of life. The paucity of data is mainly due to the limitations of existing methods of measurement, which are either invasive, use radioactivity, or require cooperation of the subject. Traditionally, nutritional status in infants has been assessed using skinfold measurements [10,11], arm muscle area, or body mass index [12,13], which are relatively insensitive; however, their accuracy in predicting fat and lean mass has been found to be limited in infants [14]. Skinfold measurements are notorious for their inter-observer variation and the inaccuracy in untrained hands [14,15], which makes them less useful in most clinical settings, with different clinicians involved in the treatment of a child. Moreover, it has been shown that skinfold thickness in infants is poorly related to total body fatness [16]. A rather accurate estimate of body water and hence FFM, and to a lesser extent TBF, can be obtained by the dilution technique using labeled water [17]. However, this technique is too expensive and cumbersome for measuring the large numbers of infants needed for the calculation of accurate reference centiles.

Recently measurement of total body electrical conductivity (TOBEC) has emerged as an accurate, precise, and reproducible method for the estimation of FFM and TBF in infants [18-20]. A TOBEC measurement is rapid, safe, easy to perform, and suitable for measurement of large numbers of infants. The instrument has been commercially available since 1989. At present TOBEC is the most reliable convenient method for routine estimations of infant body composition, but is not widely used due to the (still) relatively high price of a TOBEC instrument (approximately $ 45,000), and the fact that the instrument is large and difficult to move and therefore not suitable for field studies. However, its good reproducibility, precision, and accuracy justifies the use of TOBEC as a reference method.

We present for the first time centile standards for TBF and FFM by gender for infants aged 1 to 12 months. We recently published various simple, TOBEC derived anthropometric prediction equations for TBF and FFM [14] which can be used in conjunction with the centile standards presented in this study. Because changes in body composition are associated with changes in length and weight, centiles for TBF and FFM were constructed against length and weight as well as against age. Regression equations for the P50 and the residual SD, depending on age, weight, or length, are provided for constructing charts and calculating standard deviation scores.

**Table 7.1.** Characteristics of the study group.

| | boys (n=221) | | girls (n=202) | | |
|---|---|---|---|---|---|
| | mean | range | mean | range | |
| Infants | | | | | |
| age (months) | 5.79 | *0.8-12.3* | 6.2 | *.47-12.6* | NS [1] |
| weight (kg) | 7.56 | *3.77-11.9* | 7.20 | *3.41-10.8* | $p<0.001$ |
| length (cm) | 67.7 | *54.0-83.0* | 67.1 | *51-82.4* | $p<0.001$ |
| TBF (kg) | 1.85 | *0.16-4.06* | 1.85 | *0.37-3.59* | NS |
| TBF (%) | 23.4 | *3.79-36.5* | 24.7 | *9.84-36.7* | $p=0.005$ |
| FFM (kg) | 5.71 | *3.30-8.88* | 5.34 | *3.01-8.00* | $p<0.001$ |
| head circumference (cm) | 43.2 | *36.0-51.2* | 42.5 | *35.5-48.1* | $p<0.001$ |
| Parents | | | | | |
| length mother (cm) | 169 ± 6 [2] | *152-185* | 169 ± 6 [2] | *156-189* | NS |
| length father(cm) | 182 ± 7 | *161-203* | 183 ± 7 | *165-204* | NS |
| weight mother (kg) | 66.4 ± 11.0 | *44-110* | 66.4 ± 11.5 | *46-120* | NS |
| weight father (kg) | 80.8 ± 10.5 | *59-117* | 79.9 ± 12.1 | *56-135* | NS |

TBF=total body fat; FFM=fat-free mass.
[1] Differences between boys and girls were tested for infant parameters by ANOVA with age and age$^2$ as covariable, and for parental parameters by Student *t* test (NS = not significant).
[2] Mean ± SD

## 7.3 METHODS

### *7.2.1. General protocol*

In cooperation with the local child health clinics of the Rotterdam Home Care Foundation, a random sample of 2000 infants (living in the Rotterdam metropolitan area and aged between 1 and 12 months) was drawn from their database. These families were sent a letter with detailed information on the study, in which they were invited to participate in the study. A total of 601 parents responded. For reasons of anonymity, non-responding families could not be checked for socioeconomic status, birth weight, and so on. To ensure an optimal representation of the general population, no selection on the 601 infants was made on the basis of length, weight, or body fatness; selection was only made on the basis that the mother and infant were healthy. All infants with no history of chronic illness, born from healthy mothers with no history of major pathology during pregnancy or delivery and not under chronic medication, were enrolled in the study. After enrolment the parents were sent an invitation to attend for the measurement and a questionnaire to record parental weight, height, health, socioeconomic state, nationality, family constitution, and other

details. Information on pregnancy, labor, the infant's birth weight, early growth, feeding, and state of health was also obtained. The data of all healthy Caucasian infants ($n$ = 423) were selected for the present study. Written informed consent was obtained from the parents. The study protocol was approved by the ethics review boards of the Erasmus University / University Hospital Rotterdam and the Rotterdam Home Care Foundation.

*7.3.2. TOBEC measurements.*

Details about the TOBEC method, accuracy, reproducibility, the calibration equation used and the calculation of TBF and FFM have been published before [18-23]. Briefly, the TOBEC instrument (Model HP-2; EM-SCAN Inc., Springfield IL, USA) is a large solenoidal coil driven by a 2.5 MHz oscillating radio-frequency current. The principle underlying TOBEC is that lean tissue is far more electrically conductive than fat, due to the much greater content of electrolytes dispersed in the FFM. When a conductive mass passes through the electromagnetic field, the magnetic component of the field induces small eddy currents within the conductive mass, producing a small amount of heat. The energy of the eddy currents is dissipated from the magnetic field. The total energy loss is detected as a phase change in coil impedance. This phase change serves as an index of the amount of conductive mass. The amount of fat is calculated by subtraction of the estimated conductive mass (the FFM) from body weight. Electric and magnetic field intensities are less than 0.02% and 0.4% respectively of the American National Standards Institute limits (in $^{mW}/_{cm}2$) for continuous human exposure [21].

Body temperature affects TOBEC outcome [22]; therefore infants with apparent fever or illness were measured after recovery. Infants were not fed for at least 2 hours before the measurement. To prevent cooling and to ensure geometric homogeneity between infants with respect to the introduction of the conductive mass into the electromagnetic field, infants were undressed and carefully swaddled in a large blanket, while care was taken that limbs were not flexed and did not touch each other or the trunk. Infants were placed on their back on the sledge of the instrument. A pacifier was allowed when necessary. One TOBEC reading took approximately 10 seconds. A complete TOBEC measurement consisted of 10 reliable 10-s readings which were averaged for the FFM calculation. If urination occurred, the infant was swaddled again in a dry blanket and remeasured. Movement or crying during a reading was also a reason for remeasuring the infant. In the present study background measurements averaged 39.6 (SD = 2.9) TOBEC units and the coefficient of variation (CV) of the ten 10-s readings was 1.24 % (0.58)%.

After the TOBEC measurement infants were weighed naked on an electronic baby scale (Instru Vaaka Oy, Vaany, Finland) to the nearest 1 g (0-3 kg), 2 g (3-6 kg) or 5 g (6-10 kg), and recumbent crown-heel length was measured to the next succeeding mm on a length board. Fronto-occipital head circumference was measured to the nearest mm with a 1 cm wide standard plastic measurement tape.

*7.3.3. Statistical procedure.*
Centiles were constructed from the raw data using Altman's procedure [24]. A detailed description is given in paragraph 7.6 of this chapter. TBF (kg), TBF (%), and FFM (kg) were used as dependent (Y) variables. Age (months), weight (kg), and length (cm) were used as independent (X) variables. All calculations were performed separately for boys and girls. The validity of the centiles was assessed by calculating the percentage of data points above and below the 10th and 90th centile and tested for significant deviation from the expected distribution by the $\chi^2$ test [26]. Details on the assessment of the accuracy and precision of the centiles are described in paragraph 7.6 of this chapter. An effect was assumed to be statistically significant at a p value of $< 0.05$.

## 7.4 RESULTS

*7.4.1. Subject characteristics*
Subject characteristics are summarized in **Table 7.1**. A significant difference between sexes was present for weight, length, TBF percentage, FFM, and head circumference. The distribution of body lengths and weights was in agreement with the Dutch growth charts centiles [25]. All infants were born at term without a history of serious illness, and were clinically healthy at the time of the measurement. Mean gestational age was $40.0 \pm 1.3$ w, range 37.0 - 43.3. **Table 7.2** shows the most important environmental factors that might affect infant growth and body composition.

*7.4.2. Reference centiles.*
**Figures 7.2** to **7.10** show the original data points for FFM (kg), TBF (kg) and TBF (%) against, respectively, age, weight and length by gender. The 90th, 75th, 50th 25th and 10th centile, derived from these data points, have been drawn in each plot. **Table 7.3** shows the check on the percentage of data points beyond the 10th and 90th centile. A $\chi^2$ test showed no significant deviations from the expected distribution. The regression equations of the P50 and the residual SD as depending on X are provided in **Table 7.4** [b].

## 7.5 DISCUSSION

*7.5.1. Reference centiles*
Our study is the first providing centile standards which describe the normal pattern of TBF and FFM growth in infants. Published data on age related changes in TBF and FFM in infants are scarce and only *average* values derived from carcass analysis [27,28] or

[b] Centile charts for TBF (kg), TBF (%), and FFM (kg) against age, weight, and length for boys and girls have been printed in **Appendix 2.**

**Table 7.2.** Environmental factors which may influence infant growth and body composition.

| | Percentage of total number of infants | |
|---|---|---|
| | Boys | Girls |
| Gestation/delivery | | |
| Alcohol (>1 consumption /month) | 13 | 12 |
| Smoking (> 1 cigarette /day) | 25 | 20 |
| Mild hypertension (> 85 mmHg) | 5 | 5 |
| Delivery at home | 24 | 28 |
| Elective (artificially induced) partus | 14 | 12 |
| Vacuum / forceps | 7 | 8 |
| Caecerean section | 3 | 3 |
| Phototherapy for neonatal icterus | 3 | 2 |
| Parents (father / mother) | | |
| Smoking (>5 cigarettes /day) | 43 / 36 [2)] | 46 / 29 [2)] |
| Alcohol (> 1 consumption /week) | 76 / 56 | 77 / 56 |
| Breast feeding | 66 | 68 |
| Education (father / mother) [1)] | | |
| University / higher level secondary educ. | 32 / 28 [2)] | 38 / 27 [2)] |
| Intermediate level secondary educ. | 26 / 27 | 26 / 28 |
| Elementary / lower level secondary | 42 / 45 | 36 / 45 |
| Profession (father / mother) [1)] | | |
| Professional / higher management | 24 / 10 [2)] | 35 / 11 [2)] |
| Administrative | 20 / 23 | 15 / 18 |
| Skilled/clerical | 29 / 24 | 24 / 26 |
| Semi-skilled | 23 / 10 | 23 / 12 |
| Unskilled/unemployed/housewife | 4 / 33 | 3 / 33 |
| Parity [1)] | | |
| 1st Child | 35 | 34 |
| 2nd Child | 46 | 46 |
| 3rd Child | 15 | 15 |
| 4th Child or more | 4 | 5 |

[1)] No significant differences were found in total body fat, per cent total body fat, and fat-free mass between education, profession, or parity subgroups (by one way analysis of variance).
[2)] Percentage of fathers/percentage of mothers.

calculated from indirect body composition estimates [29] have been published so far. The distribution of biological scatter in TBF and FFM has not yet been quantified for growing infants. This is due to the fact that, until recently, no body composition method was accurate, simple, and convenient enough to measure the number of infants needed for calculation of accurate reference centiles.

In **Figure 7.2** to **7.10** the widely known body composition reference values published by Fomon et al [29] have been plotted in the centile charts. Fomon's age and weight related reference curves lie within our 25th and 75th centile range. From the position of Fomon's length related curves in our centile standards, it can be seen that Fomon's infants were on average smaller in length, possessed equal amounts of fat but had relatively more FFM per unit length compared with our study population. Most probably this can be attributed to a secular trend in length growth in the past 25 years, and infant

diet: whereas Fomon's infants were bottle fed (old fashioned formula), over half of the infants from the present study were breast fed.

Fomon's reference values for TBF (kg and percent) fall from above P50 to below P50. An artifact caused by TOBEC is not likely, since De Bruin et al showed that FFM and TBF measurements obtained from TOBEC and isotope dilution are strictly linearly related and not significantly different throughout the entire first year of life [20]. The difference might be accounted for by several factors. 1) Fomon used weight, length and total body water data for his 0-4 months population from formula fed infants, and he used 1979 NCHS data for his 3-10 years population; he then interpolated the TBF values proportionally to truncal skinfold thicknesses for 4 months through 3 years of age. 2) Feeding habits have changed over time; the feeding pattern of our study population are a better reflection of modern feeding habits (with a high proportion of breast feeding). Our body composition data are thus likely to be a better reflection of the average body composition of present day infants. 3) Fomon used longitudinal data, while in our study cross sectional data were used. However, the differences are so great it is unlikely they could be attributed to this.

*7.5.2. Nutritional assessment.*

Body composition data give a better insight in nutritional status and quality of growth than body weight alone, or than achieved by routine clinical examination [30]. Cross et al compared routine clinical examination by a pediatrician with upper arm circumference as the standard measure of nutritional status in infants [30]. We recently showed, however, that upper arm circumference in infants is very poorly correlated with TBF and FFM [14]. Skinfold measurements and Quetelet's index have also been found to be poorly correlated with TBF [14, 16, 18, 31]. So we can conclude that in infants these *local* anthropometric measurement, used in children and adults as a proxy for total body composition, are a poor reflection of the actual *total* energy and protein stores of the infant's body. Centile charts of these variables [10-12] are therefore of limited value in infants. Measurement of *total* body composition, represented by TBF and FFM, will provide better estimates of nutritional reserves than regional anthropometric measurements and may provide a more accurate assessment of nutritional status. For infants quantitation of TBF has been performed traditionally using the anthropometric method of Dauncey et al [32]. This method has only very recently been validated for the first time and was shown to have moderate accuracy but poor precision [18, 33]. However, this does not inevitably mean that anthropometric measurements are obsolete. We recently published - specifically for use in infants - new TBF and FFM prediction equations based on a variety of anthropometric measurements, which correlated much better with TBF and FFM [14]. Depending upon the available anthropometric data the appropriate equations can be chosen. We here give as an example:

TBF = *exp* (-0.358+1.499 [ln (weight x calf circumference / length)]) (SD=0.25, $r$=0.93).

TBF = *exp* (-6.1506+1.1453 [ln (calf circumference)] + 0.8722 [ln (weight)] + 0.4951 [ln (sum of 3 skinfolds)]) (SD=0.23, $r$=0.95).

FFM = *exp* (0.433+0.056 [$\sqrt{}$(weight x length)]) (SD=0.28, $r$=0.97).

**Table 7.3.** Percentage of all data points beyond the 10th and 90th percentile for each centile chart by gender.

| | TBF (kg) | | TBF (%) | | FFM (kg) | |
|---|---|---|---|---|---|---|
| X variable | >P90 | <P10 | >P90 | <P10 | >P90 | <P10 |
| Boys | | | | | | |
| Age | 7.7 | 8.6 | 8.6 | 8.6 | 10.9 | 9.5 |
| Length | 8.1 | 7.7 | 9.5 | 7.7 | 10.4 | 9.5 |
| Weight | 10.0 | 8.6 | 10.9 | 8.6 | 8.6 | 10.0 |
| Girls | | | | | | |
| Age | 7.4 | 10.4 | 9.9 | 10.4 | 9.4 | 9.9 |
| Length | 11.9 | 6.9 | 11.9 | 10.4 | 11.9 | 8.9 |
| Weight | 10.9 | 10.9 | 9.4 | 10.9 | 10.9 | 10.9 |

None of the distributions was significantly different from the expected (by the $\chi^2$-test).
TBF = total body fat; FFM = fat-free mass; P90, P10 = 90th centile, 10th centile.

where TBF, FFM, and weight in kg, length and calf circumference in cm and sum of 3 skinfolds (triceps, subscapular and quadriceps skinfold thickness) in mm. When no accurate body composition method (for example TOBEC or isotope dilution) is available, these new anthropometry based prediction equations are a more accurate alternative for assessing nutritional status in infants than upper arm anthropometry or skinfold thickness, and we suggest they be used for screening purposes in conjunction with the present centile standards. However, anthropometric methods are still less precise than TOBEC or isotope dilution; therefore one should remain cautious when using these data to derive individual total body composition estimations.

*7.5.3. Choice of reference method.*

TOBEC was chosen as the reference method of choice because at present it is the only accurate method that can easily supply large amounts of data on TBF and FFM in infants on a non-invasive basis [20]. The method is already widely used in human adults and in animal research. The pediatric TOBEC instrument, which has a much better coil copper winding construction and homogeneous electromagnetic field properties than the smaller TOBEC coils for animal use, is rather robust concerning changes in hydration of the FFM compartment [34], so physiological changes in FFM hydration (that is, water content of the FFM) at a given age will not seriously affect TOBEC outcome [22]. Growth related physiological changes in hydration of the FFM, which occur during the process of FFM maturation and are most evident in early life, are accounted for by the calibration procedure [35]. The pediatric TOBEC instrument has been calibrated against carcass analysis data from minipigs [23], which showed that 99.7% of the variability in TOBEC outcome could

be explained by the animals' FFM. The calibration equation showed an SD of 77 g which is consistent with an error of ±154 g (95% confidence limits). The reasons for assuming that the minipig calibration equation can be extrapolated to human infants have been outlined in detail before [19, 20, 23]. The accuracy of TOBEC has been demonstrated in two ways. No significant difference was found between body composition values derived from labeled water and derived by TOBEC in healthy term infants during the first year of life [20]. Also, a "seamless" join was found between the curves of TBF and FFM during intrauterine growth (measured by fetal carcass analysis, the gold standard) and during extrauterine growth (measured by TOBEC) [19]. At present, therefore, TOBEC is the body composition method of choice for nutritional assessment in conjunction with the present centile standards. It is to be expected that the price of the instrument will decrease in the near future when the method will be more widely used in infants.

*7.5.4. Reliability and accuracy of the centiles.*

Our centiles were derived from Caucasian babies and do not necessarily apply to non Caucasian infants. Although a limited number of infants was available, we took care that the sample was as representative as possible for the general population: only the infant's and mother's health were used as exclusion criteria. Healthy thin or obese babies, without a history of failure to thrive or chronic illness, were enrolled in the study.

It was not possible to account fully for parental socioeconomic status in this study. Firstly, it was not possible to check the socioeconomic status of the parents who did not respond, for reasons of anonymity of the randomly selected addresses. Secondly, in this study it was not possible to match each age group (for example each month) for socioeconomic status: the total number of infants would become too limited for calculation of centiles. We therefore decided to include all healthy infants meeting to the inclusion criteria. Theoretically, bias resulting from a smaller number of infants from lower socioeconomic classes might result in a slight upward shift of the centiles. However, inclusion of more infants from lower socioeconomic classes would not have lowered the P10 centile in the present study, for, although effects of socioeconomic status on maternal smoking habits and birth weight have been described, socioeconomic status was not a significant risk factor for malnutrition or obesity in this cross-sectional survey. We therefore conclude that socioeconomic effects on body composition in the first year of life are of limited importance, at least in the present study.

Because data on about 200 infants were available for the calculation of each centile chart, the present standards should be considered as the first quantitative description of the pattern of TBF and FFM growth in infants. An indication, therefore, of the accuracy of the centiles has been given in section 7.6 of this chapter. Most centile standards are not accompanied by an assessment of the errors. However, when centile standards are based on limited numbers of data points, as often occurs, the error in the estimation, especially of those centiles or standard deviation scores that lie further away from the mean, can become significant. In section 7.6 of this chapter we describe how the accuracy of the estimated

centile curves can be assessed. To give an impression of the precision of the centile curves, we provided in **Figure 7.1** the P90, P50, and P10 of TBF(%) versus age in girls together with 90% confidence intervals. This shows that precision falls at both ends of the curves. It is necessary to consider these uncertainties when using the centile charts for comparison of individual body composition data.

*7.5.5. Conclusion.*

We suggest that these centiles are a valid way of monitoring nutritional status and the effect of treatment interventions in infants. Children at either extreme of the centile curves may be at risk of obesity or undernutrition, although at present the numbers of infants were insufficient for accurately prediction of more explicit extremes, for example the 97th and 3rd centiles. Further research should disclose the relation between infants at either extremes of the centile curves and the associated risk for future health hazards. The suggested relation between malnutrition in early life and adult chronic disease [2-5] and between obesity in infancy, childhood and adulthood [7, 9] certainly adds to this challenge.

## 7.6 STATISTICAL COMMENTS.

*7.6.1. Statistical procedure*

Altman's approach based on modeling of absolute residuals was used for centile construction [24]. Data analysis was performed with the SPSS for Windows$^{TM}$ (version 6.0) statistical package. Each P50 was fitted as a polynomial by entering first through fourth powers of the X variable into stepwise linear regression. Stepping method criteria for entry and removal were $p<0.05$ and $p>0.10$, respectively, and the tolerance criterion (used to prevent against collinearity) was set at 0.00001. Residuals were examined for normality by the Lilliefors variant of the Kolmogorov-Smirnov test. In case of non-normality, Y was logarithmically transformed and the stepwise regression procedure was repeated with the transformed variable. At this stage, each residual plot was inspected visually for the presence of trends, and tested for positive autocorrelation by Durbin-Watson test [36] and for negative autocorrelation by visual inspection of the plot of the residuals against their lagged ones [36]. To allow dependence of the residual SD on X, the absolute values of the residuals were regressed on X, as suggested by Altman [24]. When a significant linear or quadratic relation was found, this relation was used to express the residual SD as a function of X and the stepwise regression procedure for the P50 was repeated once, now with $1/SD^2$ as the weighting factor. In case no significant relation of the absolute residuals with X was found, the residual SD resulting from the stepwise polynomial regression was taken to calculate the centile standards as described below. In case of a significant relation of the absolute residuals with X, the residual SD as dependent on X was estimated as the predicted mean resulting from the regression of the absolute residuals on X, multiplied by $\sqrt{(\pi/2)}$ (this factor is due to the fact that the absolute residuals

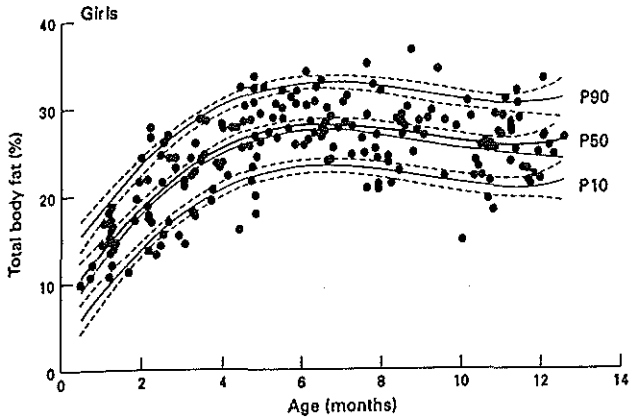


**Figure 7.1.** Example of accuracy of centile curves: 10th, 50th and 90th centiles with accessory 90% confidence intervals.

follow approximately a half normal distribution, which has a mean of $\sqrt{(\pi/2)}$ times the residual SD). Subsequently centiles were calculated as $P50 \pm k(SD)$, where $k$ is chosen as 1.282 and 0.674 to give 90% and 75% reference intervals, and as -1.282 and -0.674 to give 10 and 25 per cent reference intervals.

*7.6.2. Statistical results.*

All data groups, except for one, showed a Lilliefors/Kolmogorov-Smirnov (K-S) test statistic with $p > 0.2$, which is in agreement with a normal distribution. TBF (kg) *versus* age in males was the only variable that needed a log transformation (K-S test statistic with $p = 0.02$). In all but one regressions of TBF (kg) and FFM (kg) the residuals of Y increased with the X-variable (age, weight or length). In these cases weighted stepwise linear regression was performed, with $1/SD^2$ as weighting factor. On careful visual inspection, the difference between weighted and unweighted curves was only apparent at the edges of the P50 (that is, disappearance after weighted regression of the typical "dangling" curve ends artifact often seen in higher degree polynomial regression).

*7.6.3. Regression coefficients*

**Table 7.4** shows the regression coefficients of the P50 polynomials. For TBF(kg) against age in boys, the only dependent variable which was not approximately normally distributed and needed a log transformation, the regression is given in the form of $\ln(Y) = a + bX + cX^2 + dX^3$. When the residual SD of the regression needed to be modelled as a function of the X variable, the appropriate regression equation of the absolute residuals against the X variable, as multiplied by the factor $\sqrt{(\pi/2)}$, has been supplied in the Table. From these regression coefficients the centile charts can easily be reproduced as clarified above and standard deviation scores can be calculated by :

$$\frac{\textit{(actual Y) - (P50 value of Y for corresponding X value)}}{\textit{SD for corresponding X value.}}$$

*7.6.4. Accuracy of the centile standards*

The accuracy of an estimated centile P50 + k·SD can be judged by computing its 90% confidence interval as P50 + k·SD ± 1.65·se(P50 + k·SD). The standard error is determined as $se(P50 + k \cdot SD) = \sqrt{[se(P50)^2 + k^2 \cdot se(SD)^2]}$. The standard error of P50 is given by the usual formula for the standard error of the predicted value in multiple regression, see for instance [36]. In case SD does not depend on X, the standard error of SD is given by $SD/\sqrt{(2n)}$ [37]; otherwise this might be used as an approximate formula. As an illustration, in **Figure 7.1** the 90% confidence intervals for the 10th, 50th and 90th centile are given for TBF(%) in relation to age in girls.

**Table 7.4.** Regression coefficient of the 50$^{th}$ centile polynomials and of the regression their residual SD's.

| Independent X-variable | Dependent Y-variable | | |
|---|---|---|---|
| | TBF (kg) | TBF (%) | FFM (kg) |
| **Boys** | | | |
| Age (mo) | $\ln Y = -0.9638 + 0.5665X - 0.0595X^2 + 0.0021X^3$<br>$r^2=0.72$<br>SD = 0.26040 | $Y = 8.2231 + 6.5941X - 0.7565X^2 + 0.0268X^3$<br>$r^2=0.45$<br>SD = 3.96160 | $Y = 3.4314 + 0.4627X - 0.00903X^2$<br>$r^2=0.83$<br>SD = $0.3497+0.0033X+0.0033X^2$ |
| Length (cm) | $Y = -6.3515 + 0.1334X - 3.723*10^{-8}X^4$<br>$r^2=0.77$<br>SD = -0.6597 + 0.0158X | $Y = -171.0996 + 5.4210X - 0.0372X^2$<br>$r^2=0.42$<br>SD = 4.08511 | $Y = 1.6835 + 1.2563*10^{-5}X^3$<br>$r^2=0.94$<br>SD = -0.4862+0.0119X |
| Weight (kg) | $Y = -1.6332 + 0.5752X - 0.0143X^2$<br>$r^2=0.89$<br>SD = -0.0711+0.0462X | $Y = -44.3129 + 19.9832X - 1.6061X^2 + 0.0031X^4$<br>$r^2=0.59$<br>SD = 2.2214 + 0.1792X | $Y = 1.6190 + 0.4290X + 0.0140X^2$<br>$r^2=0.96$<br>SD = $0.2285-0.0478X+0.0068X^2$ |
| **Girls** | | | |
| Age (mo) | $Y = -0.0527 + 0.5325X - 0.0341X^2 + 4.3767*10^{-5}X^4$<br>$r^2=0.76$<br>SD = 0.1973+0.0230X | $Y = 7.3883 + 7.0665X - 0.6505X^2 + 0.001314X^4$<br>$r^2=0.55$<br>SD = 3.76954 | $Y = 3.1353 + 0.4500X - 0.0150X^2 + 3.40*10^{-5}X^4$<br>$r^2=0.87$<br>SD = 0.2693+0.0253X |
| Length (cm) | $Y = -8.6848 + 0.1840X - 8.3826*10^{-6}X^4$<br>$r^2=0.75$<br>SD = -0.1681+0.0084X | $Y = -139.9504 + 3.5352X - 2.33*10^{-4}X^3$<br>$r^2=0.49$<br>SD = 3.97150 | $Y = 1.2680 + 1.7481*10^{-5}X^3 - 6.4164*10^{-8}X^4$<br>$r^2=0.95$<br>SD = $0.8091-0.0286X+3.089*10^{-4}X^2$ |
| Weight (kg) | $Y = -1.3754 + 0.4829X - 6.7071*10^{-5}X^4$<br>$r^2=0.90$<br>SD = -0.0148+0.0380X | $Y = -42.5733 + 20.3583X - 1.6595X^2 + 0.003259X^4$<br>$r^2=0.59$<br>SD = 3.56802 | $Y = 1.3754 + 0.5171X + 6.7071*10^{-5}X^4$<br>$r^2=0.96$<br>SD = -0.0148+0.0380X |

All regressions with non-constant residual SD were obtained by weighted linear stepwise regression, using $1 / SD^2$ as weighting factor
TBF = total body fat; FFM = fat-free mass, SD = residual standard deviation .

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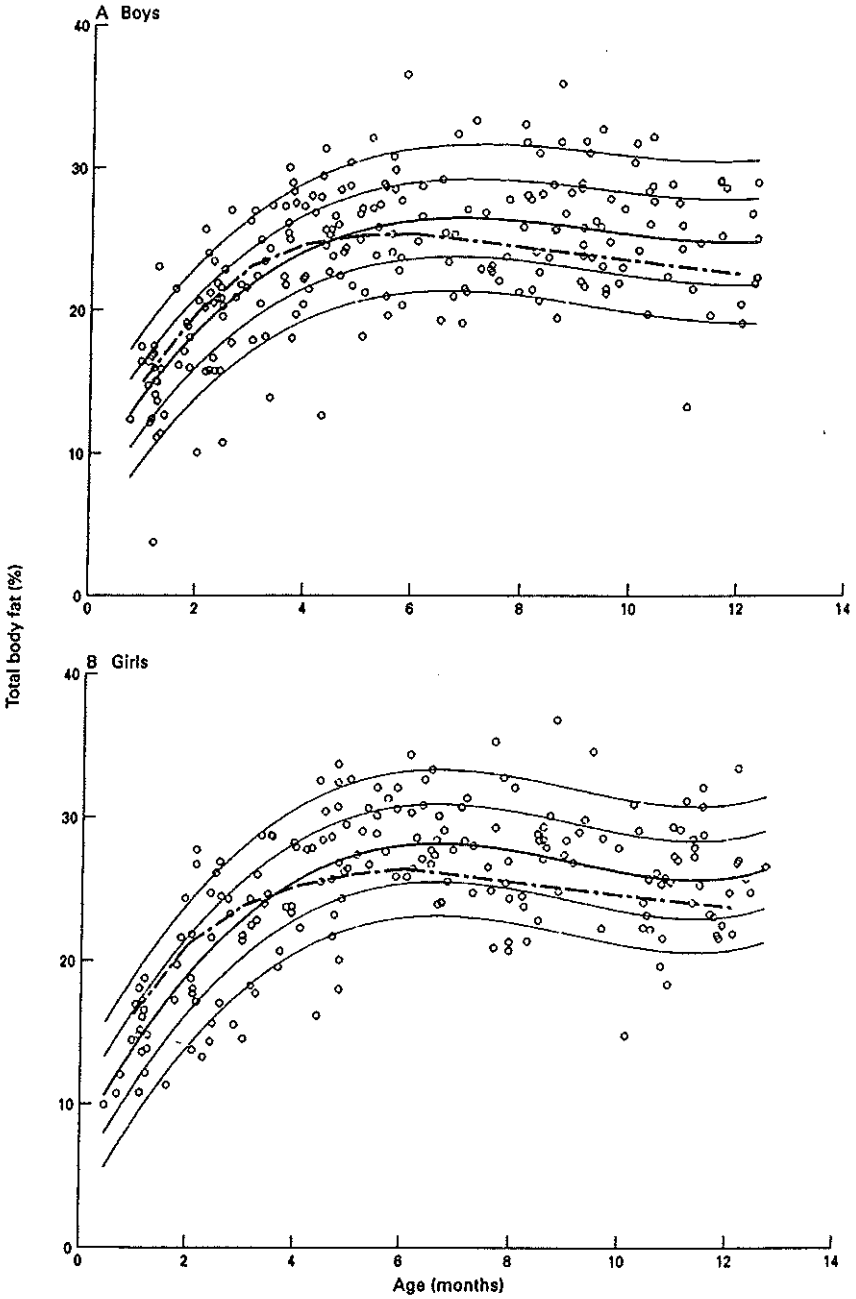


**Figure 7.2.** Individual data points and 10th, 25th 50th 75th and 90th centiles of per cent body fat plotted against age for boys and girls. Dotted line represents the reference data from Fomon et al [19].

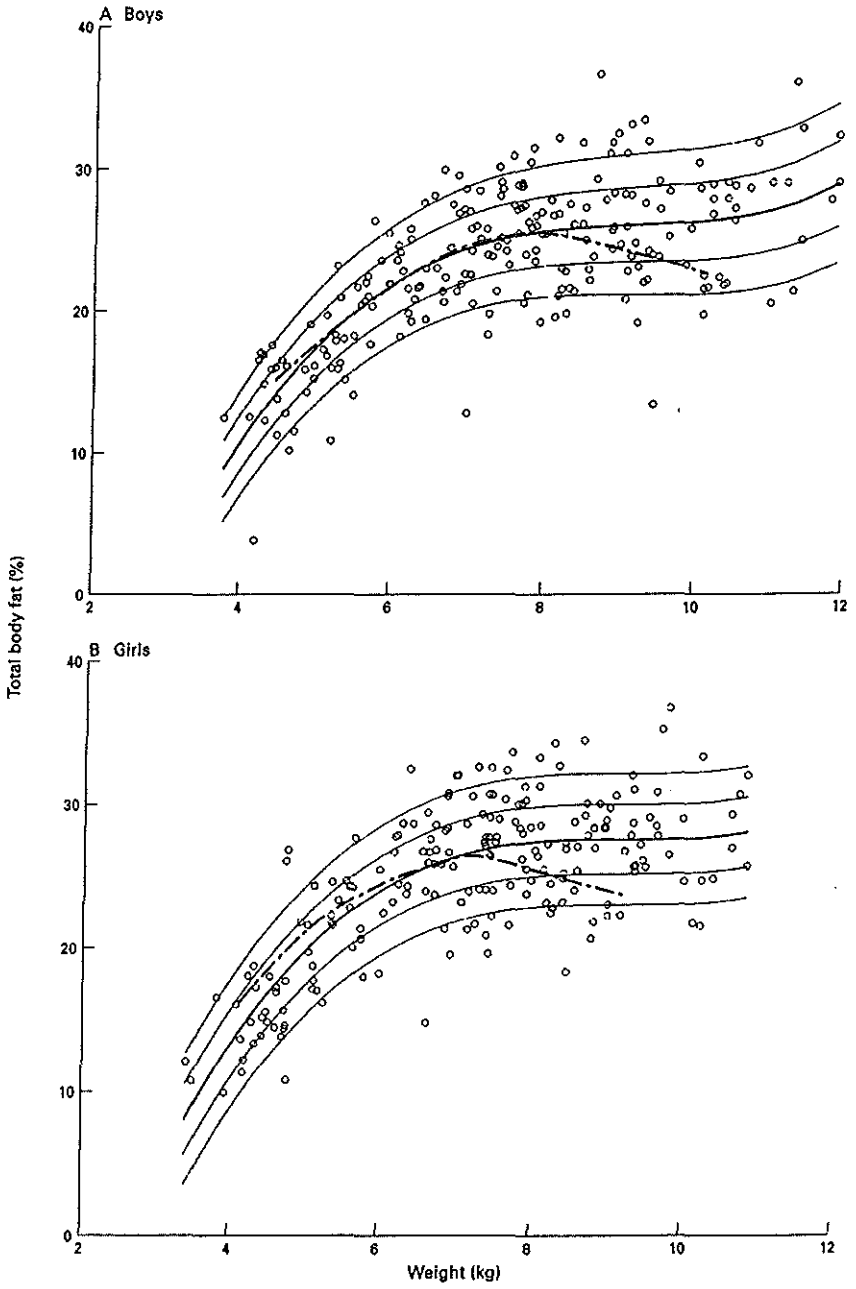


**Figure 7.3**. Individual data points and 10th, 25th 50th 75th and 90th centiles of per cent total fat plotted against weight for boys and girls. Dotted line represents the reference data from Fomon et al [19].

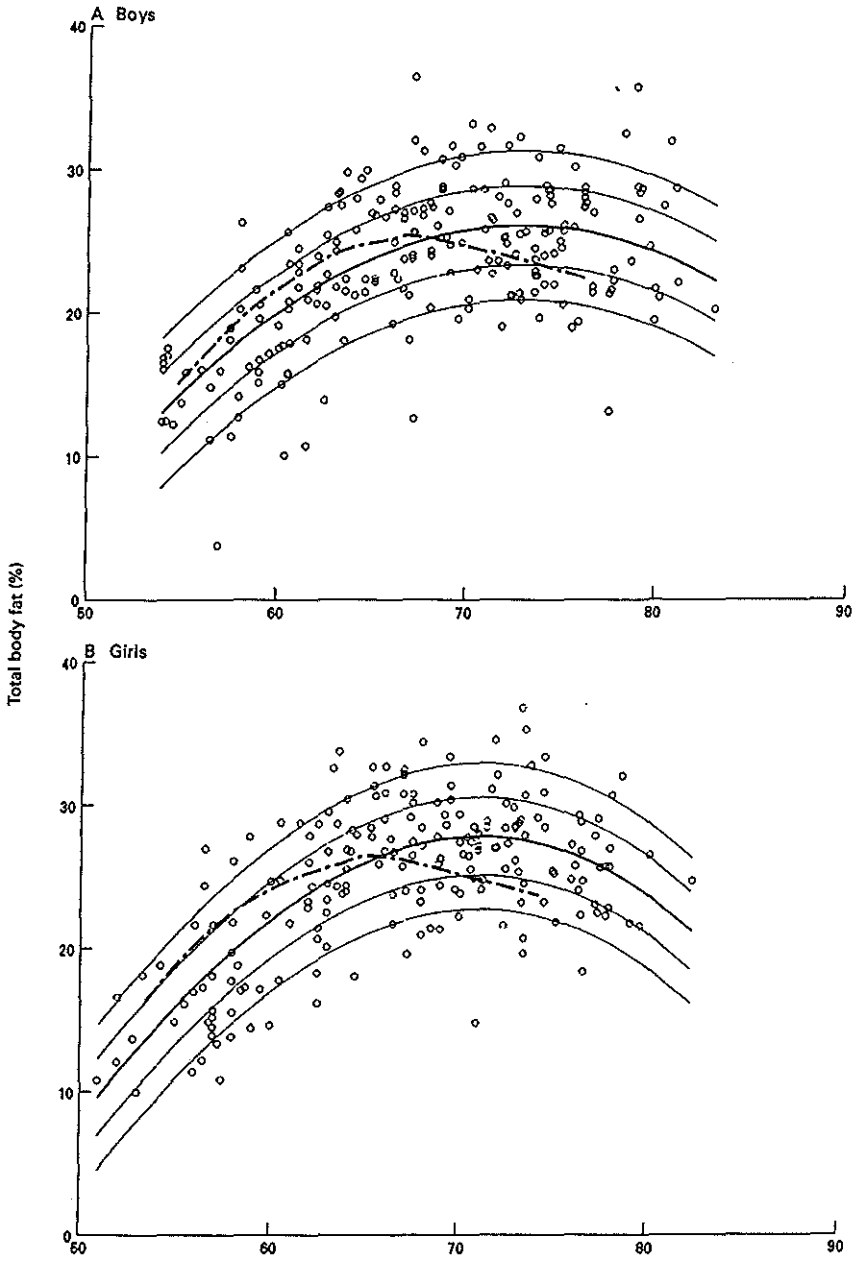


**Figure 7.4.** Individual data points and 10th, 25th 50th 75th and 90th centiles of per cent body fat plotted against length for boys and girls. Dotted line represents the reference data from Fomon et al [19].

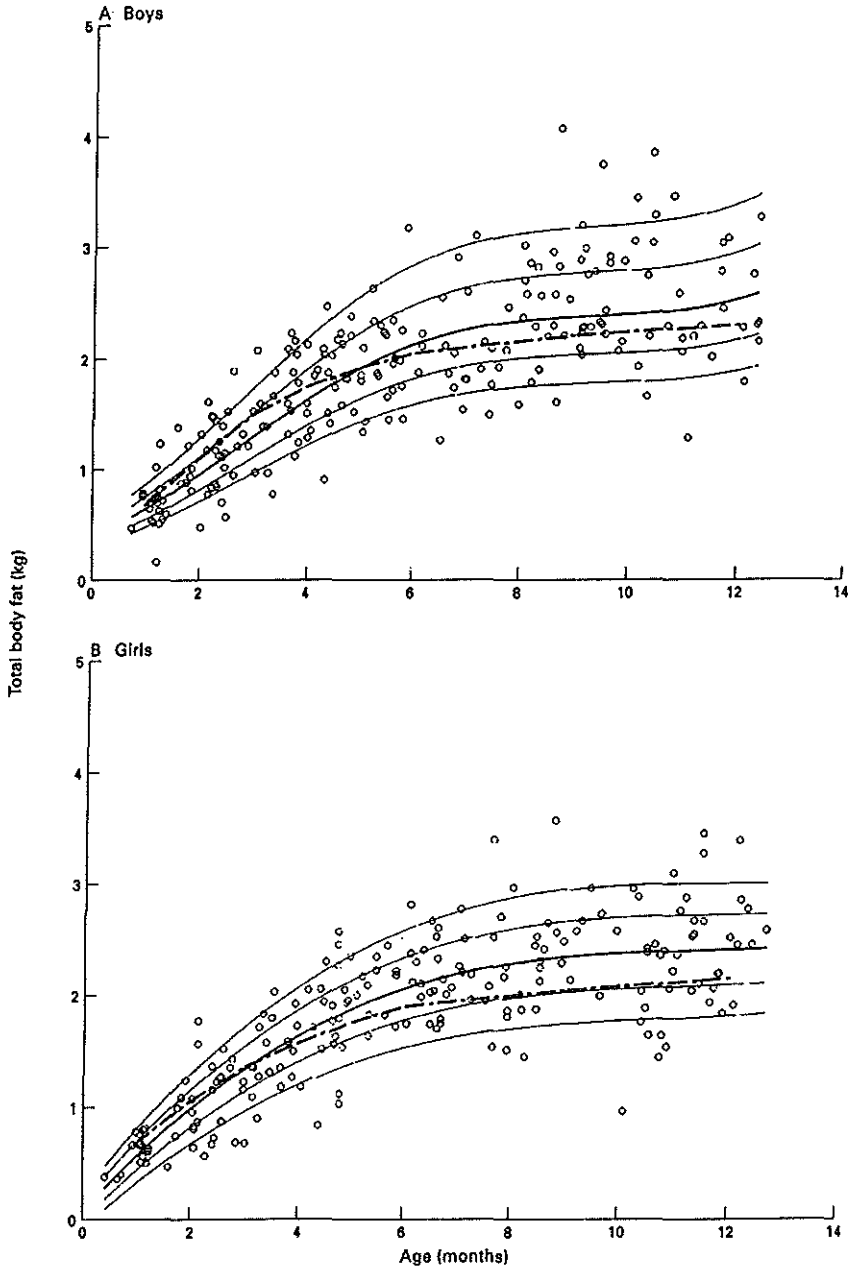


**Figure 7.5.** Individual data points and 10th, 25th 50th 75th and 90th centiles of body fat (kg) plotted against age for boys and girls. Dotted line represents the reference data from Fomon et al [19].

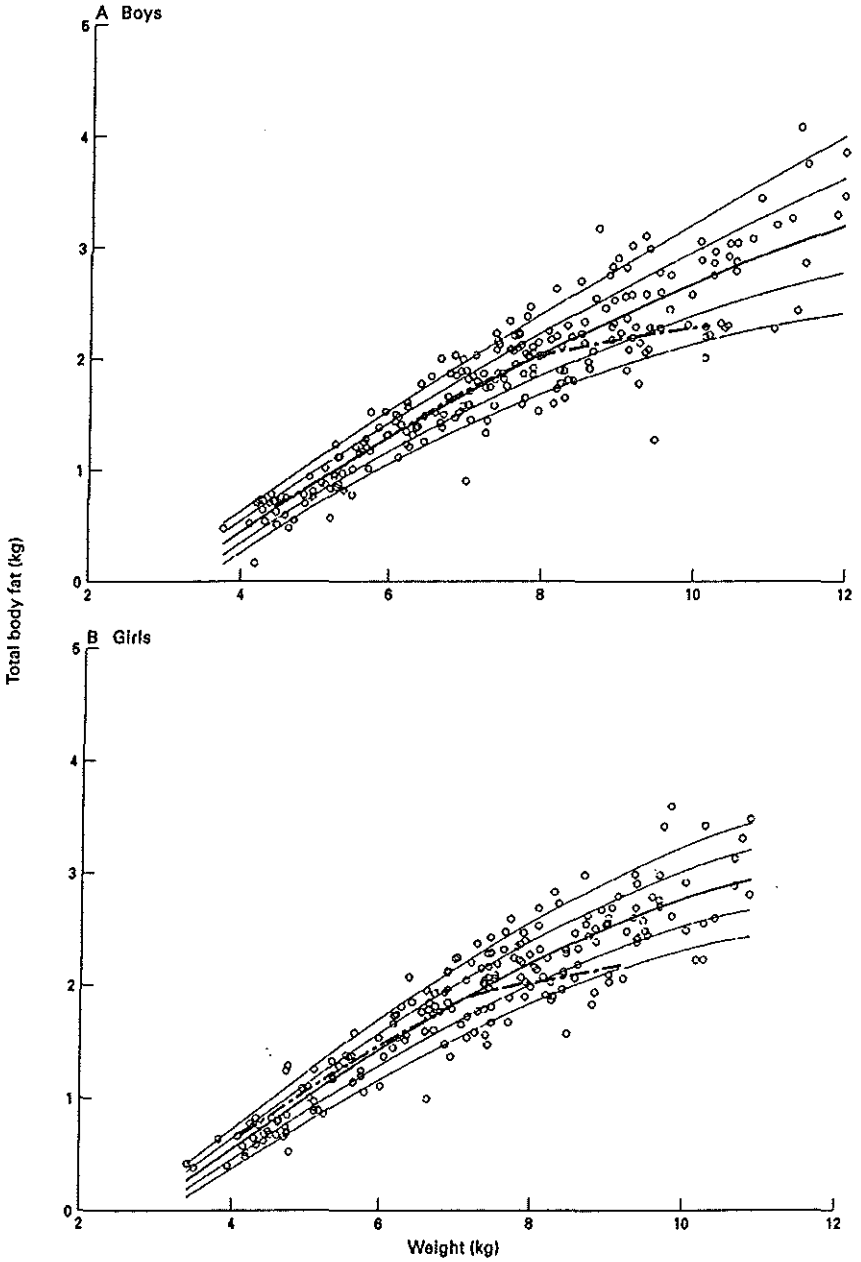


**Figure 7.6.** Individual data points and 10th, 25th 50th 75th and 90th centiles of body fat (kg) plotted against weight for boys and girls. Dotted line represents the reference data from Fomon et al [19].

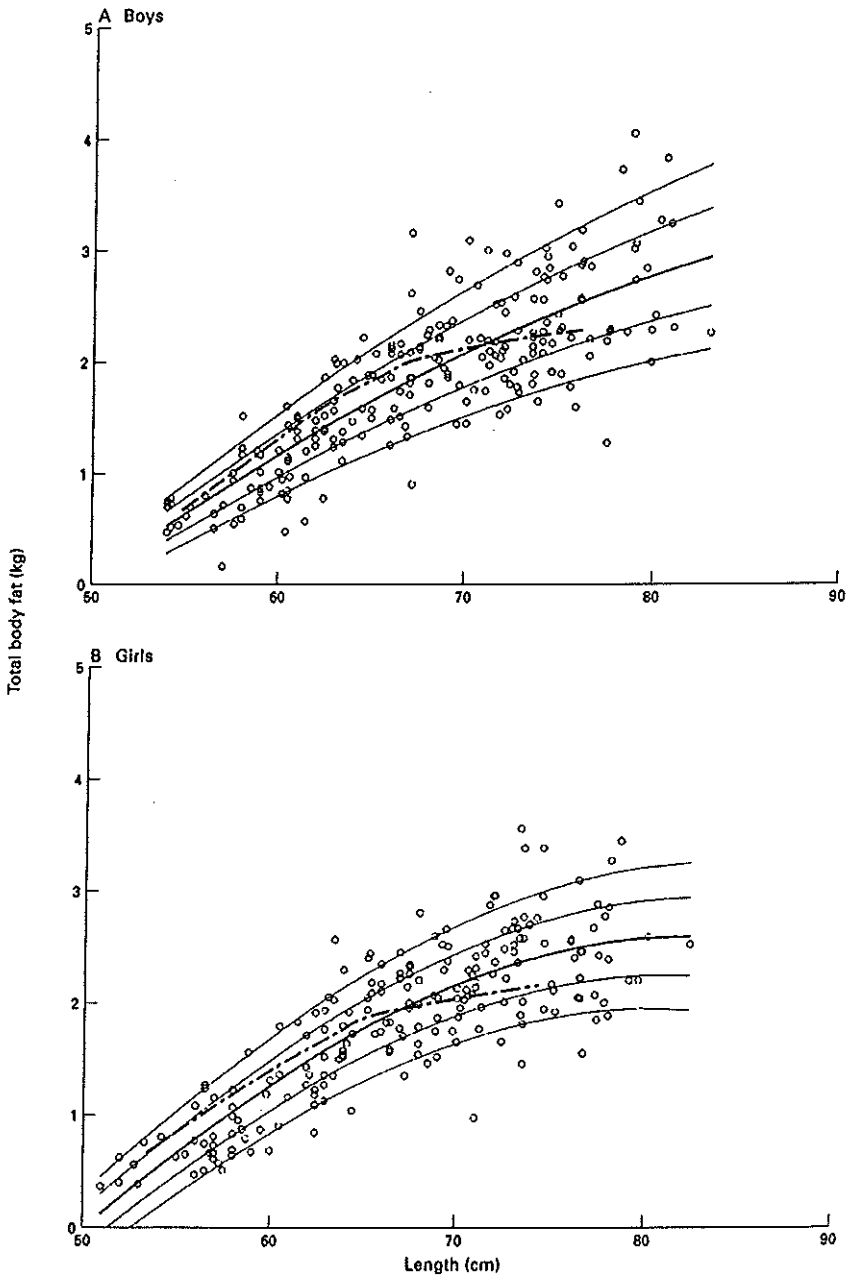


**Figure 7.7**. Individual data points and 10th, 25th 50th 75th and 90th centiles of body fat (kg) plotted against length for boys and girls. Dotted line represents the reference data from Fomon et al [19].

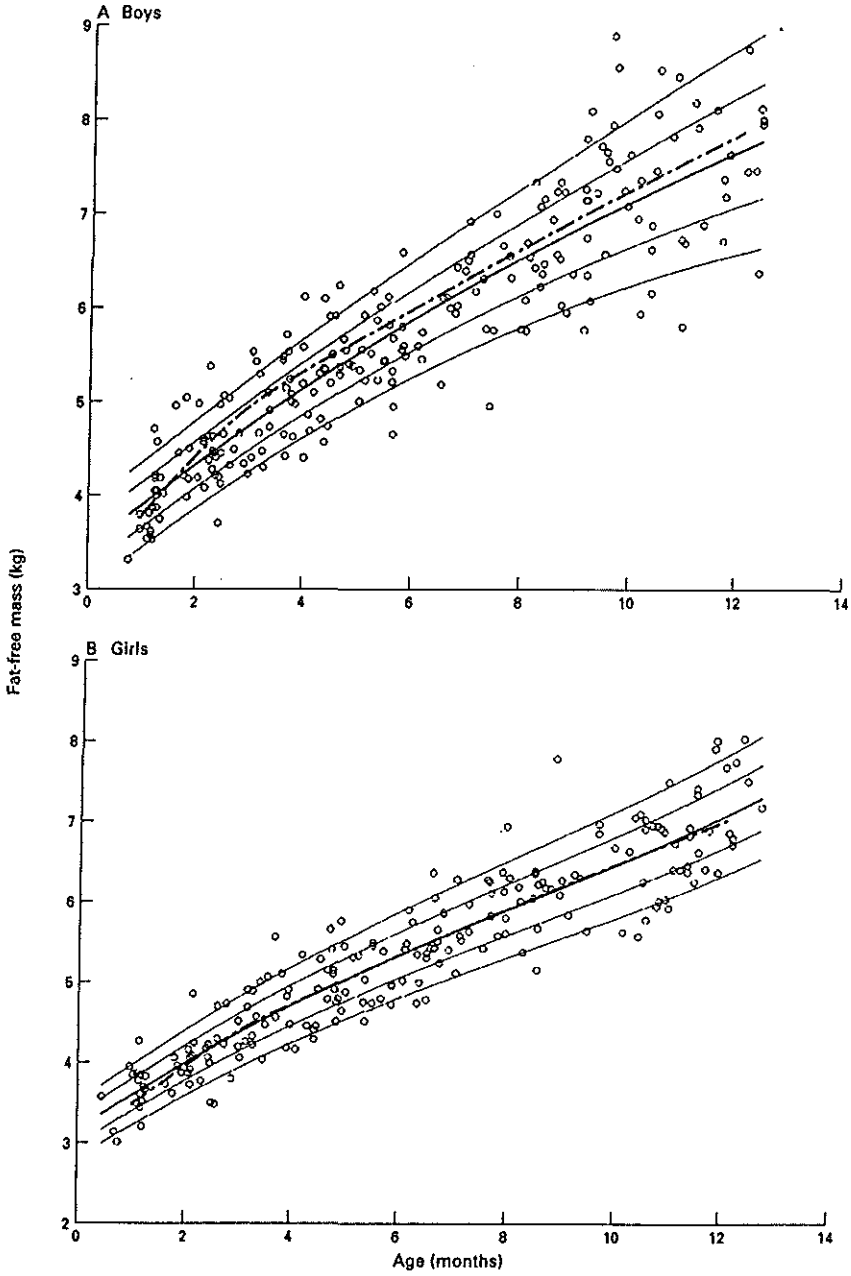


**Figure 7.8.** Individual data points and 10th, 25th 50th 75th and 90th centiles of fat-free mass (kg) plotted again age for boys and girls. Dotted line represents the reference data from Fomon et al [19].

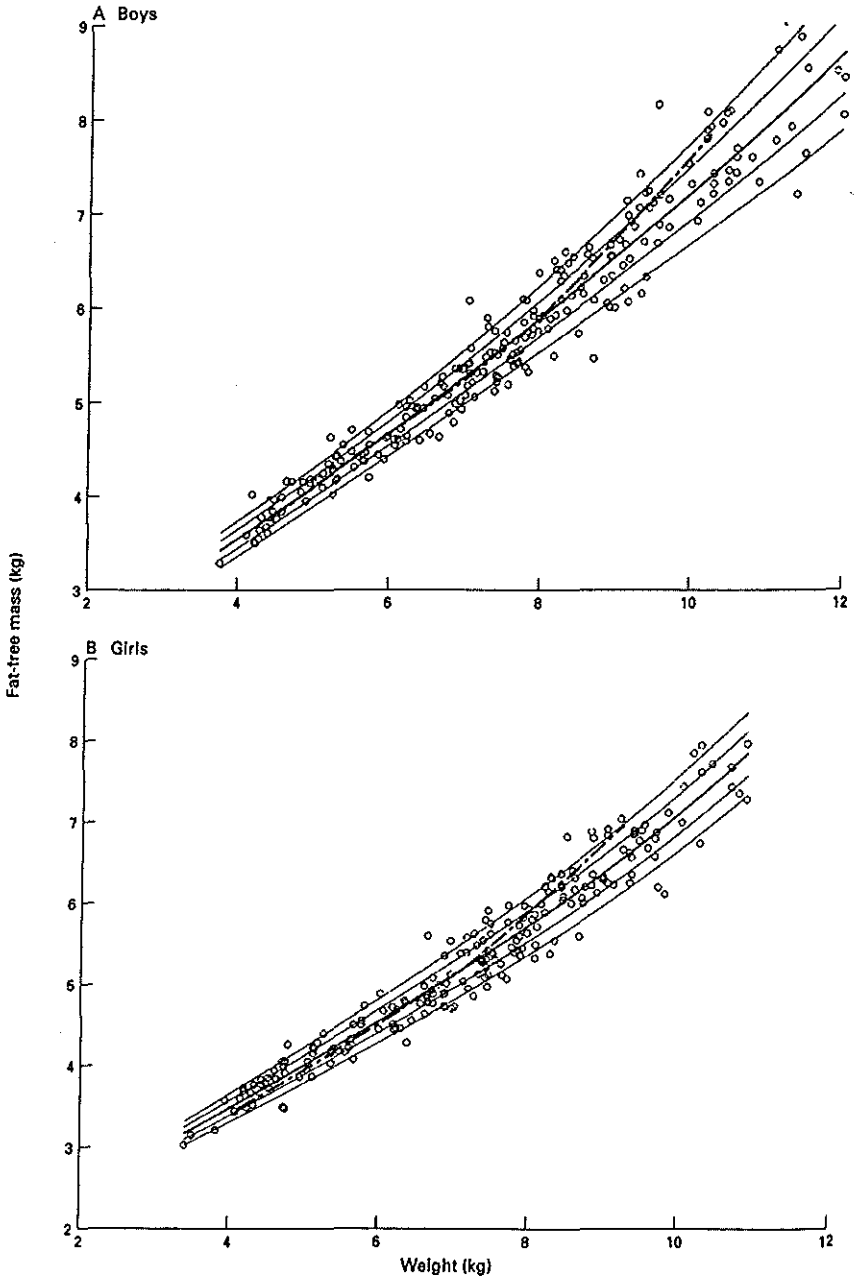


**Figure 7.9.** Individual data points and 10th, 25th 50th 75th and 90th centiles of fat-free mass (kg) plotted against weight for boys and girls. Dotted line represents the reference data from Fomon et al [19].

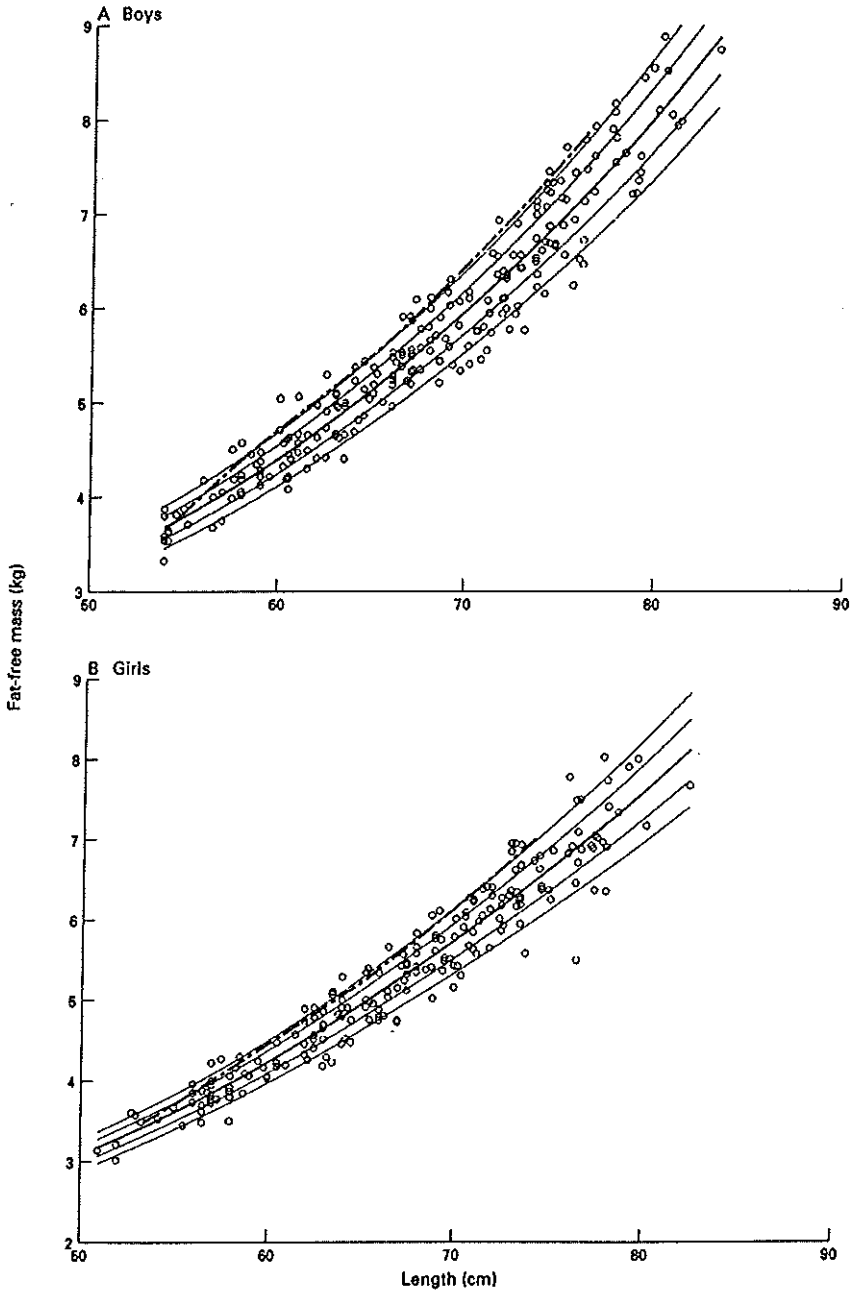


**Figure 7.10.** Individual data points and 10th, 25th 50th 75th and 90th centiles of fat-free mass (kg) plotted against length for boys and girls. Dotted line represents the reference data from Fomon et al [19].

*Part 4.*

# Study for the effect of exclusive breast feeding or formula feeding on growth and energy utilization.

# Determination of energy requirements by energy intake and by the sum of energy expenditure and energy deposition.

*Chapter 8*

# Energy Utilization and Growth in Infancy [a)]

## 8.1 Summary

This study is the first in reporting approximations of energy requirements for male and female breast-fed (BF) or formula-fed (FF) infants based on individual estimates of total daily energy expenditure (TDEE) and energy deposition derived from total body fat (TBF) and fat-free mass (FFM) gain. In 46 healthy, full-term infants the effect of at least 4 months of exclusive BF *versus* FF on macronutrient and energy intake, TDEE by the doubly-labeled water method, energy deposition and growth was investigated prospectively at 1, 2, 4, 8 and 12 mo. FFM and TBF were determined by total-body electrical conductivity (TOBEC). Metabolizable energy intake (MEI) was assessed from macronutrient intake ($MEI_{TW}$) and from the sum of TDEE and energy deposition derived from gain in TBF and FFM ($MEI_{PRED}$) . At 1-2, 2-4, 4-8 and 8-12 mo of age $MEI_{PRED}$ averaged $431 \pm 38$, $393 \pm 33$, $372 \pm 33$ and $355 \pm 21$ kJ/kg/d for boys, and $401 \pm 59$, $376 \pm 25$, $334 \pm 33$ and $326 \pm 17$ kJ/kg/d for girls. Apart from a small but significant difference in weight, TBF and FFM in 4 and 8 mo old girls (FF > BF) no significant difference between BF and FF infants was found with respect to weight, length, head circumference, TBF, FFM and TDEE at all ages, neither for gain in length, weight, TBF and FFM. $MEI_{TW}$ significantly differed between feeding groups at 1-4 mo of age (FF > BF, $p < 0.005$). This feeding effect, however, was not present in $MEI_{PRED}$. $MEI_{TW}$ differed from $MEI_{PRED}$ only in BF infants, at 1-4 mo (with $p < 0.05$ at 2-4 mo). Milk intake measured by TW did not significantly differ from milk intake by the deuterium-to-infant method. The data of this study indicate that energy requirements in infants are lower than recommended by the guidelines which are presently used.

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[a)] This chapter has been submitted for publication as: **De Bruin NC, Degenhart HJ, Gál S, Stijnen T, Westerterp KR, Visser HKA.** Energy utilization and growth in breast-fed and formula-fed infants measured prospectively during the first year of life: the Sophia study.

We extend our appreciation to the parents and infants who volunteered their time and effort to take part in this longitudinal study. We thank Professor Pieter J.J. Sauer for helpful suggestions and critical reading of the manuscript and Berdien van Houwelingen for assistance during the study.

## 8.2 INTRODUCTION

Energy intake of human milk fed and formula fed infants have been reported to differ [1]. Whether these differences result in altered energy utilization or growth has been subject to much debate for many years. A recent meta-analysis [1] showed that energy expenditure in the first year of life is affected by age and by feeding mode (BF < FF). Growth in BF infants also deviates from current reference standards [2]. The former two studies make plausible that energy utilization between BF and FF infants may deviate. However, energy intake, deposition and expenditure never have been measured in the same cohort of infants. Difficulties in the estimation of energy deposition by the inaccuracy of existing body composition methods prohibited such attempts. The appearance of total-body electrical conductivity (TOBEC) [3] as a safe and accurate infant body composition method now opens the possibility for simultaneous measurement of energy intake, expenditure *and* deposition.

Energy requirements in infancy (FAO/WHO/UNU, 1985) are based on the observed human milk intake of healthy, well-nourished, thriving infants. In BF infants energy content of human milk is estimated usually from expressed breast milk [4]. Because this approach is prone to various errors, energy requirements of infants were calculated alternatively from the sum of energy expenditure and energy deposition [5, 6]. Energy expenditure was estimated from various doubly-labeled water data combined from the literature, and energy deposition was calculated from reference values on body composition [7].

We here report the results of the Sophia study, which to our knowledge is the first to simultaneously follow nutrient intake, energy expenditure, growth and body composition prospectively during the first year of life in 46 healthy, full-term infants, *exclusively* BF or FF for at least 4 mo. Solids were introduced after 4 mo. Energy requirements were assessed from the sum of energy expenditure by the doubly-labeled water method, and energy deposition from gain in TOBEC-derived TBF and FFM.

## 8.3 SUBJECTS & METHODS

### *8.3.1. Study design*

Pregnant women who intended to exclusively breast-feed or bottle-feed their babies for at least 4 mo were recruited with the cooperation of local midwives. Within the first two weeks after delivery the mothers were contacted again, and informed consent was obtained. Selection criteria were: healthy, white infants; birth weight > 2500 g; born by non-pathological, vaginal delivery from healthy, non-smoking mothers being para 1 or 2. Mothers with history of gestational diabetes, gestational hypertension, (pre-) eclampsia, or use of tobacco, alcohol or soft/hard drugs during or after gestation were excluded, as well as infants with a history of intrauterine growth retardation, asphyxia during or after birth,

**Table 8.1** Characteristics of the study population [1].

| | *boys* | | *girls* | |
|---|---|---|---|---|
| | *BF (n = 9)* | *FF (n = 15)* | *BF (n = 14)* | *FF (n = 8)* |
| Length father (cm) | 187 ± 8 | 183 ± 8 | 183 ± 8 | 184 ± 9 |
| Length mother (cm) | 170 ± 6 | 169 ± 5 | 170 ± 7 | 168 ± 5 |
| Weight father (kg) | 84.1 ± 10.2 | 80.7 ± 14.9 | 83.5 ± 13.8 | 83.0 ± 7.3 |
| Weight mother (kg) | 64.2 ± 6.7 | 67.5 ± 14.8 | 64.6 ± 7.8 | 64.9 ± 9.9 |
| Age father (y) § | 34 ± 5 | 29 ± 4 | 31 ± 4 | 29 ± 3 |
| Age mother (y) | 30 ± 4 | 28 ± 3 | 28 ± 3 | 28 ± 4 |
| Employment father (n) | 8 | 14 | 13 | 8 |
| Employment mother (n) | 5 | 8 | 8 | 2 |
| Monthly net income (fl.) | 3773 ± 1260 | 3217 ± 1225 | 3332 ± 1019 | 3570 ± 1983 |
| Education father (n) high/ intermediate/ low | 4/4/1 | 4/8/3 | 10/3/1 | 1/3/4 |
| Education mother (n) high/ intermediate/ low | 4/4/1 | 3/5/7 | 5/6/3 | 1/4/3 |
| Parity (n) | | | | |
| 1 | 5 | 8 | 11 | 4 |
| 2 | 4 | 7 | 3 | 4 |
| Gestational age (w) | 40.8 ± 1.3 | 40.5 ± 1.2 | 40.5 ± 1.5 | 40.3 ± 1.1 |

[1] Mean ± SD.
Significant difference for mode of feeding: §= $p<0.05$.

major infections or any kind of failure to thrive during the first mo of life. Also mothers who stopped breast-feeding < 4 mo were excluded from the study. Of the 92 responding mothers 42 refused afterwards, or were excluded after delivery because of incompatibility to the inclusion criteria. Fifty infants were enrolled in the study. Four infants were excluded because the mothers failed to follow the protocol in some respect. **Table 8.1** shows the characteristics of the study population. Measurements of nutrient intake, energy expenditure, growth and body composition were planned prospectively at 1, 2, 4, 8 and 12 mo of age. The study was approved by the Medical Ethical Committee of the University Hospital Rotterdam.

### *8.3.2. Energy and macronutrient intake*

***Recording of food intake.*** Food intake was measured at home by the mothers at 1, 2, 4 and 8 mo of age by 5-d test weighing and at 12 mo of age by the 'double-portion' method for 3 days. Mothers were asked not to change the infant's feeding mode from at least 1 w

before, until the end of the measurement period. Human milk intake was measured by weighing the infant before and after a feed on an electronic integrating balance (Instru Vaaka Oy, Vaany, Finland; precision 1 g (0-3 kg), 2 g (3-6 kg) , 5 g (6-10 kg)). Mothers were instructed not to change the diaper before the second weight (after the feeding) was recorded, and not to include the weight of the bib on either of the two weight recordings. The time at which the infant was weighed was noted down by the mother. Feeding duration was defined as the period between the two weights before and after the feeding, which not necessarily equals the actual time of the infant spending at the breast! Corrections for insensible water loss (IWL) during the feeding were made assuming a value for IWL of 1.8 g/kg/h [8].

Twenty-four hour breastmilk samples were collected within 4 d after the test weighing period. Mothers mechanically expressed one or two breasts depending on their habit of breast feeding: some gave one breast per feeding while others gave a portion of both breasts as a feeding. All expressed milk was gently shaken and a subsample of ~20 ml was stored in glass jars at -20 °C directly after collection. The reminder was given as feeding to the infant by bottle. Mothers were encouraged to mechanically express their milk in the manner as they fed their babies: ie, one full breast or part of two breasts per feeding. The amount of hind-milk (and subsequently the energy content of the total amount of breast-milk), will vary by the length of the feeding time and the way the mothers breast-fed their babies. Twelve mothers who failed in this procedure were asked to mechanically express 5 ml per breast before the feeding and 5 ml afterwards. This resulted in 10 ml of breast milk when mothers gave one breast per feeding and in 20 ml of breast milk when two breasts per feeding were given. Human milk samples were transported at -20 °C to the laboratory. At completion of the study all samples were thawed to prepare pooled 24 h-samples and directly refrozen at -45 °C. Aliquots of milk were then pooled proportional to the milk intake at each feeding (as determined by 5-d test weighing), and macronutrient analysis was performed.

Intake of formula was measured by weighing the bottle before and after a feed. All formula powder came from one batch (Nutrilon Premium, Nutricia Inc., Zoetermeer, Netherlands). Mothers prepared a daily stock of which immediately after stirring of the solution 20 ml was set apart for analysis of formula density.

Milk intake was corrected for the amount of regurgitation of human milk (after the feeding) and of formula (during and after the feeding). Regurgitation of milk was assessed and recorded on a 5-point scale by the mother (tea spoon=5 ml, dinner spoon=10 ml, half a cup=20ml, one cup=50 ml, more than a cup: mothers were asked to asses how many cups).

Intake of non-milk foods and fluids was determined by test weighing using a balance with 1 g precision. Details on the type of feeding, as well as further information as mentioned above was recorded by the mother on a simple structured pre-prepared form. Nutrient composition of recorded foods was calculated using the information given by the

manufacturer and in case of fresh food using a national food table [9].

At 12 mo of age intake was assessed by the 'double portion' method for 3 days. Equal portions of all drinks and foods which the infants consumed (assessed with a balance or on visual inspection with a gram- or milliliter-scaled can) were stored in plastic containers, refrigerated at home and transported at -20 °C to the laboratory where they were stored at -45°C until laboratory analysis. It was emphasized that only the amount of food/drinks which was actually consumed by the child should be deposited into the plastic containers.

*Milk intake by the 'deuterium-to-infant method'.* The amount of human milk intake was assessed also using total water output data resulting from the doubly-labeled water technique [10]. For the correction of environmental water influx on total water milk intake a correction factor of 0.937 was used [11]. When 50 g/d [10] was used instead of the correction factor of Wells & Davies [11], a difference of only 1% in total milk intake was found, in spite of the differences in climate between the two study areas (Houston, TX and Cambridge, UK).

*Nutrient analysis.* All macronutrient analyses were performed after completion of the study. Human milk and 'double portion' samples were dried at 102 °C under vacuum. Fat was determined by the Rose-Gottlieb procedure (human milk and formula samples) or by the Weibull method ('double portion' samples), total nitrogen (TN) by the Kjehldal method, lactose by an enzymatic procedure (test kit no. 176303, Boehringer-Mannheim). Non-protein nitrogen (NPN) was assumed to be 20% of TN for human milk [4, 12] and 13% for formula [13, 14]. Protein nitrogen (PN) was taken as TN *minus* NPN. Milk protein (human milk and formula) was calculated as PN x 6.38 and protein in non-milk foods as PN x 6.25. Carbohydrates were calculated by difference. Gross energy content ($GEI_{TW}$) was calculated from fat, protein and total carbohydrate by using the factors 9.25, 5.65 and 3.95 kcal/g, respectively for human milk and formula and the factors 9.4, 5.65 and 4.15 for non-milk foods [17]. $MEI_{TW}$ was assumed to be 94% of $GEI_{TW}$ [1]. Energy content of the 12 mo 'double portions' also was assessed by means of standard bomb calorimetry, which was not significantly different from macronutrient analysis ($320 \pm 58$ vs. $325 \pm 47$ kJ/100 ml, respectively, in BF infants and $345 \pm 46$ vs. $349 \pm 45$ kJ/100 ml, respectively, in FF infants). The correlation between energy content by bomb calorimetry and macronutrient analysis was 0.86, $p < 0.001$.

### *8.3.3. Growth and body composition*

***TOBEC.*** Fat-free mass (FFM) was measured by total-body electrical conductivity (TOBEC). Total-body fat (TBF) was calculated as weight *minus* FFM. Details on the TOBEC methodology, its accuracy, reproducibility, calibration, and the calculation of TBF and FFM were discussed earlier [15-18].

***Anthropometry.*** At the time of the TOBEC measurement the infants were weighed naked on an electronic baby scale (Instru Vaaka Oy, Vaany, Finland) to the nearest 1 g (0-3 kg), 2 g (3-6 kg) or 5 g (6-10 kg). Recumbent crown-heel length was measured to the nearest mm

on a length board. Fronto-occipital head circumference was measured to the nearest mm with a 1 cm wide standard plastic measurement tape. Skinfold thickness (triceps, subscapular, quadriceps) was measured with a Harpenden caliper to the nearest 0.1 mm and read at the point of stabilization of the measurement (~15-60 s after application). Standard deviation scores (SDS) of length, weight and head circumference were based on the Dutch growth reference centiles [19]. Most of the measurements in this study (>90%) were performed by the main observer. The other measurements were performed by a second observer, who was well trained by the first observer. We measured inter-observer variation also with this second observer [3] and found no significant difference for weight, length and head circumference, and a small difference for skinfold thickness measurements (<3%).

*8.3.4. Energy expenditure by $^2H_2{}^{18}O$*

Energy expenditure was measured by the doubly labeled water method. Details on $^2H_2{}^{18}O$ dosing, urine collection, transport and storage have been described elsewhere [18].

For calculation of energy expenditure the time zero intercept two-point approach was used. A urine sample taken before administration of labeled water was used as baseline sample. Urinary tracer concentrations were corrected for additional isotope dilution caused by change of the body water compartment during the eight days of the experiment, as well as for the timing error of each urine sample caused by mixing of urine with decreasing concentrations of label in the bladder between two subsequent voids [18].

Pool sizes of $^2H$ ($N_H$) and $^{18}O$($N_O$) were calculated by extrapolation of concentrations to t=0. Both isotopes have different fractionation factors and were administered concomitantly. The ratio of $N_H/N_O$ is very narrowly defined, therefore, and used as a measure for the reliability of the urine sample. Data were excluded when $N_H/N_O$ ratio was beyond 3 SD from mean $N_H/N_O$. This ratio was normally distributed (results not shown), leading to a loss of <1% of *correct* data that will be rejected. Nineteen data points were excluded on this ground. An additional seven measurements were excluded on the basis of the fact that not all spoiled tracer could be collected (6 cases) and of unclear notation of urine collection times (1 case).

The rate of carbon dioxide production ($rCO_2$) was calculated as described by Schoeller et al [20]:

$$rCO_2 = (N/2.078) \cdot (1.01 \cdot k_O - 1.04 \cdot k_H) - 0.0246 \cdot rGf \quad (1)$$

where $k_O$ and $k_H$ are elimination rates of respectively $^{18}O$ and $^2H$, N is the total body water (TBW) volume calculated from the isotope dilution spaces at time zero: ($N_O/1.01 + N_H/1.04)/2$, and corrected for an exponential change over the observation period [21] by:

$$N = [N_{t=0} - N_{end}] / [\ln(N_{t=0} / N_{end})] \quad (2)$$

where $N_{end}$ is TBW at the end of the observation period. $N_{end}$ was calculated from the difference between body weight at the start and body weight at the end of the observation period (measured within 5 d after the post-dose urine sample and interpolated to day 9) using values for the percentage of body water in weight gain as published by Fomon et al. [7]. The rate of water loss via fractionated gaseous routes ($r$Gf ) was estimated for the present study to be $1.19(k_O - k_H)$, assuming that breath is saturated with water and contains 3.5% carbon dioxide (fractionated breath water = $1.77 \cdot rCO_2$) and that transcutaneous fractionated (non-sweat) water loss amounts to about 65% of breath water. Total daily energy expenditure (TDEE) was calculated from $rCO_2$ by TDEE = $22.4 \cdot EeqCO_2 \cdot rCO_2$ , where $EeqCO_2$ is the energy equivalent of carbon dioxide at a given RQ [22]. RQ was estimated as food quotient (FQ) from food composition [23]. A calory conversion factor of 0.85 for fat was used to correct for a lower digestibility in infants as compared to adults [23]. FQ's were corrected for fat and protein deposition [23] using data on composition of weight gain from Fomon et al. [7] which were applied on the weight gain during the observation period.

*8.3.5. Energy deposition*

Energy deposition was calculated first by subtraction of TDEE from $MEI_{TW}$. Furthermore, energy deposition was calculated from gain in TBF (fat storage) and FFM (protein storage), as measured by TOBEC, in two ways: *Method A:* By calculation of increments of TBF and FFM between 1-2, 2-4, 4-8 and 8-12 mo. *Method B:* By calculation of gain in TBF and FFM for each child by using the first derivative at 1, 2, 4, 8, and 12 mo of third degree polynomial curves through the individual values of TBF and of FFM against age. The first derivative at 1, 2, 4, 8 and 12 mo then represents gain in TBF and FFM for each child at a chosen age.

Protein gain was estimated from FFM accretion using reference data from Fomon et al. [7]. Average weights between 1-2, 2-4, 4-8 and 8-12 mo were used for expression of energy deposition on a kg body weight basis. Energy conversion factors of 9.25 for fat and 5.6 for protein were used [24]. Carbohydrate storage was assumed to be negligible. For the period of 0-1 mo of age energy deposition was calculated from weight gain. Protein and fat gain at this period was assessed from weight gain using reference values on composition of weight gain [7].

*8.3.6. Data analysis*

SPSS for Windows was used for most statistical analyses. Data were expressed as mean ± SD. An effect was considered statistically significant if $P<0.05$. For the different ages separately, differences between sexes and feeding groups were analyzed by multiple linear regression, with sex and feeding group and their interaction term as independent variables in the model. If the interaction was not significant, it was left out of the model. If it was significant, feeding groups were compared within boys and girls separately. By general

| | boys | | girls | |
|---|---|---|---|---|
| *Intake / day* | *BF (n = 9)* | *FF (n = 15)* | *BF (n = 14)* | *FF (n = 8)* |
| Milk (g) by deuterium to infant [3] | | | | |
| 1 mo † | 800 ± 209 (n= 6) | 779 ± 154 (n=12) | 607 ± 96 (n=9) | 652 ± 57 (n=7) |
| 2 mo † | 830 ± 148 (n=4) | 848 ± 89 (n=13) | 687 ± 104 (n=9) | 724 ± 72 (n=6) |
| 4 mo | 922 ± 179 (n=5) | 923 ± 73 (n=9) | 828 ± 134 (n=10) | 851 ± 129 (n=6) |
| Milk/solids (g) by test weighing [3] | | | | |
| 1 mo † | 778 ± 146 | 742 ± 104 | 636 ± 101 | 686 ± 95 |
| 2 mo † § | 812 ± 144 | 842 ± 86 | 694 ± 78 | 811 ± 111 |
| 4 mo † § | 844 ± 81 | 920 ± 92 | 759 ± 116 | 855 ± 89 |
| Protein (g) | | | | |
| 1 mo † ¶ | 8.34 ± 1.44 | 9.43 ± 1.8 | 6.82 ± 0.98 | 8.80 ± 1.59 |
| 2 mo ¶ | 7.54 ± 1.27 | 10.4 ± 1.7 | 6.74 ± 0.88 | 10.4 ± 1.3 |
| 4 mo ¶ | 7.35 ± 1.04 | 10.9 ± 1.7 | 6.76 ± 1.21 | 10.3 ± 0.8 |
| 8 mo † | 23.2 ± 6.9 | 23.6 ± 5.7 | 19.5 ± 3.9 | 19.5 ± 4.1 |
| 12 mo | 28.8 ± 4.4 | 30.0 ± 5.9 | 27.9 ± 6.3 | 23.6 ± 5.3 |
| Protein (g/kg) | | | | |
| 1 mo | 1.83 ± 0.28 | 2.06 ± 0.39 | 1.69 ± 0.36 | 2.10 ± 0.46 |
| 2 mo ¶ | 1.36 ± 0.08 | 1.91 ± 0.35 | 1.39 ± 0.19 | 2.04 ± 0.15 |
| 4 mo ¶ | 1.09 ± 0.11 | 1.62 ± 0.29 | 1.16 ± 0.26 | 1.55 ± 0.14 |
| 8 mo | 2.67 ± 0.85 | 2.77 ± 0.75 | 2.48 ± 0.51 | 2.28 ± 0.45 |
| 12 mo | 2.84 ± 0.41 | 2.96 ± 0.70 | 3.03 ± 0.71 | 2.46 ± 0.67 |
| Fat (g) | | | | |
| 1 mo ¶ | 20.5 ± 8.0 | 27.3 ± 5.5 | 19.0 ± 5.2 | 26.5 ± 5.4 |
| 2 mo ¶ | 21.6 ± 7.0 | 30.3 ± 4.6 | 19.9 ± 5.3 | 31.6 ± 3.7 |
| 4 mo ¶ | 22.5 ± 5.1 | 31.5 ± 5.3 | 20.4 ± 7.9 | 31.6 ± 2.4 |
| 8 mo | 25.0 ± 6.5 | 27.2 ± 3.9 | 27.7 ± 3.2 | 27.1 ± 5.1 |
| 12 mo | 21.6 ± 7.9 | 22.1 ± 5.1 | 21.6 ± 7.0 | 19.6 ± 4.2 |
| Fat (g/kg) | | | | |
| 1 mo ¶ | 4.50 ± 1.68 | 5.95 ± 1.02 | 4.62 ± 1.17 | 6.34 ± 1.52 |
| 2 mo ¶ | 3.85 ± 1.03 | 5.53 ± 0.57 | 4.12 ± 1.15 | 6.21 ± 0.47 |
| 4 mo ¶ | 3.35 ± 0.72 | 4.66 ± 0.72 | 3.47 ± 1.31 | 4.78 ± 0.45 |
| 8 mo $ [2] | 2.84 ± 0.74 | 3.16 ± 0.49 | 3.52 ± 0.44 | 3.16 ± 0.54 |
| 12 mo | 2.13 ± 0.81 | 2.16 ± 0.50 | 2.35 ± 0.79 | 2.02 ± 0.49 |
| Carbohydrate (g) | | | | |
| 1 mo † | 60.2 ± 12.5 | 51.8 ± 9.6 | 48.8 ± 7.5 | 49.1 ± 9.0 |
| 2 mo | 61.2 ± 12.0 | 58.2 ± 8.7 | 53.5 ± 7.5 | 58.0 ± 7.3 |
| 4 mo † | 64.3 ± 7.7 | 63.2 ± 8.2 | 58.3 ± 8.9 | 59.0 ± 5.1 |
| 8 mo | 99.6 ± 15.0 | 102.0 ± 16.6 | 98.1 ± 12.7 | 94.6 ± 19.4 |
| 12 mo † | 115.4 ± 18.8 | 114.4 ± 22.2 | 103.0 ± 21.0 | 95.2 ± 18.6 |
| Carbohydrate (g/kg) | | | | |
| 1 mo | 13.2 ± 2.4 | 11.3 ± 1.8 | 11.9 ± 1.3 | 11.7 ± 2.6 |
| 2 mo | 11.0 ± 1.2 | 10.6 ± 1.4 | 11.0 ± 1.2 | 11.4 ± 0.8 |
| 4 mo | 9.5 ± 0.9 | 9.4 ± 1.5 | 10.0 ± 1.8 | 8.9 ± 0.7 |
| 8 mo | 11.4 ± 1.5 | 11.9 ± 2.3 | 12.5 ± 1.7 | 11.0 ± 1.8 |
| 12 mo | 11.3 ± 1.2 | 11.3 ± 2.7 | 11.2 ± 2.4 | 9.8 ± 2.1 |

*(Table 2 continued)*

| | | | | |
|---|---|---|---|---|
| Gross energy (MJ) | | | | |
| 1 mo § | 1.96 ± 0.45 | 2.13 ± 0.40 | 1.70 ± 0.26 | 2.04 ± 0.39 |
| 2 mo ¶ | 2.02 ± 0.48 | 2.38 ± 0.34 | 1.81 ± 0.24 | 2.42 ± 0.29 |
| 4 mo ¶ | 2.11 ± 0.25 | 2.52 ± 0.30 | 1.91 ± 0.37 | 2.44 ± 0.17 |
| 8 mo | 3.22 ± 0.45 | 3.36 ± 0.39 | 3.21 ± 0.39 | 3.12 ± 0.49 |
| 12 mo † | 3.53 ± 0.61 | 3.56 ± 0.48 | 3.29 ± 0.64 | 2.98 ± 0.49 |
| Gross energy (kJ/kg) | | | | |
| 1 mo § | 434 ± 88 | 464 ± 75 | 414 ± 50 | 489 ± 113 |
| 2 mo ¶ | 364 ± 59 | 435 ± 50 | 372 ± 54 | 477 ± 33 |
| 4 mo ¶ | 314 ± 29 | 372 ± 46 | 326 ± 67 | 368 ± 29 |
| 8 mo $ 2) | 368 ± 46 | 393 ± 54 | 410 ± 54 | 364 ± 42 |
| 12 mo | 347 ± 54 | 351 ± 63 | 359 ± 75 | 309 ± 59 |
| Percent energy from breast milk | | | | |
| 1 mo | 100 ± 0 | 0 | 100 ± 0 | 0 |
| 2 mo | 100 ± 0 | 0 | 100 ± 0 | 0 |
| 4 mo | 95 ± 12 | 0 | 97 ± 4 | 0 |
| 8 mo | 27 ± 2 (n=3) | 0 | 31 ± 2 (n=2) | 0 |
| Percent energy from formula | | | | |
| 1 mo | 0 | 100 ± 0 | 0 | 100 ± 0 |
| 2 mo | 0 | 100 ± 0 | 0 | 100 ± 0 |
| 4 mo | 0 | 96 ± 10 | 0 | 99 ± 3 |
| 8 mo † | 35 ± 20 (n=7) | 43 ± 15 | 46 ± 13 (n=13) | 51 ± 11 |
| Food quotient | | | | |
| 1 mo ¶ | 0.89 ± 0.02 | 0.86 ± 0.01 | 0.88 ± 0.03 | 0.85 ± 0.01 |
| 2 mo ¶ | 0.91 ± 0.06 | 0.85 ± 0.01 | 0.90 ± 0.03 | 0.86 ± 0.01 |
| 4 mo ¶ | 0.89 ± 0.02 | 0.85 ± 0.01 | 0.89 ± 0.04 | 0.85 ± 0.01 |
| 8 mo | 0.88 ± 0.02 | 0.88 ± 0.02 | 0.88 ± 0.01 | 0.88 ± 0.01 |
| 12 mo | 0.89 ± 0.01 | 0.89 ± 0.02 | 0.90 ± 0.02 | 0.90 ± 0.01 |

For all values of 1 to 4 mo of age test weighing data were taken for intake volume (and not deuterium-to-infant data).
Significant effect of mode of feeding: §= $p<0.05$, ¶= $p<0.005$
Significant effect of gender: †= $p<0.05$
Significant interaction of gender by mode of feeding: $= $p<0.05$
[1] mean ± SD. Data at 1,2,4 mo of age (BF infants) corrected for insensible water loss.
[2] Significant interaction effect did not result in a significant feeding effect when tested in separate gender groups.
[3] The data for milk intake by the deuterium-to-infant method were derived from a subgroup of infants in which milk intake by test weighing was measured. Percent difference in milk intake between TW and the deuterium-to-infant (DTI) method at 1, 2 and 4 mo of age averaged respectively 3, 2 and -4 % in BF and 3, 2 and -2 % in FF infants (all not significantly different from zero by multiple-linear regression analysis).

linear mixed model regression analysis, using the procedure Proc Mixed of the SAS statistical package (SAS Institute Inc., SAS/STAT Software: Changes and ENhancements through relase 6.11, Cary, Nc: SAS Institute Inc., 1996, 1104 pp), the dependence of TDEE and $MEI_{PRED}$ on gender, age, length, weight and FFM was studied. In these repeated measures analyses covariance structure was left completely free. The periods of exclusive breast/formula feeding (0-4 mo) and after weaning (>4 mo) were treated as separate periods, because of principally different basic growth conditions in relation to feeding mode (exclusive breast/formula feeding *versus* mixed infant diet).

As a practical and financial consequence of the design of the study, which aimed at simultaneous measurement of growth, energy intake and also energy expenditure by the very expensive doubly-labeled water method, a limited number of infants could be allowed to participate in the study. Also, energy expenditure measurements could not be performed but on a subset of infants. At 8 and 12 mo of age this inevitably subverts the power of the study as far as the energy expenditure data and their derivatives are concerned. We therefore present these data only as mean values for boys and girls. Here, data have been broken down into feeding mode and gender only when significant differences or interactions were observed.

## 8.4 RESULTS

### *8.4.1. Macronutrient and energy intake*

All infants were exclusively BF and FF from birth to at least 4 mo of age, except for 7 *breast-fed* infants at the start of the measurement period at 4 mo of age: 4 who started with supplemental formula, 2 with supplemental fruit and 1 with supplemental apple/pear juice. At 4 mo of age 2 *formula-fed* infants started with supplemental fruit, 3 with supplemental apple/pear juice and 1 with supplemental vegetables. In only one infant supplemental intake exceeded 10% of total gross energy intake (24%). These solids have been incorporated in the macronutrient and energy intake estimations of **Table 8.2**. None of the infants had switched from BF to FF or *vice versa*. At 8 mo of age 5 infants were still partially BF (which averaged 28±3 % of total energy intake and 16±5 % of total protein intake from breast milk, see **Table 8.2**). At 12 mo of age none of the infants received BF. Feeding time in BF infants at 1, 2 and 4 mo averaged 164 ± 26 min/d. Feeding time decreased with age (significantly at 2 and 4 mo for both sexes). IWL at 1, 2 and 4 mo averaged 25±8 ml. If no corrections for IWL would have been made, total intake would have been underestimated with 3.6±1.1%.

At 1, 2 and 4 mo, respectively, fat concentration of breast milk was 3.0±0.9, 2.9±0.8 and 2.7±1.1 g/100 ml, nitrogen concentration was 0.206±0.024, 0.183±0.018 and 0.165±0.018 g/100 ml, protein concentration was 1.12±0.16, 0.99±0.09 and 0.86±0.10 g/100 ml, lactose concentration was 6.45±0.38, 6.43±0.26 and 6.46±0.29 g/100 ml and

**Table 8.3** Percentage of total energy intake from protein, fat and carbohydrate [1].

| | *boys* | | *girls* | |
|---|---|---|---|---|
| | *BF (n = 9)* | *FF (n = 15)* | *BF (n = 14)* | *FF (n = 8)* |
| Energy-% protein: | | | | |
| 1 mo | 10 ± 0.9 | 10 ± 1.0 | 10 ± 1.7 | 10 ± 0.3 |
| 2 mo ¶ | 9 ± 1.3 | 10 ± 1.0 | 9 ± 0.6 | 10 ± 0.2 |
| 4 mo ¶ | 8 ± 0.9 | 10 ± 1.0 | 8 ± 1.4 | 10 ± 0.2 |
| 8 mo † | 17 ± 4.2 | 17 ± 4.3 | 14 ± 1.8 | 15 ± 2.1 |
| 12 mo | 20 ± 2.3 | 20 ± 3.2 | 20 ± 3.1 | 19 ± 3.1 |
| Energy-% fat: | | | | |
| 1 mo ¶ | 39 ± 9.2 | 50 ± 1.6 | 43 ± 6.8 | 50 ± 1.2 |
| 2 mo ¶ | 41 ± 5.1 | 50 ± 1.7 | 42 ± 6.9 | 50 ± 0.9 |
| 4 mo ¶ | 41 ± 5.9 | 50 ± 1.8 | 40 ± 9.5 | 50 ± 1.8 |
| 8 mo † | 30 ± 5.8 | 32 ± 4.5 | 34 ± 2.0 | 34 ± 4.6 |
| 12 mo | 23 ± 6.0 | 24 ± 5.4 | 25 ± 5.5 | 25 ± 4.2 |
| Energy-% carbohydrate: | | | | |
| 1 mo ¶ | 51 ± 8.8 | 41 ± 1.2 | 48 ± 6.0 | 40 ± 0.9 |
| 2 mo ¶ | 51 ± 4.0 | 41 ± 1.3 | 50 ± 6.6 | 40 ± 0.7 |
| 4 mo ¶ | 51 ± 5.1 | 41 ± 1.4 | 52 ± 8.7 | 40 ± 1.9 |
| 8 mo | 52 ± 6.1 | 50 ± 5.1 | 51 ± 2.2 | 51 ± 4.7 |
| 12 mo | 55 ± 5.6 | 54 ± 5.9 | 53 ± 5.3 | 54 ± 4.9 |

[1] Mean ± SD.
Significant effect of mode of feeding: ¶= $p<0.0001$.
Significant effect of gender: †= $p<0.01$.

carbohydrate concentration was 7.9±0.6, 7.9±0.5 and 7.5±0.6 g/100 ml. Energy concentration calculated from fat, protein and carbohydrate concentrations at 1, 2 and 4 mo respectively, was 271±33, 265±33 and 249±46 kJ/100 ml (which equals 65±8, 63±8 and 60±11 kcal/100 ml).

Nutrient intake and FQ's are summarized in **Table 8.2**. Except for carbohydrate intake, FF infants showed higher macronutrient and gross energy intakes during the exclusive BF and FF period. The difference was most striking at 2 and 4 mo of age. At 4 mo of age no difference between sexes or feeding groups was found in milk intake volume by the deuterium-to-infant method. **Table 8.3** shows that the percentage of $GEI_{TW}$ from protein and fat was lower for BF as compared to FF infants.

*8.4.2. Growth and body composition*

Birth weights were not significantly different between subgroups. Sum of 3 skinfolds was higher in FF infants of both sexes at 1 and 4 mo and in girls at 2 mo. Significant differences by feeding mode for weight were found in girls at 4 and 8 months (BF < FF: 5.9±0.7 vs. 6.6±0.5 kg at 4 mo, and 7.9±0.5 vs. 8.6±0.6 kg at 8 mo of age) but not in boys (6.8±0.8 kg at 4 mo and 8.7±0.8 kg at 8 mo). No significant differences by feeding mode in length and head circumference were observed (**Figure 8.1**). A significant difference by mode of feeding in TBF and FFM was found only in girls of 4 and 8 mo of

age. On average FFM was higher in boys at all ages (**Table 8.4**). Weight gain was higher in boys at 0-1 mo (27 g/d *vs.* 20 g/d in girls, $p<0.05$). Differences in weight gain by feeding mode were observed only in girls at 2-4 mo (FF>BF: 24 *vs.* 18 g/d, $p<0.01$). Length gain was not significantly influenced by gender or feeding mode. Fat gain was significantly higher in FF girls at 1-4 mo (FF>BF: 15 vs 11 g/d at 1-2 mo and 12 vs. 9 g/d at 2-4 mo, $p<0.05$). FFM gain was higher in FF infants only between 2-4 mo in girls (12 vs. 8 g/d, $p<0.01$).

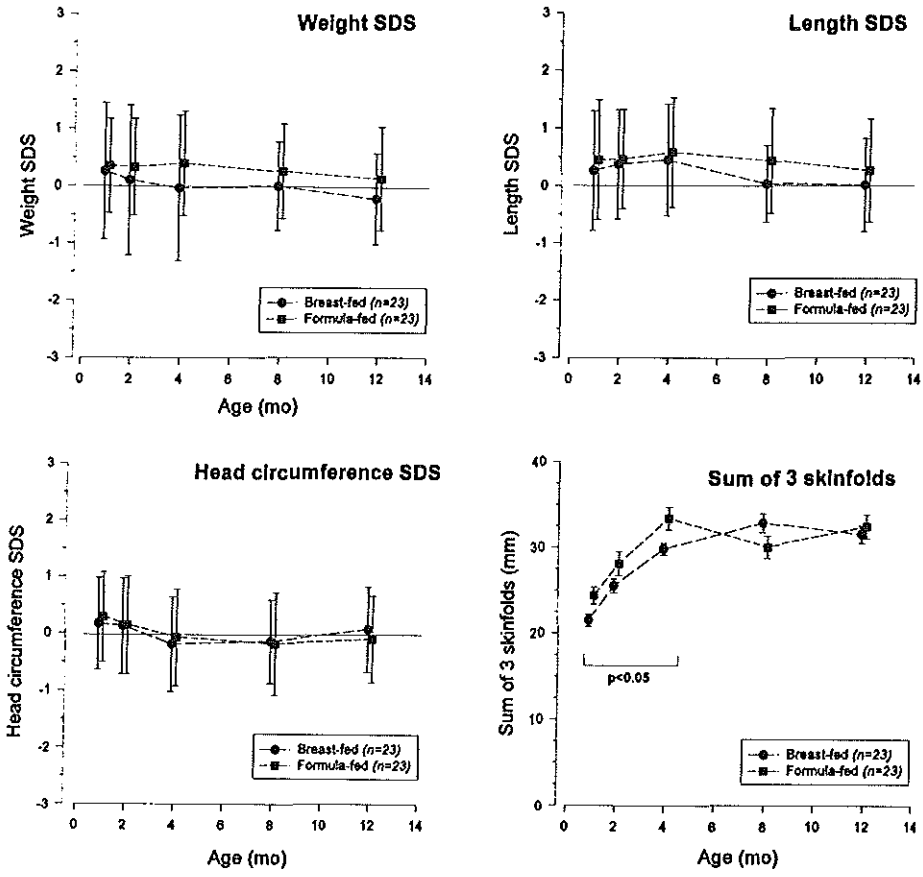


**Figure 8.1** Standard deviation scores of weight, length, head circumference and average values for sum of skinfolds. All data are mean ± SD, except 'sum of three skinfolds' : mean ± SE.

**Table 8.4** Body composition [1].

| | *boys* | | *girls* | |
|---|---|---|---|---|
| | *BF (n = 9)* | *FF (n = 15)* | *BF (n = 14)* | *FF (n = 8)* |
| Total body fat (%) | | | | |
| 1 mo | 15.2 ± 3.1 | 14.6 ± 4.2 | 14.4 ± 3.8 | 14.2 ± 2.2 |
| 2 mo | 20.6 ± 5.0 | 18.9 ± 3.3 | 19.1 ± 3.2 | 20.4 ± 2.9 |
| 4 mo | 24.7 ± 5.6 | 24.1 ± 3.7 | 25.0 ± 2.5 | 27.2 ± 1.9 |
| 8 mo | 26.7 ± 3.3 | 25.4 ± 3.7 | 25.5 ± 3.2 | 27.3 ± 3.4 |
| 12 mo $ | 26.6 ± 1.9 | 23.7 ± 5.4 | 24.9 ± 3.0 | 26.7 ± 3.6 |
| Total body fat (kg) | | | | |
| 1 mo | 0.70 ± 0.20 | 0.68 ± 0.24 | 0.61 ± 0.20 | 0.60 ± 0.11 |
| 2 mo | 1.17 ± 0.40 | 1.05 ± 0.28 | 0.95 ± 0.24 | 1.03 ± 0.15 |
| 4 mo | 1.71 ± 0.51 | 1.65 ± 0.42 | 1.49 ± 0.28 | 1.80 ± 0.14 |
| 8 mo | 2.35 ± 0.42 | 2.21 ± 0.48 | 2.02 ± 0.32 | 2.33 ± 0.31 |
| 12 mo | 2.71 ± 0.31 | 2.45 ± 0.73 | 2.30 ± 0.34 | 2.62 ± 0.46 |
| Fat-free mass (kg) | | | | |
| 1 mo † | 3.87 ± 0.40 | 3.91 ± 0.36 | 3.51 ± 0.42 | 3.63 ± 0.34 |
| 2 mo ‡ | 4.38 ± 0.41 | 4.43 ± 0.39 | 3.95 ± 0.47 | 4.04 ± 0.36 |
| 4 mo ‡ | 5.08 ± 0.46 | 5.13 ± 0.39 | 4.43 ± 0.48 | 4.83 ± 0.42 |
| 8 mo † | 6.43 ± 0.52 | 6.43 ± 0.47 | 5.86 ± 0.37 | 6.23 ± 0.53 |
| 12 mo ‡ | 7.46 ± 0.60 | 7.79 ± 0.64 | 6.92 ± 0.44 | 7.14 ± 0.55 |

Significant effect of gender: †= $p<0.05$, ‡= $p<0.005$
Significant interaction of gender by mode of feeding: $= $p<0.05$
[1] Mean ± SD

*8.4.3. Energy expenditure*

Energy expenditure (MJ/d) was not significantly different between BF and FF infants, except at 1 mo of age (**Table 8.5**). TDEE per kg FFM significantly increased with age from 1 through 8 mo of age ($p<0.05$, repeated measures ANOVA) and stabilized thereafter. TBW determined by $^{18}O$-labeled water has been reported before [18], and was not significantly different between feeding groups. Dose of $^{2}H_{2}{}^{18}O$ administered, $k_H$, $k_O$, $k_O/k_H$-ratio and $N_H/N_O$-ratio did not differ between study groups (**Table 8.6**).

TDEE was regressed against age (mo), weight (kg) or FFM (kg), sex (boys=0, girls=1):

TDEE (kcal /d) = 119 + 19.0 FFM + 6.44 $FFM^2$ + 41.7 Age - 2.21 $Age^2$ - 34.7 Sex
TDEE (kcal /d) = 97.3 + 48.8 Weight + 19.3 Age - 41.5 Sex
TDEE (kcal /kg FFM /d) = 78.0 + 7.46 Age - 0.400 $Age^2$ - 6.74 Sex

When quadratic terms of FFM, age or weight were significant, they were included in the equation. TDEE was not significantly affected by feeding mode.

**Table 8.5** Energy expenditure [1].

| | *Boys* | *Girls* | *All* |
|---|---|---|---|
| Energy expenditure (MJ / d) | | | |
| 1 mo † ¶ [3] | 1.36 ± 0.21 (n=18) | 1.19 ± 0.20 (n=16)[2] | 1.28 ± 0.22 (n=34) |
| 2 mo ‡ | 1.72 ± 0.22 (n=17) | 1.38 ± 0.22 (n=15) | 1.56 ± 0.28 (n=32) |
| 4 mo ‡ | 2.12 ± 0.29 (n=14) | 1.78 ± 0.23 (n=17) | 1.94 ± 0.31 (n=31) |
| 8 mo † | 2.91 ± 0.37 (n=12) | 2.56 ± 0.23 (n=10) | 2.75 ± 0.35 (n=22) |
| 12 mo † | 3.57 ± 0.23 (n=8) | 3.08 ± 0.36 (n=8) | 3.32 ± 0.38 (n=16) |
| (kJ / kg /d) | | | |
| 1 mo | 298 ± 46 (n=18) | 288 ± 42 (n=16) | 293 ± 44 (n=34) |
| 2 mo † | 315 ± 36 (n=17) | 286 ± 31 (n=15) | 301 ± 37 (n=32) |
| 4 mo $ [4] | 319 ± 42 (n=14) | 292 ± 40 (n=17) | 304 ± 43 (n=31) |
| 8 mo § [5] | 343 ± 42 (n=12) | 320 ± 35 (n=10) | 333 ± 40 (n=22) |
| 12 mo $ [6] | 341 ± 35 (n=8) | 323 ± 27 (n=8) | 332 ± 31 (n=16) |
| (kJ /kg FFM /d) | | | |
| 1 mo | 351 ± 53 (n=18) | 336 ± 50 (n=16) | 344 ± 51 (n=34) |
| 2 mo | 393 ± 44 (n=17) | 357 ± 48 (n=15) | 376 ± 49 (n=32) |
| 4 mo | 422 ± 51 (n=14) | 395 ± 56 (n=17) | 408 ± 55 (n=31) |
| 8 mo § [7] | 486 ± 54 (n=12) | 438 ± 40 (n=10) | 455 ± 49 (n=22) |
| 12 mo | 450 ± 40 (n=8) | 441 ± 32 (n=8) | 445 ± 35 (n=16) |

Significant effect of mode of feeding:§= $p<0.05$, ¶= $p<0.005$.
Significant effect of gender: †= $p<0.05$, ‡= $p<0.005$.
Significant interaction effect (gender by mode of feeding): $ = $p<0.05$.

1) Mean ± SD
2) Significant effect of mode of feeding (FF>BF) in girls only: $p<0.005$.
3) 1.30±0.13 (n=6), 1.40±0.23 (n=12), 1.07±0.16 (n=9), 1.34±0.13 (n=7) MJ/d, respectively, for BF boys, FF boys, BF girls and FF girls.
4) 305±48 (n=5), 326±40 (n=9), 308±33 (n=11), 262±37 (n=6) kJ/kg/d, respectively, for BF boys, FF boys, BF girls and FF girls.
5) 357±47 (n=5), 334±40 (n=7), 338±35 (n=6), 293±11 (n=4) kJ/kg/d, respectively, for BF boys, FF boys, BF girls and FF girls.
6) 323±21 (n=3), 351±40 (n=5), 340±25 (n=4), 306±15 (n=4) kJ/kg/d, respectively, for BF boys, FF boys, BF girls and FF girls.
7) 489±58 (n=5), 453±49 (n=7), 459±41 (n=6), 408±4 (n=4) kJ/kg FFM /d, respectively, for BF boys, FF boys, BF girls and FF girls.

### *8.4.4. Energy deposition*

Energy deposition calculated as TDEE *minus* $MEI_{TW}$ showed an extremely large error (data not shown). In several cases TDEE exceeded $MEI_{TW}$ and negative values for energy deposition were found. Using method A, **Table 8.7** shows higher energy deposition in boys at 0-1 mo and no differences thereafter. At 1-2 mo an interaction effect between sex and feeding mode was observed. The low values of energy deposition found between 0-1 mo (calculated partly with use of Fomon's reference data) as compared to 1-2 mo of age were not present at 1 mo and 2 mo as calculated by method B ("first derivative" method). Using method B, no significant differences between study groups were found.

### *8.4.5. Predicted metabolizable energy intake*

We checked for both methods A and B the 95% confidence intervals of the *p*-values of the

**Table 8.6.** Fractional turnover rates and ratio of dilution spaces of hydrogen and oxygen [1].

| | *1 mo* | *2 mo* | *4 mo* | *8 mo* | *12 mo* |
|---|---|---|---|---|---|
| dose (g) | 8.2 ± 0.9 | 9.9 ± 1.3 | 11.1 ± 2.1 | 15.1 ± 3.0 | 19.1 ± 2.8 |
| $k_H$ | 0.240 ± 0.040 | 0.237 ± 0.018 | 0.229 ± 0.023 | 0.202 ± 0.035 | 0.775 ± 0.032 |
| $k_O$ | 0.279 ± 0.041 | 0.280 ± 0.019 | 0.274 ± 0.026 | 0.252 ± 0.036 | 0.224 ± 0.034 |
| $k_O/k_H$ | 1.168 ± 0.028 | 1.182 ± 0.020 | 1.200 ± 0.022 | 1.250 ± 0.038 | 1.288 ± 0.052 |
| $N_H/N_O$ | 1.025 ± 0.005 | 1.026 ± 0.006 | 1.027 ± 0.006 | 1.026 ± 0.006 | 1.028 ± 0.008 |
| TBW (kg) | | | | | |
| boys | 3.04 ± 0.30 [2] | 3.43 ± 0.31 [2] | 3.99 ± 0.28 [2] | 4.93 ± 0.26 [2] | 6.15 ± 0.19 [2] |
| girls | 2.78 ± 0.30 | 2.93 ± 0.26 | 3.55 ± 0.42 | 4.50 ± 0.32 | 5.34 ± 0.56 |

[1] Mean ± SD.
[2] Significant effect of gender ($p<0.005$).
No significant difference between feeding modes or gender were present.

multiple linear regressions at 8 and 12 mo of TDEE and its derivative parameters and found wide ranges including zero. The power of all tests involving these parameters will undoubtedly be unsatisfactory due to the limited number of infants in which doubly labeled water experiments were performed. **Figure 8.2** shows that in most instances $MEI_{PRED}$ was significantly higher in boys as compared to girls (except at 1-4 mo when normalized for weight). A significant interaction effect between sex and feeding mode was present for $MEI_{PRED}$ expressed as kJ/kg/d at 1, 2 and 8 mo of age ($p<0.05$). For BF boys and girls, and FF boys and girls, respectively, $MEI_{PRED}$ was: 490±46, 448±54, 401±67, and 501±38 kJ/kg/d at 1 mo; 442±50, 414±35, 384±39, and 422±33 kJ/kg/d at 2 mo, and 336±16, 355±33, 358±31, and 309±11 kJ/kg/d at 8 mo of age (for corresponding *n* see Tables).

$MEI_{PRED}$ (MJ/d) was linearly related to age (**Figure 8.2**). Using data from method B the relation could be described as follows (with $MEI_{PRED}$ in kcal/d, which for clinical purposes is still the most customary unit):

$$MEI_{PRED}\ (kcal/d) = 467 + 31.2\ Age - 77.1\ Sex$$
$$MEI_{PRED}\ (kcal/d) = 191 + 62.6\ Weight - 32.4\ Sex$$
$$MEI_{PRED}\ (kcal/d) = -244 + 13.2\ Length - 53.8\ Sex$$
$$MEI_{PRED}\ (kcal/d) = 237 - 14.8\ Age + 1.257\ Age^2 + 59.8\ Weight - 33.7\ Sex$$

where sex is 0 for boys and 1 for girls, age in months, weight in kg, length in cm. When quadratic terms of age, weight or length were significant, they were included in the equation.

In **Figure 8.3** $MEI_{TW}$ is compared to $MEI_{PRED}$ by method B in the same subgroup of infants. It shows that $MEI_{TW}$ was increasingly underestimated in BF infants at 1-4 mo of age.

**Table 8.7.** Total daily energy deposition calculated from gain in fat and protein as derived from TOBEC body composition estimates [1].

| | boys (n=24) | girls (n=22) | All (n=46) |
|---|---|---|---|
| A. Energy deposition [2] | | | |
| (kJ/d) | | | |
| 0-1 mo [3] † | 288 ± 108 | 226 ± 83 | 259 ± 100 |
| 1-2 mo $ [5] | 585 ± 209 | 527 ± 176 | 556 ± 192 |
| 2-4 mo | 447 ± 146 | 443 ± 100 | 447 ± 125 |
| 4-8 mo | 231 ± 94 | 217 ± 107 | 224 ± 99 |
| 8-12 mo | 127 ± 136 | 127 ± 84 | 127 ± 112 |
| (kJ/kg/d) | | | |
| 0-1 mo [3] | 71 ± 29 | 60 ± 23 | 66 ± 27 |
| 1-2 mo $ [6] | 114 ± 36 | 116 ± 38 | 115 ± 36 |
| 2-4 mo | 72 ± 20 | 80 ± 17 | 76 ± 18 |
| 4-8 mo | 31 ± 13 | 31 ± 16 | 31 ± 15 |
| 8-12 mo | 13 ± 14 | 14 ± 10 | 14 ± 12 |
| B. Energy deposition [4] | | | |
| (kJ/d) | | | |
| 1 mo | 660 ± 270 | 648 ± 192 | 656 ± 230 |
| 2 mo | 552 ± 184 | 539 ± 125 | 548 ± 155 |
| 4 mo | 368 ± 79 | 355 ± 79 | 359 ± 79 |
| 8 mo | 150 ± 109 | 142 ± 96 | 146 ± 100 |
| 12 mo | 134 ± 234 | 138 ± 192 | 134 ± 213 |
| (kJ/kg/d) | | | |
| 1 mo | 142 ± 54 | 155 ± 43 | 148 ± 49 |
| 2 mo | 100 ± 29 | 109 ± 23 | 104 ± 25 |
| 4 mo | 54 ± 11 | 58 ± 15 | 56 ± 13 |
| 8 mo | 18 ± 13 | 18 ± 12 | 18 ± 13 |
| 12 mo | 12 ± 23 | 14 ± 20 | 13 ± 21 |

1) mean ± SD
2) calculated with average weights of 1-2, 2-4, 4-8 and 8-12 mo.
3) Calculated from actual weight gain data and reference data on composition of weight gain [Fomon et al., 1982].
4) Calculated for each child from the first derivative at each age of a third degree polynomial curve through either the values of TBF and FFM against age.
5) 644±247, 543±180, 472±171, 619±150 kJ/d, respectively, for BF boys, FF boys, BF girls and FF girls.
6) 125±46, 109±33, 104±33, 134±38 kJ/d, respectively, for BF boys, FF boys, BF girls and FF girls.

Significant effect of gender: † = $p<0.05$.
Significant interaction of gender by mode of feeding: $ = $p<0.05$

## 8.5 DISCUSSION

### *8.5.1. Predicted metabolizable energy intake*

Our study is the first describing simultaneous measurements of energy intake, TDEE and body composition in individual infants and direct calculation of energy requirements from TDEE and energy deposition. In our study population $MEI_{PRED}$ in MJ/d was not affected

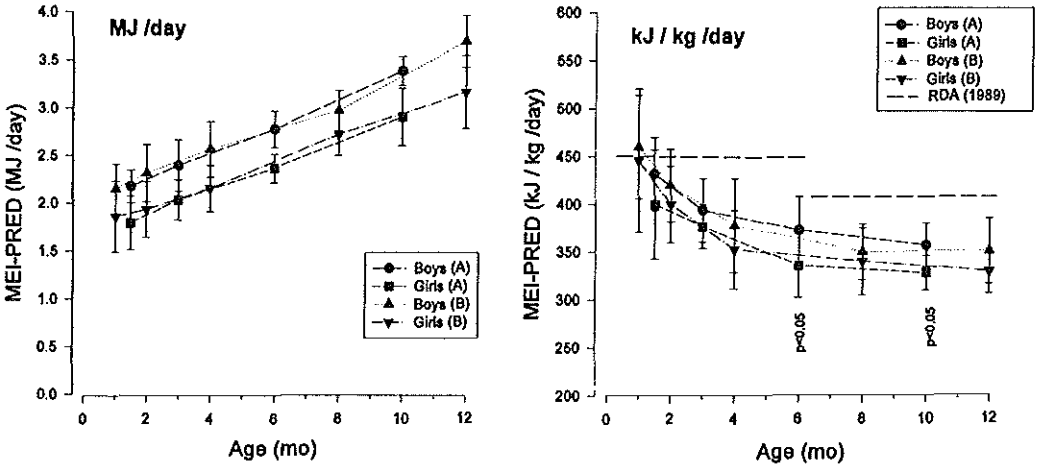


**Figure 8.2** Predicted metabolizable energy intake, calculated by adding total-daily energy expenditure and energy deposition (derived from body composition data). All data are mean ± SD. Dotted horizontal lines represent the recommended dietary allowances (RDA, 1989). *Method A:* Predicted metabolizable energy intake calculated using energy deposition from body-composition-differences between months. *Method B:* Predicted metabolizable energy intake calculated using energy deposition from "first derivative" method. For *n* see corresponding tables.

by feeding mode and was higher in boys. When $MEI_{PRED}$ was normalized by weight (kJ/kg/d) significant interaction effects arose, due to small body composition differences by feeding mode in girls. Former estimates of $MEI_{PRED}$ [1, 5, 6] were derived from compiled literature data on TDEE and reference data on composition of weight gain [7]. **Figure 8.4** shows that $MEI_{PRED}$ of the present study agreed with data of Prentice et al. [5] and of Whitehead [6], who based $MEI_{PRED}$ estimations on TDEE data of Prentice et al. Recently Butte [1] summarized TDEE data from various studies and summed these with energy deposition extracted from Fomon's reference data [7]. Averaged for gender and mode of feeding, Butte's estimates of $MEI_{PRED}$ at 0-2 mo and 9-12 mo deviate from our data and the other estimates of $MEI_{PRED}$ [5, 6]. A trend for lower energy deposition in the first month of life as observed by Butte [1] was present in our study as well, and might be due to the applied reference values for composition of weight gain [7] or to the physiological postnatal weight loss with subsequent lower energy deposition.

### *8.5.2. Energy intake by test weighing*

Our data on gross energy intake by TW ($GEI_{TW}$) agreed with Butte et al. [25, 37]. They

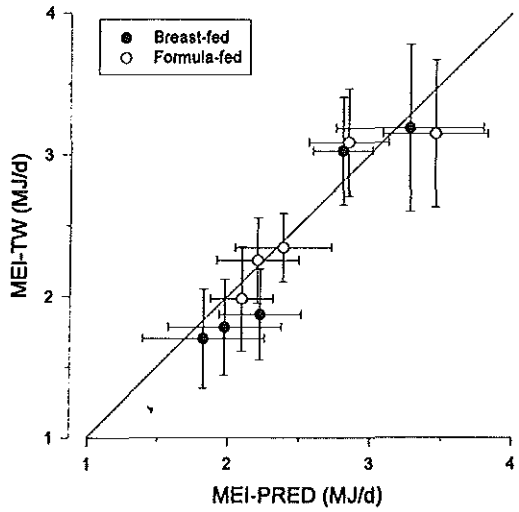


**Figure 8.3.** Comparison of metabolizable energy intake by test weighing and as predicted from energy expenditure and composition of weight gain. The difference is significant in breast-fed infants at 2 and 4 mo of age (Wilcoxon non-parametric test: p=0.03 and p=0.005, respectively), and in formula-fed infants at 1 mo of age (Wilcoxon nonparametric test: p=0.03).

found values of 422±67 *vs.* 493±71 and 301±38 *vs.* 364±46 kJ/kg/d for BF *vs.* FF infants at respectively 1 and 4 mo of age. However, the magnitude of the gender differences could not be adequately calculated from their paper. Except for some values in FF infants our data on energy intake agreed with a recent meta-analysis on energy intake of BF and FF infants [1]. In the DARLING study [26] $GEI_{TW}$ values were found at 3 mo of age in BF *vs.* FF infants of 359±50 *vs.* 405±59 and 359±38 *vs.* 418±63 kJ/kg/d in girls and boys, respectively. These values agree with the average of our 2 and 4 mo values for $GEI_{TW}$. For BF infants (but not FF infants) $GEI_{TW}$ at 8 mo in our study was higher than in the DARLING study at 9 mo, which may be due to the larger number of infants in the DARLING study still receiving BF at that age. Stuff and Nichols [27] reported $GEI_{TW}$ values of 301±88 and 263±75 kJ/kg/d at 8 mo for infants who received exclusive breast feeding until 5 and 6 mo of age, respectively. These values are lower than found in our study at 8 mo of age. Like in our study, the above mentioned studies [26,27] did not find a gender difference in $GEI_{TW}$ per kg body weight. In our study as well as in these latter two studies differences in $GEI_{TW}$ (at least from birth to 4 mo of age) did not result in changes in length growth or weight gain.

We are aware of the fact that the methodology of protein determination from nitrogen

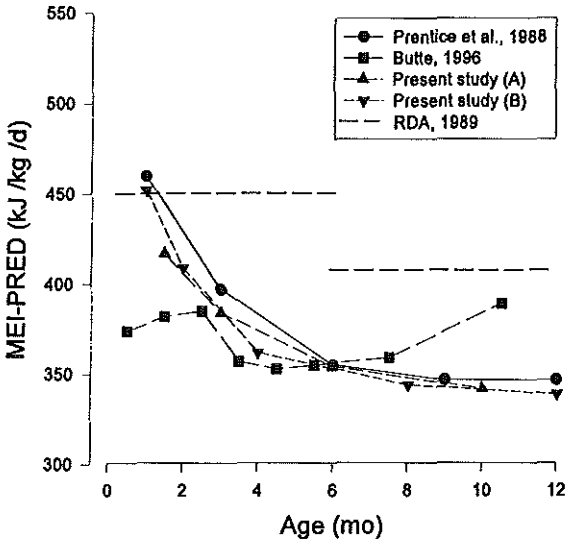


**Figure 8.4** Predicted metabolizable energy intake: data of the present study compared with earlier estimations of Prentice et al. [5] and Butte [1]. Dotted lines represent the recommended dietary allowances (RDA) [4]. *Method A:* Predicted metabolizable energy intake calculated using energy deposition from body-composition-differences between months. *Method B:* Predicted metabolizable energy intake calculated using energy deposition from "first derivative" method. For *n* see corresponding tables.

with assumption of NPN has its limitations. Different NPN values have been reported. However, because of the substantial inter-individual variability in NPN and the fact that our protein values are in agreement with other reports we suggest that the assumed value of 20% for NPN [12] is acceptable for our population and methodology.

The Rose-Gottlieb method used for breast milk fat determination might be a source of error in the discrepancy between the BF-FF differences found in $MEI_{TW}$ and not in $MEI_{PRED}$. The Rose-Gottlieb method measures triglycerides and not free fatty acids. Due to continuous lipase activity, true fat content might be increasingly underestimated when samples are held in storage for a longer period. However, the discrepancy would be anticipated to be more at 1-2 mo and less at 2-4 mo, while the opposite was true. Another explanation for the increasing discrepancy between $MEI_{TW}$ and $MEI_{PRED}$ from 1 -4 mo might be a difference in the amount of hind milk pumped by the mothers compared to the average amount normally suckled by the child. We asked the mothers to take the same time

for expression of milk per breast as they did for letting the baby drink on each breast. Data on total volume of milk pumped by the mothers was not recorded. We are not able to confirm that the mothers indeed pumped as much hind milk as they usually would have given to their babies. This difference indeed might become more exaggerated at 2 and 4 mo in mothers who might become more hastily and inaccurate in following the exact study protocol. Corrections for insensible water loss (IWL) during feeding time were made assuming a value for IWL of 1.8 g/kg/h [8]. Butte [1] recently summarized published IWL values, which averaged 1.62 ± 0.90 g/kg/h. The difference is too small to account for the discrepancy between $MEI_{PRED}$ and $MEI_{TW}$ at 1-4 mo of age.

The percentage of caloric intake from fat (fat en-%) at 12 mo of age averaged 25%, which was slightly lower than the two Dutch food intake surveys conducted in 1986 [28] and 1992 [29]. In these surveys the percentage of caloric intake from fat at 1 year of age averaged 31,7 ± 6.9 % in 1986 (n=100) and 29.9 ± 7.5 % in 1992 (n=101), respectively, which was lower than values at ages 2 through 5 y. At 12 mo none of our study infants had shown any sign of chronic non-specific diarrhea, which has been associated with low fat intake [30]. In addition to growth, which was normal according to the Dutch growth percentiles (**Figure 8.1**), also psychomotor development was normal: Bayley tests on psychomotor development at 18 mo of age were performed in all our infants and showed no difference between genders or feeding modes after correction for parental education (unpublished data).

### *8.5.3. Difference between $MEI_{PRED}$ and $MEI_{TW}$*

In the exclusively BF infants $MEI_{TW}$ was consistently lower than $MEI_{PRED}$. Estimation of milliliters of milk intake by TW equalled estimates of water intake (converted to milk intake) by deuterium-to-infant method. At 8 mo test weighing and subsequent self-reporting by the mothers overestimated $MEI_{TW}$ in all infants. Parents might be inclined to 'rounding off upwards' their baby's food intake. At 12 mo the 3 day 'double portion' measurement of energy intake well matched the $MEI_{PRED}$ assessment of 8-12 mo (if extrapolated to 12 mo of age) in all infants. The 'double portion' method might be preferred above self-reporting by parents of test-weighed food intake in older infants with mixed diets.

### *8.5.4. Energy content of breast milk*

Lucas et al assessed the metabolizable energy content of breast milk ($MEC_{BM}$) by an alternative approach with use of $MEI_{PRED}$ [24, 31]. Using this approach $MEC_{BM}$ in our study was 63±2 kcal/100ml and 67±3 kcal/100ml between 1-2 and 2-4 mo respectively. Lucas et al. found lower values: 57±5 and 60±5 kcal/100 ml at respectively 5 and 11 weeks of age and 59±5 kcal/100 ml in an earlier study at both 4-6 and 10-12 weeks of age [24, 31]. Due to the errors involved in each of the various steps of this method, however, these values can be interpreted only as a rough indication of breast milk energy content. To check the validity of this approach the metabolizable energy content of *formula*

($MEC_{formula}$) may serve as an internal check. $MEC_{formula}$ calculated from $MEI_{PRED}$ equalled $MEC_{formula}$ by test weighing with subsequent laboratory determination of energy content (67±2 kcal/100 ml *vs.* 69±2 kcal/100ml, respectively). $MEC_{formula}$ as stated by the manufacturer was 62 kcal/100 ml. The difference between the manufacturer's value and our observed $MEC_{formula}$ might be due to higher powder concentrations used by the mothers. Fat determination by the manufacturer and our study was performed by equal laboratory procedures [personal information].

*8.5.5. Growth and body composition*

No consistent differences were found in growth and body composition between BF and FF infants. However, when girls were analyzed separately, BF girls slightly lagged behind in growth and body composition at 4 and 8 mo of age, due to a reduced weight gain between 2-4 mo of age. At 2-4 mo of age FF girls had higher weight gains, resulting in higher amounts of TBF and FFM at 4 and 8 mo of age. Although this effect was small and the physiological significance may be questioned, it is interesting to see that in the DARLING study [32-34] as well as in animal studies in primates [35, 36] similar feeding effects were found which were more apparent in females. Further studies should find out whether this indeed is a physiological phenomenon.

Although not significant we observed the same trend in weight and length Z-scores as found in a recent meta-analysis [2]. Most studies in this area focussed on prolonged breast feeding. Growth data of our study were not different from others when restricted to ≤ 4 mo. A progressive increase in protein and energy intake and skinfold thickness in FF infants as compared to BF infants was found in the DARLING study. However, as in our study, length growth was not different, while only a small difference in weight gain was present, predominantly in girls [32-34]. Comparable differences of $MEI_{TW}$ in the period of exclusive BF or FF between feeding groups were found in the present study. Like in the DARLING study also in our study a steeper rise in skinfold thickness in FF infants as compared to BF infants was found at 1-4 mo, however, this phenomenon could not be observed in the *whole*-body composition measurements by TOBEC, ie, although skinfolds in FF infants were higher as compared to BF infants, *total* fat was not different.

*8.5.6. Conclusions*

In healthy infants exclusively BF or FF for 4 mo, significant differences in energy intake between feeding groups (determined by test weighing) were not followed by accompanying differences in energy expenditure, growth or body composition. In a subset of infants in which doubly labeled water measurements could be performed, metabolizable energy intake predicted from the sum of energy expenditure and energy deposition (by direct TOBEC body composition measurements) did not differ between feeding groups, but the study groups are small, especially at 8 an 12 mo. No differences in volume of milk intake (ml per day) were found between deuterium-to-infant method and test weighing. The discrepancy

between $MEI_{PRED}$ and $MEI_{TW}$ may be due to methodological problems in the accuracy and reproducibility of the estimation of breast milk energy and fat content by milk expression. The advantage of the assessment of $MEI_{PRED}$ from energy expenditure and composition of weight gain is that this approach gets around such methodological obstacles. $MEI_{PRED}$ estimations can be used alternatively to assess energy requirements in BF and FF infants. The data of the present study are in line with data from other studies [1,5,6] and can be used for future new guidelines for energy requirements in infants.

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*Part 5.*

# General Discussion Summary

# *Chapter 9*
# General Discussion

[8] But Daniel resolved not to defile himself with the royal food and wine. (...)
[15] And at the end of the ten days they looked healthier and better nourished than any of the young men who ate the royal food.
[16] So the guard took away their choice food and the wine they were to drink and gave them vegetables instead

*Daniel 1*

## 9.1 Introduction

In biblical times Daniel observed that men who ate vegetables and drank water thrived better than those eating the king's food and drinking wine. This ancient example of nutritional assessment illustrates that from time immemorial man has linked food consumption with health.

The central theme of the studies in this thesis was the measurement of body composition and its role in nutritional assessment and in the determination of energy utilization during the period of infancy. Because an accurate body composition method was lacking, much emphasis was laid on the measurements of body composition in infants (Part 1 and 2). The process of energy utilization can be summarized by the amount of energy consumed as related to the amount of energy 1) excreted with urine and feces , 2) expended by daily maintenance, growth, thermoregulation and activity, and 3) deposited in newly synthetized tissues (growth) (**Figure 9.1**). Because of the law on energy preservation pathological conditions on either of these energy utilization paths will influence the other paths.

This chapter opens with a short review on the present state of the art of body composition methods, with emphasis on their applicability in infants, illustrating why TOBEC was chosen as standard body composition method. The objectives of the present thesis which will be discussed here, were (as resumed from chapter 1):

1. Is TOBEC suitable for accurate measurement of body composition throughout the first year of life?

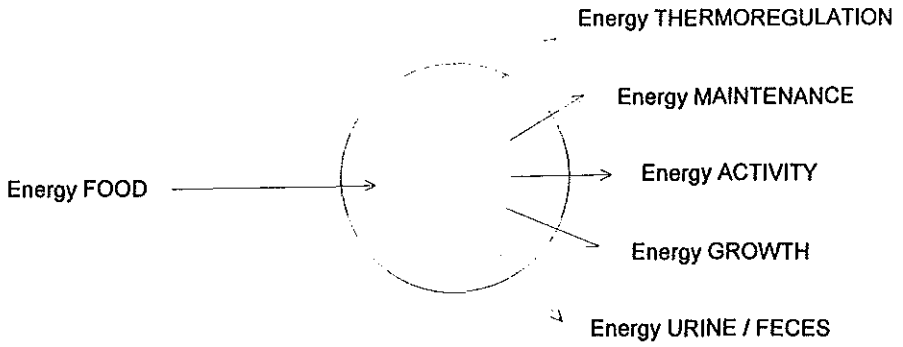


**Figure 9.1.** The process of energy utilization

2. What is the value of anthropometry for nutritional assessment in infants? Construction of reference centiles on infant total body fat and fat-free mass.

3. What is the effect of exclusive breast-feeding or formula-feeding on growth, body composition and energy utilization.
   Prediction of energy requirements from the sum of energy deposition (by TOBEC) and energy expenditure.
   How well do traditional methods assess energy consumption, especially in breast-fed infants?

## 9.2 SHORT REVIEW ON BODY COMPOSITION METHODS.

Body composition methods for use in infants have to be non-radioactive, non-invasive, without the need for active cooperation of the subject. This paragraph summarizes the most important indirect body composition methods with emphasis to their (potential) applicability in infants. Several body composition reviews have been published [Lukaski, 1987; Sheng & Huggins, 1979; Sheng & Nichols, 1991; Ellis & Nichols, 1993], and an excellent book on

**Table 9.1** Summary of literature data on carcass analysis of human infants and children.

| Author(s) [ref.] | Subjects | Wt g | FFM % | TBF % | TBW % | Prot. % | Other % |
|---|---|---|---|---|---|---|---|
| Iob et al. [1934] | 1 full term neonate | 2915 | - | 6.7 | 75.5 | - | 3.15 |
| Widdowson et al. [1951] | boy 4.5 y (tuberculous meningitis) | - | - | 22.7 | 53.8[3)] | 18.5[3)] | - |
| Widdowson et al. [1951] | 6 full full term neonates | - | - | 16.2 | 68.8[4)] | 12.0[4)] | - |
| Widdowson et al. [1964] | 7 full term infants > 2500 g | 3433 | 85.3 | 14.7 | 70.7[5)] | 11.9[5)] | 2.7 |
| Fomon [1967] | 6 male, still births >2400 g body weight [1)] | 3477 | 89.0 | 11.0 | 75.1 | 11.4 | 2.5 |
| Widdowson [1968] | from reference: Apte et al. [1972] | 3000 | 88.0 | 12.0 | 72.7 | 11.5 | 3.8 |
| Apte et al. [1972] | 12 term still births >2250 g[2)] | 2635 | - | 11.2 | 76.2 | 9.3 | 2.2 |

Wt = weight, FFM = fat-free mass, TBF = total body fat, Prot. = protein
1) Data were corrected for water loss between time of delivery and chemical analysis, assumed to be 277 g loss of water.
2) These infants were born from low-economic class mothers from India, many of them suffered from malnutrition.
3) On a fat-free basis: protein=23.8%, water=69.5%
4) On a fat-free basis: protein=14.3%, water=82.0%
5) On a fat-free basis: protein=14.0%, water=82.8±1.3%

this subject has been written by Gilbert B. Forbes [1987].

*9.2.1 Carcass analysis: the 'gold standard'.*

As Klish[1989] stated: "the only accurate way of measuring the composition of the human body and allowing for individual variation is by direct chemical analysis, but, since this is not practical, we are stuck with indirect methods". Widdowson and Dickerson [1964] extensively reviewed data on human carcass analysis. Most data were on fetuses. Very few carcass analysis data of infants and children were found at that time, and not much has been added on direct chemical analyses since. **Appendix 3** summarizes the body composition of the 'reference fetus' [Ziegler et al, 1976] and the 'reference child' [Fomon et al, 1982]. **Table 9.1** presents an overview of the literature data on human carcass analysis from full-term birth into childhood.

*9.2.2 Total body fat.*

*In vivo* estimation of the TBF compartment has been notoriously difficult. In our laboratory *absorption and desorption of non-radioactive xenon* has been used to measure TBF [Mettau et al, 1977]. The technique is based on the dilution principle. After an incubation period the amount of TBF is calculated from the clearance rate of the fat-soluble gas xenon. Results with this technique in animals were comparable with carcass analysis in laboratory animals. The xenon-method has been updated and validated in our laboratory for use in preterm infants. Other fat-soluble substances, like cyclopropane and krypton, have been used in the past by other investigators, all with unsatisfactory results.

A promising new body composition method, and recently described also for use in infants, is *dual-energy X-ray absorptiometry (DEXA)* [Brunton et al, 1993]. The DEXA technique consists of the use of two X-ray beams of different energies (40 and 80 keV), which are attenuated to different degrees as they pass through the body, dependent upon the quantity and nature of the tissue. Besides TBF, also FFM and bone mineral content can be measured and segmental body composition measurements can be performed. FFM can be predicted by DEXA with reasonable accuracy in small subjects. However, TBF estimations in small animals using the recently developed infant whole-body software showed a considerable systematic error [Picaud et al, 1996]. When the investigators performed a new calibration with piglets, the total standard error of the estimate (SEE) was 72 g [Picaud et al, 1996], which is similar to the error of the TOBEC calibration described in this thesis. Ellis and Nichols [1993] state that several technical and calibrational aspects of this method as, for example, beam hardening, body thickness and body geometry effects, fat composition and fat-free mass hydration have yet to be resolved.

Another technique, based on *inelastic scattering of neutrons* has an unacceptably high radiation dose for use in infants, and has been used for post-mortem analysis in preterm infants only [Ellis et al, 1994].

*9.2.3 Fat-free mass.*

The assumption that, for each group of age, FFM has a relatively constant composition, as regards to density, water, protein, potassium and bone, has led to the development of techniques that measure whole-body density, water, potassium or electrical conductivity, as indices of FFM. Body fat is hence calculated by subtraction of FFM from body weight. The gradual change, however, in hydration of FFM (especially during infancy) always should be taken into account when evaluating these methods. Boileau et al. [1984] calculated a decrease in FFM hydration of 0.38% per year in subjects between 8 and 30 years of age. Therefore FFM-hydration resulting from FFM maturation should either be incorporated into the calibration, or estimated individually and corrected for. Inter-individual variations and short-term shifts in water-content of the FFM have been found to be relatively small in healthy and sick adults [Beddoe et al, 1985] and will not be further discussed here.

***Body density and body volume.*** Although densitometry by underwater weighing is used as a standard method in adults [Behnke et al, 1942; Keys & Brozek, 1953], it has not been a

method of choice in infants due to the fact that underwater weighing needs active cooperation of the subject to be measured. Two alternatives for underwater weighing for use in infants have been developed. The first measures body volume by *air-displacement,* as calculated from the differential pressure changes between two identical chambers, one empty and one containing the infant [Taylor et al, 1985; Dell et al,1987]. This method has also been tested in our laboratory [data not shown]. We found that, although body density in inanimate objects could be predicted with reasonable accuracy, measuring living infants resulted in very considerable errors. *Acoustic plethysmography* [Sheng et al, 1988] measures body volume by the Helmholtz principle (resonance frequency is inversely related to the volume of the resonating chamber). Both methods are still in an experimental stage for use in infants.

*Body water.* Among the most frequently used body composition methods is the estimation of the various body water compartments by the dilution principle. Traditionally extracellular water is determined from 'exchangeable' chloride measured by bromide dilution. Total body water in infants is determined using isotopes of hydrogen [Friis-Hansen, 1957, Edelman et al. 1952] or (more recently) of oxygen [Trowbridge et al, 1984], while an indication of the amount of intracellular body water has been derived from the difference between total and extracellular body water. Usually FFM is calculated from TBW data using the reference values for FFM hydration calculated by Fomon et al. [1982], and TBF is derived by subtraction from body weight. As stated above, the propagation of errors into the calculation of TBF will be substantial, which will deteriorate the accuracy of the individual TBF estimate by isotope dilution.

*Anthropometry.* Weight for age and length, and length for age centiles are used in screening surveys. For more sensitive nutritional assessment the use of body dimensions and skinfold thickness measurements at various places on the body as an index or proxy for body fatness is ubiquitous [Lohman, 1981]. In adults Quetelet Index, waist-to-hip ratio and upper arm anthropometry [Jelliffe et al, 1969; Gurney et al, 1973] are most popular. Reference values for body dimensions have been collected for children and adults [Frisancho, 1990]. For the period of infancy, reference data on skinfold thickness and upper arm anthropometry have been collected [Tanner et al, 1975; Oakley et al, 1977; Sann et al, 1988]. Not much is known, however, on the validity of traditional anthropometric variables in infants. Subscapular and triceps skinfold thicknesses are poor predictors of infant body fatness [Davies & Lucas, 1990]. Also Quetelet's Index is a poor predictor of body fatness in infants [Davies & Lucas, 1989]. These studies showed that revision of traditional anthropometric parameters for use in infants was needed. Quantitation of the absolute amount of TBF has been performed by anthropometric prediction equations, from which those by Dauncey et al [1979] and by Weststrate and Deurenberg [1989] have been calculated specifically for use in infants. However, also these methods have never been validated appropriately, except from the studies described in this thesis (chapter 5 and 6).

*$^{40}$K-counting.* Potassium is the major intracellular cation. Measurement of the amount of $^{40}$K (which *natural* abundance in the FFM of the human body is 0.012%) by special low-

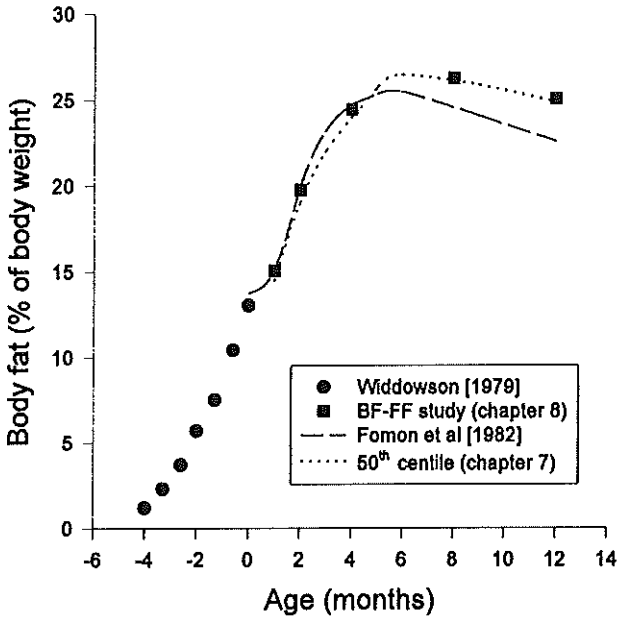


**Figure 9.2.** Percentage body fat in the intrauterine and extrauterine period, as determined by carcass analysis of human fetuses [Widdowson, 1979] and by TOBEC (data from chapter 8), respectively. Reference data from the present thesis (chapter 7) and from Fomon et al [1982] have been added.

background whole-body $^{40}$K-counters has been used for several decades to assess lean body mass [Forbes & Hursh, 1963], even in preterm neonates [Ellis & Shypailo, 1992]. The method is cumbersome and very expensive.

*Methods in an experimental stage, or not (yet) applicable to infants.* Because of the unacceptable dose of radiation, *computed tomography* (CT-scan) is not a method of choice in infants. *Near-infrared spectroscopy* has been reported to be inaccurate in infants [Kabir et al. 1994]. Estimation of TBF by *nuclear magnetic resonance* (NMR) has been reported for infant primates [Lewis et al, 1986], as well as *magnetic resonance imaging* (MRI) for use in adults [Fuller et al, 1985]. A technique based on inelastic scattering of neutrons by body carbon (*neutron activation analysis*) have been developed for use in adults. Because of unacceptable radiation doses, the method has been used only for post-mortem body composition measurements in preterm infants [Ellis et al, 1994].

*Methods using electrical conductivity.* These techniques are based on the principle that lean tissue better conducts an electrical current than fat. *Bioelectrical impedance analysis* (BIA) is a simple bedside method for FFM estimation with reasonable accuracy in adults

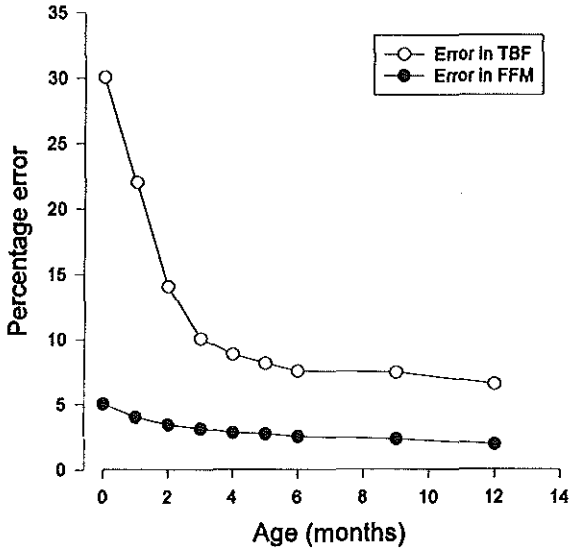


**Figure 9.3** Percentage error of individual total-body electrical conductivity (TOBEC) measurements individual estimation of total body fat and fat-free mass. Calculations based on mean body composition data from Fomon et al. [1982] and the estimation of the error in a TOBEC measurement from Chapter 3.

and older children. For children below 3-4 years of age the method has shown to lack accuracy [Mayfield et al, 1991]. When several electrodes are placed on the body's extremities, BIA measures electrical resistance when a very small amount of current is sent through the body. Because electrical current takes the route of least resistance, BIA outcomes appear to be confounded by the presence of e.g. adiposity or ascites. In contrast, *total-body electrical conductivity (TOBEC)* measurements are whole-body measurements, not dependent upon direct current flows, but on FFM-induced changes in the electro-magnetic field properties. TOBEC appears not to be confounded by adiposity [Fiorotto, 1991] or large extraperitoneal fluid injections [Cochran et al, 1989]. Details on the TOBEC technique have been extensively decribed in the preceding chapters, especially chapters 2 and 3.

## 9.3 BODY COMPOSITION MEASUREMENT IN INFANTS

### *9.3.1 Is TOBEC suitable for body composition estimation in infants?*

Part 1 of this thesis shows that the technique of total body electrical conductivity (TOBEC) is safe, accurate and reproducible to estimate FFM and TBF in infants. The accuracy of the

method has been established now in several ways. First, as described in chapter 3, the method has been thoroughly calibrated, independently in two centers, against data obtained from analysis of minipig carcasses. These animals show a change in body geometry and FFM maturation comparable to human infants [Fiorotto et al., 1987]. A very tight relation between FFM and TOBEC was found: 99.7% of the variability of the TOBEC outcome was explained by FFM as measured by carcass analysis. Second, regression of TOBEC-derived FFM against $^{18}O$-derived FFM(methods which are based on widely divergent methodological principles) was not significantly different throughout the entire first year of life (see chapter 4). Third, as was also found by Fiorotto [1991], when the changes in the actual amount of TBF present during the fetal period (measured by carcass analysis of human fetuses) and after full-term birth (measured by TOBEC) are plotted against age, the lines of the intrauterine and extrauterine period coincide around the time of birth (see **Figure 9.2**). While the minipig calibration equation is valid for minipigs onto 1 kg body weight, for human infants below ~4 kg body weight, as mentioned in chapter 3, TOBEC is likely to gradually overestimate FFM, because the standard curve is not linear below ~4 kg body weight. This has been observed first by Brans et al. [1992] and has been found by us recently in a 18 mo follow-up study in 76 preterm infants [data not published]. The geometry, shape on cross section and electrolyte composition of human preterm infants gradually more deviates from that of minipiglets. Calibration of the pediatric TOBEC is needed for the weight segment below 4 kg body weight, using carcass analysis of another species, or an other reference technique, preferentially a method directly measuring the (in preterm infants much smaller) TBF compartment.

The TOBEC method has not been thoroughly tested in sick infants. Our study described in chapter 2 on TOBEC measurements in non-human objects showed that errors arising from changes in temperature and major deviations from the normal cross-sectional area of the subject are likely to disturb TOBEC-derived body composition estimates (so care must be taken to measure TOBEC in infants with fever). Due to tissue autolysis and the drop in body temperature, TOBEC measurements after death are susceptible to considerable errors. We showed, however, that changes in FFM electrolyte concentrations will not disturb the TOBEC outcome, except from significant changes in bicarbonate levels (e.g. in the case of diabetic ketoacidosis and renal failure). Ions bound in a crystalline structure (such as in bone) will not elicit a significant TOBEC signal, nor wil deviations in plasma levels of protein and amino acids affect TOBEC outcome. As for the precision of TOBEC, in chapter 3 we estimated the precision of an individual measurement to be on average 0.154 kg of FFM. The error appeares to be a fixed value throughout the first year of life and becomes $<5\%$ above 3 kg FFM. As holds true for all indirect estimations of TBF, the propagation of this error of the larger compartment (FFM) into the subsequent estimate of the smaller compartment (TBF) is substantially higher. **Figure 9.3** is a plot of the percentage error in FFM and TBF by TOBEC in the first year of life (using the TOBEC error, described in chapter 3, of $\pm 150$ g and the body composition of the 'reference child' of Fomon et al. [1982]). No other method with equal measurement accuracy and safety is available yet.

As shortly discussed in chapter 2, in theory the em-field is more sensitive to eddy currents at the surface of a cylindrical object than those at depth. The effect of superficial eddy current distribution is highly significant at frequencies exceeding ca. *60 MHz* [Harpen 1989], increasing gradually with frequency [Harker 1973]. In our laboratory we have confirmed this with TOBEC experiments using cylindrical samples filled with a saline solution. Inclusion of empty cylinders of increasing diameter inside the saline-filled solenoids showed that the TOBEC HP-2 instrument was able to discriminate the smaller empty cylinders when placed excentrically inside the saline-filled solenoid, but not when placed centrally [data unpublished]. Sutcliffe et al. [1995] also found that the electromagnetic field of their experimental instrument predominantly responded to eddy current of the outer layers of a coaxial saline phantom. Although apparently eddy currents are elicited preferentially in the more outer layers of the body, it has been shown empirically that with the present *2.5 Mhz* TOBEC-HP2 coil FFM carcass analysis very well correlated with the transformed TOBEC value $\sqrt{(E\# \cdot L_{con})}$ ($r^2$=0.997, see chapter 3). This paradox is likely to be due to the fact that, on cross section, most of the non-subcutaneous (internal) body fat, as the peri-renal fat and the omental fat, is situated excentrally and *nòt exactly* in the midline of the body. Also, the above experiments were performed with saline cylinders which act as conductors, while in the body numerous cells with lipid membranes merely serve as capacitors, which will result in different dielectric behaviour. However, future studies using direct measurements of TBF (like the xenon method or DEXA) should give more insight in this matter.

Because several authors [Sutcliffe 1995; Brans 1992; Rallison 1993; Battistini 1993] 'misuse' TOBEC for estimation of body water it must be emphasized again that TOBEC measures FFM and not TBW. TOBEC correlates better with FFM than with TBW [Fiorotto 1991, Fiorotto, De Bruin et al 1995]. The good correlation of TOBEC with TBW is a result of the high correlation of TBW with FFM! One study (on small animals with the TOBEC SA model for small animals - see discussion below) could not confirm this better relation of TOBEC with FFM as compared to TBW [Bell 1994].

Recently several studies in rats have been published which showed only a moderate accuracy and a large geometry dependence of TOBEC [Bell 1994; Stenger 1995; Baer 1993]. These studies, however, were all carried out with the TOBEC SA device designed as a handy field instrument for small animals. This apparatus does not have the same precise solenoid copper windings producing a homogeneous em-field as is found in the pediatric HP-2 instruments. In our laboratory we have performed several experiments with the

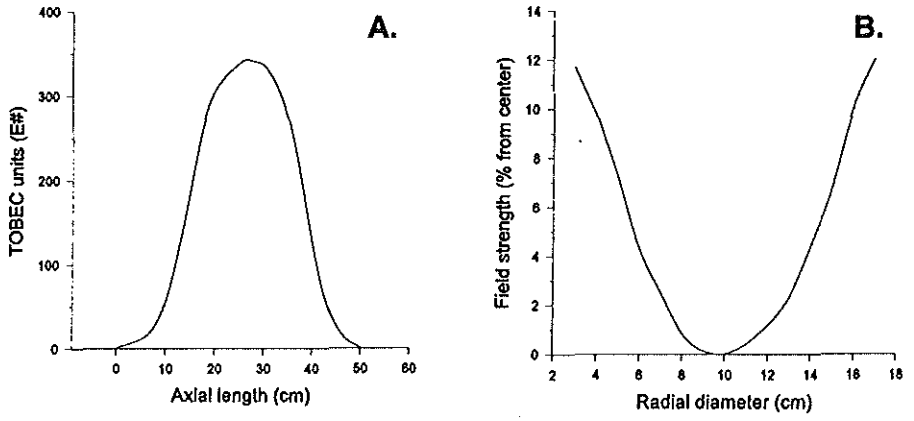


**Figure 9.4.** Electromagnetic field strength of a TOBEC SA-3203 total-body electrical conductivity instrument, plotted against coaxial length (**A**) and radial diameter (**B**). Data are personal observations (data unpublished) with a coaxial saline phantom. **A.** Electromagnetic field strength is in actual TOBEC units. **B.** Electromagnetic field strength is the percentage of the electromagnetic field strength measured at the center (at 10 cm).

TOBEC-SA model. **Figure 9.4** shows a plot of the TOBEC SA field strength against its coaxial and radial distance. Experiments in our laboratory with a TOBEC SA instrument showed that the instrument demonstrated a large intra-measurement drift in em-field strenth (data unpublished). Measurements were very sensitive to small changes in geometry and way of introduction of the object into the em-field. Therefore, the published results as concerning the SA instrument cannot be applied to the more sophisticated pediatric HP-2 model.

*9.3.2 The place of anthropometry in nutritional assessment in infants.*

Upper arm circumference is widely used as a simple proxy for body fatness, and is also used in infants [Jelliffe et al, 1969; Gurney et al, 1973]. Trowbridge et al. [1982] reported that in children from 2 to 6 years of age, urinary creatinine excretion correlated more closely with arm muscle area (calculated from triceps skinfold thickness and upper arm circumference) than with height or with arm circumference. As described in chapter 6, in infants upper arm circumference is very poorly correlated to TBF and FFM. Also skinfold

measurements and Quetelet's index have been found to be poorly correlated to TBF [Davies & Lucas, 1989, 1990; Kabir et al, 1993; Sheng et al, 1993]. It can be concluded therefore that in infants these traditional local anthropometric measurements, used in children and adults as a proxy for total body composition, poorly reflect the actual total energy and protein stores of the infant's body. Centile charts of these variables [Tanner et al, 1975; Oakley et al, 1977; Sann et al, 1988; Frisancho 1990], therefore, are of limited value in infants. Measurement of total body composition, represented by the body's TBF and FFM, will provide better estimates of nutritional reserves than regional anthropometric measurements and may provide a more accurate assessment of nutritional status. For infants quantitation of TBF has been performed traditionally using the anthropometric algorithm as described by Dauncey et al. [1977]. In Chapter 5 we showed a moderate accuracy and a very poor precision of Dauncey's method. We conclude that Dauncey's method is not suitable for measurement of TBF in the individual patient due to the large error.

The foregoing validations do not inevitably mean that anthropometric measurements are obsolete, however. As the vast majority of anthropometric parameters and indexes appeared to be invalid for infants, we decided to calculate new prediction equations for TBF and FFM. In Chapter 6 we calculated the best combinations of 2 or 3 anthropometric parameters and described the accessory prediction equations for TBF and FFM. For TBF no improvement in the predictive value was seen beyond inclusion of 3 parameters and for FFM beyond inclusion of 2 parameters. Depending upon anthropometric data available the appropriate equations can be chosen. When no accurate body composition method (like e.g. TOBEC or isotope dilution) is available, these new anthropometry-based prediction equations are a more accurate alternative to assess nutritional status in infants than upper-arm anthropometry or skinfold thickness. Anthropometric methods, however, are obviously less precise than TOBEC or isotope dilution methods (see chapters 4-6), therefore one should bare into mind the substantial error for the individual measurement when using these methods for body composition assessment on an individual basis.

Chapter 6 also described the validation of the new indexes and prediction equations in a second population of slightly older children. All measurements were performed by a second well-trained observer. While the precision of the various equations was equal to that in the first population, it appears that equations with skinfolds incorporated show the largest deviation in accuracy from TBF by TOBEC and FFM by TOBEC. We therefore estimated the interobserver variation separately for all anthropometric parameters. For skinfolds the estimated interobserver variation was ~10% (on all 5 locations), for upper and lower arm length ~6%, and for circumferences of calf, arm, thigh and chest ~2%. No interobserver difference was found for head circumference and total length. We concluded that the new indexes and prediction equations with calf circumference, weight and length therefore are least prone to interobserver variation, and are advised to be used in combination with the TBF- and FFM centile standards.

*9.3.3 Conclusions on the applicability of TOBEC and anthropometry.*
We conclude that TOBEC is a very suitable method for rapid, safe and accurate measurements of infant fat-free mass and hence body fat. The method has been thoroughly calibrated independently in two centers against carcass analysis data of minipigs, which animals show a change in geometry of their conductive mass and of FFM-electrolyte maturation comparable to human infants. Moreover, regression of FFM derived from TOBEC and $^{18}O$-dilution (both methods being based on widely divergent methodological principles) was not significantly different throughout the entire first year of life. Traditional anthropometric prediction equations and indexes were validated against TOBEC and were found to be invalid for use in infants, except from weight by length and calf circumference (for TBF) and length and weight (for FFM). Centile standards were assessed for TBF and FFM versus age, weight and length, while also new, infant-specific anthropometric prediction equations and anthropometric indexes were calculated which can be used in conjunction with these centile standards.We suggest that the present centiles are a valid way for monitoring nutritional status. Children at either extreme of the centile curves might be regarded as possibly at risk for obesity or undernutrition, although the number of infants were insufficient to accurately predict more explicit extremes as e.g. P97 and P3 centiles.

*9.3.4 Suggestions for future research.*
The direction of future research dealing with the TOBEC methodology should be on: 1) the comparison of TOBEC with new infant body composition methods, specifically dual-energy X-ray absorptiometry (DEXA) which presently is emerging as a new promising body composition method, 2) development of a new TOBEC device for use in preterm infants, and 3) the introduction of multi-frequency TOBEC measurements to discriminate between intra- and extra-cellular fluid compartments, using the different dielectric properties of extracellular and intracellular compartments at various frequencies of an em-field.

Future reseach topics on anthropometry, as related to the studies described in this thesis, could consist of, among others: 1) validation of the new prediction equations and indexes in sick infants and in other ethnic groups, 2) investigation of the usefulness of calf circumference and of its derivatives (e.g. analogous to the upper arm anthropometry) as simple screening tool for infant nutritional assessment e.g. in large population surveys or in developing countries, 3) reference centiles on calf circumference for infants. Further research should disclose the relation between infants at either extremes of the centile curves presented in chapter 7 on infant body composition and a possible associated risk for future health hazards. The suggested relation between malnutrition in early life and adult chronic disease [Widdowson & McCance, 1963; Barker & Winter, 1989; Lechtig, 1991; Osmond et al, 1993; Susser & Stein, 1994] and between obesity in infancy, childhood and adulthood and risk for coronary heart disease and cancer [Rolland-Cachera et al, 1984; Serdula et al, 1993; Dietz, 1994; Agras et al, 1990], certainly adds to this challenge.

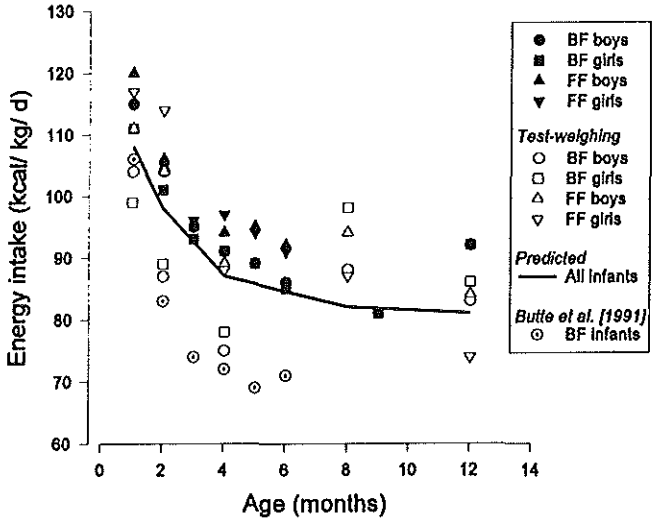


**Figure 9.5** Gross energy intakes of breast-fed (BF) and formula-fed (FF) infants. *Closed dots:* test weighing (Fomon et al [1993]). *Open dots:* test weighing (present thesis, chapter 8). *Open cross-dots:* test weighing (Butte et al [1991]). *Bold line:* Metabolizable energy intake predicted from energy expenditure and body composition (present thesis, chapter 8).

## 9.4 ENERGY UTILIZATION AND GROWTH IN TERM INFANTS.

### *9.4.1 Why revision of infant energy requirements?*

Recommended infant energy requirements have been derived generally from energy levels found in (expressed) breast milk. This concept is now challenged in two ways. First, usually no distinction is made in energy recommendations between different subgroups of infants. Evidence arises that various subgroups of infants may differ in energy needs. This has been suggested for breast-fed *vs.* formula-fed infants [Prentice et al., 1988; Butte et al. 1990] and for healthy *vs.* sick infants [Brooke et al. 1974; Parra et al. 1973]. Also, as is true for preterm infants in the direct postnatal phase, it is not unlikely that full-term and preterm born infants differ in energy requirements during the first months of life *corrected age.*

Second, controversy has arisen about the validity of the until recently generally accepted nutrient and energy density of breast milk, obtained by manual or mechanical expression [Whitehead, 1995]. Insight into the process of energy utilization, not only from the point of

view of energy intake, but also of energy expenditure and growth (as depicted **Figure 9.1**) must help to elucidate whether indeed there is a need for updating nutrient recommendations during the first year of life. To come to proper recommendations the normal patterns of energy expenditure, growth and energy deposition (i.e. fat and lean tissue accretion) for these subgroups should be estimated [a]. We studied these parameters in healthy full-term BF and FF infants (chapter 8). Although different growth patterns for infants receiving breast feeding for 9 months or more (Butte [1996] have been reported as compared to FF infants, our study could not confirm these observations. Our infants were exclusively BF or FF for at least 4 months. Only 5% of the infants were partially BF at 8 months of age. Differences in energy intake between BF and FF infants at 1 through 4 months of age were mainly due to differences in fat intake and to a lesser extent to protein intake. The magnitude of these differences, however, was not reflected in the estimates of energy expenditure and energy deposition.

*9.4.2 Assessment of energy intake from data of expressed breast milk and test weighing.* The principal difficulty in measuring milk intake has been one of obtaining representative breast-milk samples. Two problems appear. First, milk fat (a major determinant of energy content) varies considerably among individuals and changes diurnally and throughout lactation. Moreover, both milk fat concentration and milk flow rate may change continuously during the course of a feed from each breast. Lucas et al. have demonstrated that milk gained from manual or mechanical expression of the breasts does not have the same composition as milk obtained during normal suckling [Lucas et al 1980]. Milk fat concentration rises during the course of a feed. Therefore the actual energy intake of the infant may either be over- or underestimated, depending upon feeding habits and suckling behaviour of the infant. With equal milk volume intake, a habit of fully emptying one breast per feed will result in a higher energy intake than feeding half of both breasts, which is often advised to reduce the change for puerperal mastitis. **Figure 9.5** shows gross energy intake by test weighing as compiled from different studies, including our own data. Metabolizable energy intake predicted from TDEE and energy deposition also has been plotted in the graph. Fomon [1993] published energy intake data for BF and FF infants averaged from various literature data [Dewey and Lonnerdal, 1983; Whitehead et al. 1982; Dewey et al, 1991]. The author writes that it is troubling that considerably lower energy intakes have been consistently reported by the Houston group of researchers, from which the data of Butte et al. [1991] are a representative example. The energy intake data at the exclusive BF period (0-4 months) of our present study (chapter 8) are in better agreement with the Houston data as compared to the data of Fomon [1993]. The reason for this discrepancy is not known. However, as pointed out earlier it is most likely that different feeding practices of the mother (the way the mother is feeding the child - the content of one

[a] Psychomotor development is also an important parameter to consider in this respect, but is beyond the scope of this thesis.

breast fully given *versus* the content of both breasts partially offered to the infant) either over- or underestimate true energy intake from fully-expressed breast milk. Also fixed study protocols by various research groups with respect to the way of breast milk expression, feeding advices and laboratory nutrient /energy determination (e.g. bomb calorimetry versus macronutrient analyses and use of various conversion factors and correction factors for nutrient digestibility) will add to a consistant discrepancy. Moreover, Lucas et al. [1980] also reported that early in lactation suckled breast milk has a lower fat and energy content than had been previously reported in expressed breast milk.

As for the second half year of life limited data on energy intake of non-breast fed infants are available. Most of the available data have been been collected by dietary interviews, which are known to have an inclination for overreporting. Fomon [1993] summarizes available data and arrives at an average energy intake in this period of approximately 91-100 kcal/kg/d, which is higher (most likely due to the dietary interview methods used) than found by us at 12 months, both with the double portion method and as predicted from energy expenditure and body composition (predicted metabolizable energy intake: $MEI_{PRED}$). As discussed in chapter 8, our values of $MEI_{PRED}$ are comparable with $MEI_{PRED}$ data of Prentice et al. [1988]. It is also obvious from **Figure 9.5** that in our study home-reporting at 8 months results in overreporting.

*9.4.3 The relation of energy intake to total-daily energy expenditure and body composition.* Reduction of energy intake at normal energy expenditure levels will inevitably result in less synthetis of new tissue, while excess energy intake will increase fat storage and probably also energy expenditure (see **Figure 9.1**). An increase in energy expenditure at higher energy intakes, usually interpreted as a mechanism to dispose of surfeit energy, has been clearly demonstrated in preterm infants (see review by Sauer [1991]). This mechanism only becomes apparent when besides energy intake also protein intake is increased [Van Aerde, 1991]. An increase in energy intake while protein intake was kept constant did not show an effect on energy expenditure in preterms [Kashyap et al, 1986, 1988; Schulze et al, 1987]. Sauer [1991] calculated the relation for preterm infants of TDEE with protein and energy intake as: (TDEE-kcal/kg/d) = 17.6 + 5.3 (protein intake-g/kg/d) + 0.2 (energy intake-kcal/kg/d), $r = 0.8$, $p < 0.00005$. Whether the ability to dispose of surfeit energy by an increase of metabolic rate continues into infancy and childhood is still speculative due to lack of sufficient data. Fomon et al. [1971] observed that reducing protein concentration of formula to 9.9 g/l decreased growth velocity before, but not after, 2 months of age. Butte et al. [1990] found increased energy expenditure per kg body weight in FF infants, which consumed higher amounts of energy and protein. However, two remarks should be made on the paper of Butte et al [1990]: first, in this cross-sectional study the difference in TDEE between BF and FF might well be a statistical artifact. As it is known that TDEE differs between genders, accounting for the effect of gender on TDEE only by entering gender as a

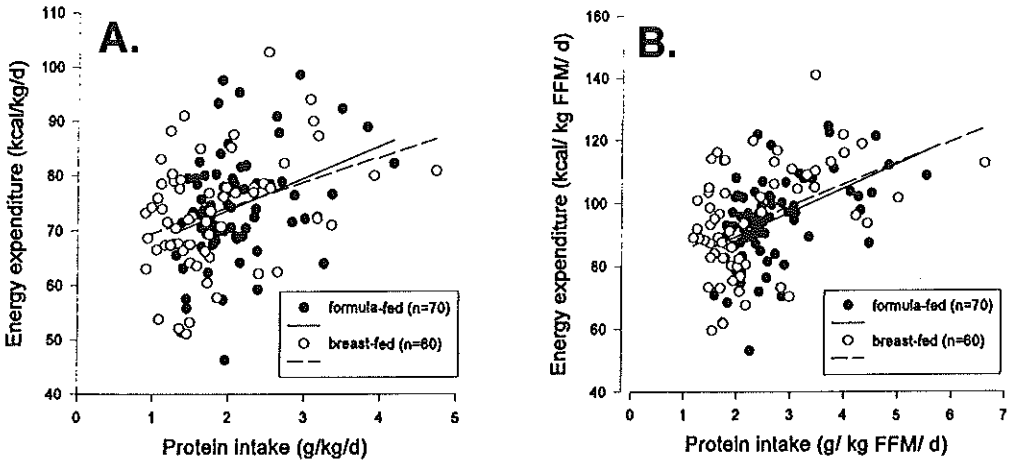


**Figure 9.6** Energy expenditure as a function of protein intake in the healthy, full-term, BF and FF infants aged 1 to 12 mo of our study (chapter 8). (**A**) Normalization for body weight. (**B**) Normalization for fat-free mass. For regression statistics see Table 9.2.

dichotome covariable into the ANOVA, seems insufficient, especially when boys and girls are largely unequally distributed over the study groups, as in this study. Secondly, energy intake was measured by test-weighing, and we demonstrated in chapter 8 that this method is likely to be unreliable for BF infants.

For the infants of our study described in chapter 8, **Figure 9.6** shows the relation between energy expenditure and protein intake when normalized by body weight or FFM. The relation of metabolic rate against protein intake, energy intake, age and gender, as determined by multiple linear regression, is summarized in **Table 9.2**. This table shows that energy expenditure in full-term infants during the fist year of life is mainly related to protein intake and barely to energy intake. The effect became even more clear when expressed per kg FFM.

As for the relation between energy *intake* and growth, in the 79 of the study infants of chapter 8 in which all energy intake and expenditure measurements were performed, energy intake by test weighing was positively correlated with gain in length ($r=0.27$, $p=0.014$), weight ($r=0.43$, $p<0.001$), TBF ($r=0.27$, $p<0.018$) and FFM ($r=0.33$, $p<0.003$) after normalization per kg body weight and controlling for age, gender and feeding mode (using partial correlation analysis). Comparable correlations with energy intake were found without normalization for body weight. These findings are in agreement with those of Heinig et al.

**Table 9.2** Multiple linear regression models to predict total-daily energy expenditure (TDEE) from protein and/or energy intake in healthy, full-term, breast-fed (BF) and formula-fed (FF) infants of our study (chapter 8) aged 1 to 12 mo.

| Feeding group | Regression equation: TDEE = | *P* | *r* | SD |
|---|---|---|---|---|
| Kcal/ kg/ d | | | | |
| BF (n=60) | 72.1 + 0.01E | 0.9 | 0..02 | 10.4 |
| FF (n=70) | 64.5 + 0.1E | 0.1 | 0.2 | 9.9 |
| BF (n=60) | 66.8 + 0.5P | 0.002 | 0.5 | 9.3 |
| FF (n=70) | 69.4 + 0.4P | 0.02 | 0.3 | 9.7 |
| BF (n=60) | 73.4 + 5.8P - 0.1E | 0.008 | 0.4 | 9.7 |
| FF (n=70) | 57.1 + 5.4P + 0.06E | 0.008 | 0.4 | 9.4 |
| BF (n=60) | 77.4 + 5.5P - 0.1E - 2.4S | 0.002 | 0.4 | 9.7 |
| FF (n=70) | 67.0 + 4.8P + 0.05E - 5.9S | 0.0009 | 0.5 | 9.1 |
| BF (n=60) | 65.1 - 1.3P + 0.1E - 3.1S + 1.7A | 0.0004 | 0.6 | 8.9 |
| FF (n=70) | 57.4 - 1.2P + 0.2E - 6.8S + 1.3A | 0.0003 | 0.5 | 8.8 |
| Kcal/ kg FFM/ d | | | | |
| BF (n=60) | 78.4 + 6.9P | 0.0004 | 0.4 | 15.7 |
| FF (n=70) | 74.2 + 7.7P | <0.0001 | 0.5 | 12.0 |
| BF (n=60) | 71.5 - 4.2P + 0.2E - 4.9S + 3.7A | <0.0001 | 0.7 | 13.0 |
| FF (n=70) | 66.8 - 3.0P + 0.3E - 7.4S + 2.8A | <0.0001 | 0.6 | 10.6 |

BF=breast-fed, FF=formula-fed, TDEE=total-daily energy expenditure, P=protein intake (g/kg/d), E=energy intake (kcal/kg/d), S=sex (1:boys, 2:girls), A=age (mo)

[1993].

Energy *expenditure* was negatively correlated with gain in FFM ($r=-0.20$, $p=0.069$) and was not significantly correlated with gain in length, fat or weight. Without normalization per kg body weight no significant correlation between energy expenditure (kcal/d) and gain (g/d) in weight, fat or FFM was found. The much better correlations of tissue accretion with energy intake as compared to energy expenditure might be an indication that the full-term infant disposes of surfeit energy by deposition and not by elevating the level of energy expenditure, although differences in methodological precision should also be considered here.

*9.4.4 Energy requirements derived from doubly labeled water and TOBEC measurements*

The doubly labeled water method is unique in its ability to measure all energy expended by the individual. The measurement can be performed in a field situation, with much convenience for the infant and with relative ease. The expended energy which is measured not only covers resting metabolism and energy expended by activity, but also the energy

used during the synthetic processes involved in growing. The only thing not measured is the intrinsic energy deposited in the new tissue itself [Whitehead, 1995]. With the emergence of more accurate infant body composition methods as TOBEC, also the latter can be determined now, and thus metabolizable energy intake can be predicted from the sum of *total* energy expenditure and energy deposition. The accuracy of the estimation of energy requirements from doubly labeled water and TOBEC body composition is dependent upon the accuracy of the sub-calculations used. The accuracy and precision of TOBEC have been discussed in detail in the preceding chapters, and in paragraph 10.3.1. The possible errors involved in the TDEE estimation by the doubly labeled water technique will be discussed in this paragraph.

***Technical comments and error estimation.*** The doubly labeled water method was first described by Lifson et al. in 1955. Because of the costs of $^{18}O$-labeled water the method became economically feasible for use in humans not before 1975 with the emergence of extremely sensitive isotope ratio mass spectrometers [Lifson et al, 1975]. As listed by Lifson and McClintock [1966] the most relevant assumptions underlying the doubly labeled water technique are *1)* body water volume remains constant during the measurement period, *2)* rates of water flux and $CO_2$ production are constant through time, *3)* the isotopes label only the $H_2O$ and $CO_2$ in the body, *4)* the isotopes leave the body only as $H_2O$ and $CO_2$, *5)* the specific activities of the isotopes in $H_2O$ and $CO_2$ leaving the body are the same as in body water, *6)* labeled or unlabeled water or $CO_2$ in the environment does not enter the subject via respiratory or skin surfaces, and *7)* isotope background levels remain constant during the measurement period. For our study group of growing infants some of these assumptions are not corroborated. Due to growth the body water compartment does not remain constant and hydrogen is incorporated into newly gained tissue, mainly into fat. In infants also the amount of perspiration differs from children and adults, as well as the amount of nonsweat transcutaneous water loss (due to different proportion of skin area exposed). Finally, water turnover is higher in infants, which shortens the measurement period, as Schoeller states that the metabolic period must lie between one and three biological half lives of the isotopic tracers [Schoeller et al. 1987].

Based on experiments with $^3H_2{}^{18}O$ in animals under (for human circumstances) extreme conditions, Nagy [1980] reports that the following sources of error are *negligible* in most situations: *1)* use of an equation that does not correspond to the pattern of change in total body water, *2)* variations in rates of water or $CO_2$ flux through time, *3)* use of $H_2{}^{18}O$ dilution space as a measure of body water volume, *4)* exchange of $^{18}O$ between water and organic compounds in animals (including excrement), *5)* incomplete mixing of isotopes in the animal, and *6)* input of unlabeled water via lungs or skin. However, he found that under the following circumstances analytical errors of $\pm 1\%$ in isotope concentrations can cause *evident errors* exceeding 70% in calculated rates of $CO_2$ production: *1)* little decline in isotope concentration during the measurement period, *2)* final isotope concentration closely approaching background levels, and *3)* high rate of water flux relative to the rate of $CO_2$ production. In our study some samples met these last criteria. These samples also showed a

deviation in $N_H/N_O$ ratio beyond 3 SD, which indicates an error in sample handling or storage, and were excluded from further data analysis (see chapter 8).

The effects of isotope fractionation are more important in young infants compared with adults because of the high water turnover. We used fractionation constants adapted for infants by Jones et al. [1987] and adopted by Westerterp [1991]. Changes in isotopic background during the measurement period are not likely to have occurred, for it was emphasized towards the parents (and controlled for) that diets (especially at times of weaning at 4 and 8 months of age) should not be changed one week prior to and during the measurement period.

Due to readily exchangeable nonaqueous hydrogen the hydrogen pool size has been experimentally estimated to be enlarged compared with the body water compartment by approximately 3% in adults [Schoeller, 1980], which was lower than the theoretically calculated value of 5.22% by Culebras and Moore [1977]. This value can be derived from the $N_H/N_O$ ratio. In the infants of our study (chapter 8) we found an $N_H/N_O$ ratio of 1.027, which is consistent with the 3% enlargement of hydrogen pool size found for adults. No significant difference between gender, feeding mode or age was found. Besides readily exchangeable nonaqueous hydrogen, which enlarges the hydrogen pool size as compared to the body water compartment, also sequestration of hydrogen (mainly) into the body fat tissue of rapidly growing subjects occurs. From the data of Haggarty [1991] the relation of the percentage error in $rCO_2$ and weight gain (g/d) in infants can be calculated [b]. At maximum weight gain at 1-2 months of age the error from incorporation of hydrogen into fast growing fat tissue will not exceed -0.5%.

An error of 1-4% has been estimated as arising from the use of FQ (food quotient) instead of RQ for the calculation of TDEE from $rCO_2$ [Butte, 1990]. FQ will not equal RQ in conditions of energy imbalance, because part of the nutrient intake is used for growth. We therefore adjusted FQ for fat and protein accretion as derived from weight gain during the measurement period and reference data on weight gain composition [Fomon et al. 1982]. Black et al. [1986] calculated that complete ignorance of this correction leads to an error of -3.5% in the final TDEE calculation in infants between 1 to 4 mo of age. The *total* error in TDEE by the doubly labeled water method was estimated at ±5% under field conditions and with use of corrected FQ values [Black et al. 1982].

It must be noted however that this 5% error is based on the multipoint approach. Black and collegues from the laboratory of Dr. Coward (Dunn Nutritional Unit, Cambridge UK) use a multipoint urine sampling strategy, while in our study the two-point strategy was used (adhered to by the laboratories of Dr. Schoeller and Dr. Westerterp). Cole et al. [1990] calculated the combined accuracy and precision of the two-point approach to be 3.6% for adults. A 5% error for the two-point method therefore seems reasonable. Although there is still debate by the two 'camps' on the subsequent pro's and contra's of these strategies, it has been accepted that, when laboratory sample analyses are precise and there is little doubt

[b] %error in $rCO_2$ = -0.1176-0.0114 (weight gain), $r^2$=0.98, SE=0.42%.

**Table 9.3** Weight, length, total body fat (TBF) and fat-free mass (FFM) velocity of breast-fed (BF) and formula-fed (FF) boys and girls at 2 to 4 months of age.

| Growth velocity at 2 to 4 months | Boys | | Girls | |
|---|---|---|---|---|
| | BF (n=9) | FF (n=15) | BF (n=14) | FF (n=8) |
| Weight (g/d) $ | 24 ± 7 | 22 ± 5 | 18 ± 3 | 24 ± 4 ** |
| Length (mm/d) † | 1.03 ± 0.16 | 0.92 ± 0.23 | 0.85 ± 0.12 | 0.87 ± 0.16 |
| TBF (g/d) | 10 ± 4 | 10 ± 3 | 9 ± 2 | 12 ± 3 * |
| FFM (g/d) †$ | 13 ± 3 | 12 ± 3 | 8 ± 2 | 12 ± 2 ** |

† Effect of gender by multiple linear regression: $p < 0.05$
$ Interaction effect of gender by feeding mode: $p < 0.05$
* Effect of feeding mode in subgroup of girls only: $p<0.05$
** Effect of feeding mode in subgroup of girls only: $p<0.01$

on the validity of the samples, the two-point method is more robust, cheaper and less time consuming than the multipoint approach. Because only average values were used for the estimation of energy requirements the possible errors from the probably less precise two-point samples will be averaged out.

An increased methodological error however will also increase the SD of the $MEI_{PRED}$ estimations beyond the level of biological scatter. As a consequence of this, the advised safety margin in recommendation on energy requirement of +2SD will be exaggerated; therefore, as Fomon [1993] already pointed out, a safety margin of +1SD on prediction equations like those given in chapter 8 will be sufficient.

In a recent summary by Fomon [1993] most reports showed no significant difference in gastrointestinal fat absorption by BF and FF infants. We found one study with fat absorption in BF > FF, in which stool total lipid content was determined indirectly from the sum of nonsoap lipids and soap fatty acids [Quinlan et al. 1995]. If digestibility nevertheless would be a cause in the discrepancy between predicted metabolizable energy intake and energy intake by test weighing (described in chapter 8), the energy conversion factors used for calculation of energy deposition from TBF and FFM accretion will not be fully appropriate and should be reevaluated.

*9.4.5 Body composition and growth in healthy breast-fed and formula-fed infants.*
The infants described in chapter 8 were exclusively breast-fed (BF) and formula-fed (FF) from birth through at least 4 months of age. It was anticipated that an effect from mode of feeding could be expected approximately from 2-3 months onward [Hitchcock et al. 1981; Chandra et al. 1981] and therefore gain in weight, length TBF and FFM in the period of 2-4 months had our special attention (see **Table 9.3**). No significant differences between BF and FF infants were found by us using ANOVA or multiple linear regression techniques. In the period of 2-4 months of age a significant statistical interaction effect of gender by feeding

mode was present (i.e. conflicting direction of significant differences between subgroups) for gain in weight and FFM. This was due to a decrease in rates of gain in FFM and weight in BF girls. Although the effect was small and the number of infants in our study was limited, it is interesting to notice that several other authors also found the decrease in growth velocity with lower energy and/or protein intakes to be more predominant in females than in males [Heinig et al 1993; Lewis et al 1989 ; Owen et al 1984]. Shepherd et al [1988] also found a gender-specific feeding effect on body composition; they found that formula feeding promoted greater fat accretion in boys and greater FFM accretion in girls. Confusingly, however, and conflicting with these latter observations, Salmenperä et al. [1985] as well as Ahn et al. [1980] found, predominantly in boys, differences in length but not in weight for (prolonged) BF *versus* FF.

Probably due to selective referencing of literature in many papers, several authors have already taken the position that growth differences between BF and FF infants are established now [Fomon, 1993]. Various studies have reported equal growth between BF and FF infants [Evans, 1978; Saarinen and Siimes, 1979; Whitehead and Paul, 1981; Butte et al., 1984; Persson, 1985; Stuff and Nichols, 1989; present study]. Many reports, which have focused on prolonged breast-feeding ultimately find late growth differences in the second half year of life [Ahn et al. 1980; Owen et al. 1984; Salmenperä et al. 1985; Dewey et al. 1992]. A recent meta-analysis on BF growth studies found a difference in growth of BF infants from 3 through 8-12 mo, as compared to reference centiles [Dewey et al, 1995].

When body composition between healthy BF and FF infants is considered, also here conflicting data arise. Salmenperä et al. [1985] found higher skinfold thickness values for BF infants, while Dewey et al. [1993] and our study (chapter 8) present higher skinfold thickness values for FF infants. Recently, Butte et al. [1995] published data of a cross-sectional study on body composition in BF and FF infants at 1 and 4 months of age. Their data were comparable with ours. TBF and FFM as measured by TOBEC and $^{18}O$-dilution did not differ between feeding groups when expressed in absolute terms. When expressed per kg body weight, at 4 months their BF infants were significantly leaner than FF infants.

After so many conflicting studies, is seems reasonable to suggest that growth is *not directly* related to nutrition, but either multifactorial, or related by some kind of confounding factor. Several factors which have an effect on both growth and nutrient intake may come to mind (genetic and social climate). Parental weight and height for example is known to be correlated with growth ($r$ = 0.44 [Salmenperä et al. 1985]).

In our study (chapter 8) significant "confounding" background factors were, when controlled for gender, for *fat gain*: gestational age (at 1-2 mo: $r$ = 0.42), father's age (at 1-2 mo: $r$ = - 0.31), father's length (at 4-8 mo: $r$ = - 0.32) and birth weight (at 4-8 mo: $r$ = - 0.39), for *fat-free mass gain*: father's age (at 4-8 mo: $r$ = 0.37), and gestaional age (at 4-8 mo: $r$ = - 0.37), for *length gain*: number of cigarette smoking (at 1-2 mo: $r$ = - 0.36), mother's weight (at 2-4 mo: $r$ = - 0.34), for *weight gain*: mother 's length (at 1-2 mo: $r$ = 0.32), father's length (at 4-8 mo: $r$ = - 0.33), father's age (at 4-8 mo: $r$ = 0.36) and birth weight (at 4-8 mo: $r$ = - 0.35). In our study birth weight was negatively correlated with

weight gain and TBF gain at 4 to 8 months of age ($r$ = -0.35, $p < 0.05$). Persson et al. [1985] also found the effect of birth weight on growth velocity (negative correlation) being present in the first months of life. A clear regression to the mean effect for weight and length after birth was shown by Persson et al. [1985]. The additive effect of psychosocial stimulation and nutritional supplementation on psychomotor development has been demonstrated by the Jamaica Study in infants aged 9-24 months [Grantham-McGregor et al., 1991]. For somatic growth, supplementation but not stimulation was beneficial [Walker et al. 1991]. The effect of social environment and parental education on psychomotor development is well established. Poor social environmentment affects gestational age and birth weight, like emotional deprivation may result in dwarfism. The relation of this type of factors on the study of breast feeding outcomes is known and are potential confounding factors for growth and nutritional studies in BF and FF infants.

*9.4.6 Suggestions for future research.*

Larger studies should confirm the presently found discrepancy between energy intake calculated from the sum of energy expenditure and body composition on the one hand and test-weighing and expression of breast milk on the other hand. More studies are needed to confirm the different fat and protein concentrations published by Lucas et al. [1980] as derived from suckled breast milk using the nipple shield method. To come to new recommendations for energy intake, especially focussed on special subgroups of infants, more studies are needed, e.g in full-term and preterm infants (small-for-dates as compared to appropriate- or large-for-dates) during the first year of life. Studies by Dr. Lewis in Iowa in primates showed (by carcass analysis) that preweaning diet influences childhood as well as adolescent body composition [Lewis et al. 1984, 1986, 1989]. These long-term prospective studies have never been carried out in humans. Lewis et al. also showed an effect in primates of energy intake on hormonal status [Lewis et al. 1992, 1993]. Except from a few recent papers for young adults [Gertner, 1993; Travers et al. 1995; Ling et al. 1995; Hoffman et al. 1995; Mårin et al. 1995], to our opinion not much is known in this respect, for growing humans.

## 10.5 FINAL CONCLUSIONS.

We conclude that at present TOBEC is a suitable and robust method for rapid, safe measurements of body composition throughout the first year of life. Acccuracy has been indirectly proven in several ways. TOBEC-derived FFM regressed against $^{18}O$-derived FFM was not significantly different throughout the entire first year of life (whereas both methods are based on widely divergent methodological principles).

Traditional anthropometric prediction equations and indexes were validated against TOBEC and were found to be invalid for use in infants, except from weight by length and calf circumference (for TBF) and length and weight (for FFM). Calf circumference

appeared a new promising anthropometric parameter for body fatness and will replace the use of upper arm circumference for screening purposes in infants.

Centile standards were assessed for TBF and FFM versus age, weight and length, while also new, infant-specific anthropometric prediction equations and anthropometric indexes were calculated which can be used in conjunction with these centile standards.

It is likely that the energy content of breast milk cannot be accurately estimated from expressed breast milk samples. Estimates of energy intake from breast-fed infants by traditional methods may need reconsideration. New estimations of energy intake for the first time have been experimentally derived by us as predicted from the sum of total energy expenditure and energy deposition from direct accurate body composition measurements. The values were in agreement with more theoretical estimates based on the same principle of Prentice et al. [1988]. No differences were found for breast-fed or formula-fed infants. Boys showed higher energy requirements than girls. We provide simple linear regressions for prediction of energy requirements (chapter 8 and 9). These data serve as an approximation of energy requirement in male and female infants during the first year of life. Our data indicate that energy requirements in infants are lower than recommended by the guidelines which are presently used.

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# *Chapter 10*
# Summary

Accurate determination of body composition in infants is important for assessment of nutritional status and quality of growth in infants. To avoid the errors involved in the estimation of energy requirements from energy intake of breast-fed infants, body composition data in conjunction with estimates of energy expenditure are needed to come to new recommendation of energy requirements in the first year of life.

## 10.1. CHAPTER 1

After a short outline of the history of body composition measurements in humans, Chapter 1 summarizes the objectives of the present thesis which aimed at 1) studying the validity of the TOBEC technique for accurate estimation of body composition in infants throughout the first year of life, 2) examining the validity of anthropometry in infancy for clinical and scientific purposes, 3) using the TOBEC technique to determine for the first time reference standards for body fat and fat-free mass during infancy, and, 4) measuring growth and energy utilization (i.e. energy intake, energy expenditure *as well as* energy depostion) and assessing energy requirements from energy expenditure and energy deposition in a prospective pilot study in healthy breast-fed and formula-fed infants.

## 10.2. CHAPTER 2

In Chapter 2 the effect of alterations in physical and chemical characteristics on body composition estimates by the TOBEC technique was validated with non-human models.

The effect of electrolyte type, concentration and volume on TOBEC was determined using 2, 3 and 5 liter solutions of six different chlorides and sodium bicarbonate. Equimolar concentrations yielded TOBEC values in accordance with known ion-conductivities: $H^+ >> Ca^{2+} > Mg^{2+} > K^+ > Na^+ > Li^+$, and $Cl^- > HCO_3^-$. The behaviour of these solutions was described very accurately over a wide range of concentrations (1 to 200 mM) by a simple exponential law. Dissolved egg-white protein, glycine and L-glutamine elicited no TOBEC signal.

*In vitro*, using polyethylene bottles filled with physiologic saline, in the interval of 2 to

45°C a linear relation was observed between temperature and TOBEC. Below the freezing point no TOBEC signal was elicited. The effect of tissue autolysis and body temperature on TOBEC was examined by repeated measurements of TOBEC and temperature in seven fresh infant minipig cadavers. Five minipigs were allowed to cool. Shortly after death TOBEC decreased by 2.5% per °C. Two animals were kept at constant temperature. The TOBEC signal showed a gradual increase of 9% after seven hours due to autolysis.

We conclude that *in vivo* TOBEC measurements are affected by ion-concentration (e.g. non-isotonic hydration changes), geometry (e.g. deviations in body shape), temperature (e.g. fever, skin-cooling) and tissue autolysis (measurements after death). Proteins, molecules with strong dipole moments, and ions trapped in crystalline structures do not significantly affect the TOBEC reading. Practical consequences of the present study could be summarized as follows: 1) the reproducibility of TOBEC measurements is excellent. Variability of instrument response is only a minor source of error in the estimate of body composition 2) errors arising from changes in temperature will undoubtly disturb TOBEC-derived body composition estimates, 3) most physiological changes in FFM electrolyte concentrations will not disturb the TOBEC outcome although changes in bicarbonate levels may affect the TOBEC signal in the case of diabetic ketoacidosis and renal failure, 4) deviations in plasma levels of protein and amino acids will not affect the TOBEC outcome, 5) ions bound in a crystalline structure (such as in ice and bone) will not elicit a significant TOBEC signal, 6) major deviations from the normal cross-sectional area of the measured subjects may have a significant effect on TOBEC, 7) due to tissue autolysis and body temperature decline, TOBEC measurements after death are susceptible to considerable errors.

Finally, this study showed that electrolyte solutions in a TOBEC electromagnetic field behave in accordance with data on ion conductivity. This is of much importance to the TOBEC methodology: it suggests that much of the knowledge of the behaviour of electrolytes, cells and tissues in an electromagnetic field as has been studied in the past can be transferred to the field of body composition measurements by total body electrical conductivity.

## 10.3. CHAPTER 3

In Chapter 3 we compared precision, day-to-day variability, and magnetic field profile of three TOBEC HP-2 (pediatric) instruments. We also derived a new calibration equation that relates the fat-free mass to the total body electrical conductivity measurements in piglets, and compared it with the equation provided currently by the manufacturer which was derived on a prototype TOBEC instrument.

The performance of the instruments was generally similar, although a significant difference in the magnetic field of one instrument was identified. A strategy for identification of dissimilarities compared to our instruments was given in this chapter. The coefficient of variation of inanimate phantom measurements varied from $\pm 0.2$ to $\pm 0.5\%$, and the day-

to-day variability was generally similar. Such measurement error is significant (±0.035 to 0.078 kg FFM) for small subjects.

The new calibration equation was similar to the original equation; therefore, all the data were pooled to generate a new equation that is linear at least to 10 kg.

Thus, the HP-2 total body electrical conductivity instruments, which can be safely and easily used to measure FFM and fat in infants through 1 year of age, proved to be reliable and precise, and results obtained from different instruments can be confidently compared.

## 10.4. CHAPTER 4

In Chapter 4 fat-free mass of healthy infants (149 measurements (boys n=76, girls n=73) in 50 infants aged 1-12 months) was measured by two body composition methods based on widely divergent principles: the TOBEC and the isotope dilution technique. Isotope dilution techniques are widely used to estimate total body water and calculate fat-free mass. The hypothesis was tested that the TOBEC calibration equation based on minipig carcass analysis data, and validated earlier in infants to 4 mo of age, would yield accurate estimates of FFM also in the second half year of life. At this age body geometry of minipigs progressively deviates from human infants.

TOBEC-derived fat-free mass and fat mass were in excellent agreement with Fomon's reference data. Strictly linear relationships with slopes not significantly different from one were found between fat-free mass by TOBEC and fat-free mass by $^{18}O$ isotope dilution ($r$ = 0.98, SD = 0.29 kg for boys, $r$ = 0.98, SD = 0.32 kg for girls). When body water is calculated from the raw isotope dilution data, body water is underestimated by the extrapolation to t=0 approach and an overestimated by the plateau approach.

The second objective was to validate the extrapolation approach against TOBEC. Fat-free mass by the $^{18}O$ isotope dilution technique was slightly but significantly lower than with use of the TOBEC method, the difference being on average 0.18 (±0.24) kg for girls and 0.08 (±0.21) kg for boys (ie, 4 % (SD = 4.5%, $p < 0.0001$) and 1.5 % (SD = 3.9%, $p$ = 0.004), respectively).

We concluded *1)* that the TOBEC calibration equation is accurate for the entire first year of life, and *2)* that isotope dilution using the extrapolation to t=0 approach is suitable for body water and fat-free mass estimations in groups of infants.

## 10.5. CHAPTER 5

In Chapter 5 the agreement was assessed of two anthropometry-based methods for the estimation of body fat, namely the methods of Dauncey et al. [1977] and Weststrate et al. [1989] *versus* the TOBEC method. The study group consisted of 435 healthy, full-term infants, aged 21-365 days.

In infants aged <4 months Dauncey's method *overestimated* body fat on average by 0.135 kg (SD =0.254). In infants aged >4 months Dauncey's method *underestimated* body fat on average by 0.202 kg (SD =0.471). Only a moderate correlation between Dauncey's method and body fat by TOBEC was found ($r^2$=0.61). We modified Dauncey's method using physiological arguments by including quadriceps skinfold and halving all skinfolds. This improved the correlation to $r^2$=0.75, but the agreement remained poor.

The method of Weststrate better correlated with TOBEC-derived body fat than did Dauncey's modified method ($r^2$=0.85 *versus* $r^2$=0.75 in boys, and $r^2$=0.90 *versus* $r^2$=0.75 in girls respectively). Independent from age, Weststrate's method overestimated body fat with approximately 0.5 kg.

This study showed the limited applicability of anthropometry-based methods to assess absolute amounts of body fat in infants. The results do not implicate that skinfold thickness measurements and other anthropometric measurements or combinations of anthropometric measurements have lost their value in body composition studies. However, as this study shows, at present the large errors associated with anthropometric measurements prohibit its use of these two methods for accurate quatitation of body fat in the individual infant.

## 10.6. CHAPTER 6

Little is known in infants about the validity of anthropometric indexes for body fat and fat-free mass. In Chapter 6 we compared estimations of body fat and fat-free mass derived by the TOBEC method with the most commonly used anthropometric indexes. The study group consisted of the same infants as in chapter 5 (435 healthy infants aged 21-365 days).

Body fat was best correlated with 'weight by length' and with calf circumference. Fat-free mass was best correlated with body weight. Upperarm anthropometry, skinfold thickness, Quetelet's and Ponderal index were poorly correlated with total body fat and fat-free mass.

New, better correlating, anthropometry-based prediction equations specifically for use in infants were calculated, as well as new simple indexes (analogous to for example Quetelet's index). For body fat these indices were: (weight · calf circumference / length), and for fat-free mass: √(weight · length). The new prediction equations and indexes were cross-validated in a second population by a second observer. We calculated that interobserver variation was largest for equations with skinfolds included.

It was concluded that anthropometry can be used for rough estimations of body composition, although different indexes are preferred than used in children and adults.

## 10.7. CHAPTER 7

Data on body composition in conjunction with reference centiles are helpful to identify the severity of growth and nutritional disorders in infancy and to evaluate the adequacy of

therapeutic interventions during this important period of rapid growth. In Chapter 7 cross-sectional age,- weight,- and length-related centile standards are presented for total body fat and fat-free mass. as derived from TOBEC measurements in 423 non-hospitalized healthy, full-term, white infants aged 14 -379 days.

Centiles were calculated using Altman's method which is based on polynomial regression and hence modeling of the residual variation. Percentage body fat steeply increased during the first half year of life, and slowly declined beyond this age. The new, simple, TOBEC-derived anthropometric prediction equations for body fat and fat-free mass (chapter 6) can be used in conjunction with these standards for groups of infants or rough screening purposes. Regression equations for the $50^{th}$ centile and the residual SD as depending on age, weight or length are provided for purpose of construction of centile charts and calculation of standard deviation scores. We suggest that the present centiles are a valid way for monitoring nutritional status and the effect of therapeutic interventions in infants.

## 10.8. CHAPTER 8

In Chapter 8 a pilot study is described which reports approximations of energy requirements for male and female breast-fed (BF) or formula-fed (FF) infants based on individual estimates of total daily energy expenditure (TDEE) and energy deposition derived from total body fat (TBF) and fat-free mass (FFM) gain.

In 46 healthy, full-term infants the effect of at least 4 months of exclusive BF *versus* FF on macronutrient and energy intake, TDEE by the doubly-labeled water method, energy deposition and growth was investigated prospectively at 1, 2, 4, 8 and 12 mo. FFM and TBF were determined by total-body electrical conductivity (TOBEC). Metabolizable energy intake (MEI) was assessed from macronutrient intake ($MEI_{TW}$) and from the sum of TDEE and energy deposition derived from gain in TBF and FFM ($MEI_{PRED}$) .

At 1-2, 2-4, 4-8 and 8-12 mo of age $MEI_{PRED}$ averaged $431 \pm 38$, $393 \pm 33$, $372 \pm 33$ and $355 \pm 21$ kJ/kg/d for boys, and $401 \pm 59$, $376 \pm 25$, $334 \pm 33$ and $326 \pm 17$ kJ/kg/d for girls. Apart from a small but significant difference in weight, TBF and FFM in 4 and 8 mo old girls (FF > BF) no significant difference between BF and FF infants was found with respect to weight, length, head circumference, TBF, FFM and TDEE at all ages, neither for gain in length, weight, TBF and FFM. $MEI_{TW}$ significantly differed between feeding groups at 1-4 mo of age (FF > BF, $p < 0.005$). This feeding effect, however, was not present in $MEI_{PRED}$. $MEI_{TW}$ differed from $MEI_{PRED}$ only in BF infants, at 1-4 mo (with $p < 0.05$ at 2-4 mo). Volume of milk intake measured by TW did not significantly differ from milk intake by the deuterium-to-infant method.

The data of this study indicated that energy requirements in infants are lower than recommended by the guidelines which are presently used.

# *Chapter 11*
# Samenvatting

Afwijkingen in groei en voedingstoestand die ontstaan gedurende de eerste maanden van het leven kunnen belangrijke korte èn lange termijn gevolgen hebben voor de lichamelijke en geestelijke gezondheid. Nauwkeurige bepaling van de lichaamssamenstelling bij zuigelingen is daarom van belang om de voedingstoestand en de kwaliteit van de groei te kunnen vervolgen. Tevens spelen lichaamssamenstelling gegevens, met name betreffende de vetmassa en bot /spiermassa (vetvrije massa), een belangrijke rol in de bepaling van de gemiddelde dagelijkse energiebehoefte van zuigelingen. Preventie van ondervoeding is vooral van belang tijdens de eerste levensmaanden wanneer een groot deel van de voeding direct wordt gebruikt voor de groei.

## 11.1 Hoofdstuk 1

In dit hoofdstuk wordt een kort overzicht gegeven van de geschiedenis van het meten van de lichaamssamenstelling bij de mens. De doelstellingen van de in dit proefschrift beschreven studies worden uiteengezet. De doelstellingen zijn:

1) Bepaling van de validiteit van de techniek van total-body electrical conductivity (TOBEC) om de lichaamssamenstelling van zuigelingen te meten. Hiervoor werden metingen verricht met fysische modellen, een diermodel en werd een transversale studie bij zuigelingen tussen 1 en 12 maanden oud opgezet.

2) Bepaling van de validiteit van antropometrie als maat voor de lichaamssamenstelling bij zuigelingen voor zowel klinisch als wetenschappelijk gebruik,

3) Constructie van referentie percentielen ('groeicurven') voor lichaamsvet en vetvrije massa (bot /spiermassa). Deze percentielcurven bestonden tot op heden nog niet. Bovengenoemde doelstellingen werden met behulp van een transversale studie onderzocht.

4) Onderzoek naar verschillen in groei, lichaamssamenstelling en "energiehuishouding" (inname, opslag en verbranding van calorieën) bij borst gevoede en fles gevoede zuigelingen,

5) Het bepalen van de gemiddelde energiebehoefte van zuigelingen vanuit energieverbruik en lichaamssamenstelling gegevens. Voor de laatste twee doelstellingen werd een prospectieve studie bij gezonde borst gevoede en fles gevoede zuigelingen opgezet.

## 11.2 HOOFDSTUK 2

Met de TOBEC techniek kan de vetvrije massa van het lichaam worden gemeten. In hoofdstuk 2 werd met behulp van fysische modellen en een diermodel het effect onderzocht van veranderingen in fysische en chemische samenstelling van de vetvrije massa op de uitkomsten van de TOBEC methode. Allereerst werden 2, 3 en 5 liter oplossingen van de chloridezouten van zes verschillende elektrolyten en van natrium-bicarbonaat gebruikt om het effect van de concentratie en de hoeveelheid van elke oplossing op de TOBEC uitkomst te bepalen. De TOBEC uitkomsten van equimolaire concentraties en gelijke volumes waren in overeenstemming met de uit de natuurkunde bekende ion-geleidingssnelheden. De volgorde (van hoog naar laag) op de TOBEC uitkomst was: $H^+ >> Ca^{2+} > Mg^{2+} > K^+ > Na^+ > Li^+$, and $Cl^- > HCO_3^-$. Over een groot concentratiebereik (1-200 mM) kon het gedrag van deze oplossingen in het elektromagnetisch veld nauwkeurig omschreven worden met een eenvoudige, nieuwe, exponentiele formule. Het TOBEC signaal bleek (empirisch) tevens gerelateerd te zijn aan de $4^e$ macht van de straal (op dwarsdoorsnede) van het gemeten object. Oplossingen van eiwit, glycine en L-glutamine veroorzaakten geen TOBEC-signaal.

Met fysiologisch zout gevulde polyethyleen flessen lieten *in vitro* een lineair verband zien tussen temperatuurveranderingen van 2 tot 45 °C, en het TOBEC-signaal. Onder het vriespunt werd geen TOBEC-signaal verkregen.

Het effect op de TOBEC uitkomst van weefsel-autolyse en van *in vivo* veranderingen van de lichaamstemperatuur werd onderzocht door herhaalde TOBEC metingen uit te voeren bij zeven verse kadavers van minibiggen. Bij vijf kadavers daalde de lichaamstemperatuur na overlijden tot kamertemperatuur. Het TOBEC signaal daalde hier geleidelijk met 2,5% per graad Celsius temperatuurdaling. Bij twee kadavers die na insłapen door middel van uitwendige verwarming op lichaamstemperatuur werden gehouden, liet het TOBEC-signaal als gevolg van weefsel-autolyse een stijging van in totaal 9% zien gedurende de meettijd van ongeveer 7 uur.

De conclusies waren: 1) De reproduceerbaarheid van de TOBEC methode bleek uitstekend te zijn. Meetfouten vanuit het apparaat zelf ('instrument variability') blijken een te verwaarlozen bron van fouten in de uiteindelijke bepaling van de lichaamssamenstelling door TOBEC. 2) Veranderingen van lichaamstemperatuur veroorzaken een significante fout in de bepaling van lichaamssamenstelling door TOBEC. 3) De meeste (fysiologische) veranderingen in de concentratie van elektrolyten in het lichaam hebben geen effect op de TOBEC uitkomst. Forse veranderingen in de bicarbonaat spiegels zoals bijvoorbeeld in het geval van diabetische ketoacidose en nier insufficiëntie, zullen echter het TOBEC signaal wel kunnen beïnvloeden. 4) Verschuivingen in eiwit- en aminozuurconcentraties in het lichaam hebben geen effect op het TOBEC signaal. 5) Ionen in een vaste kristalstructuur (zoals ijs en bot) veroorzaken in het geheel geen TOBEC signaal. 6) Duidelijke afwijkingen van de normale lichaamsvorm geven dienovereenkomstige afwijkingen in het TOBEC signaal, met name daar waar het een duidelijke verandering van de dwarse diameter van romp of extremiteiten geldt (omphalocèle, groot hemangioom, etc.). 7) Na overlijden ontstaan niet verwaarloosbare meetfouten in de TOBEC meting door weefsel autolyse en temperatuurdalingen. 8) Als laatste kon empirisch

worden bevestigd dat elektrolyten-oplossingen zich in het elektromagnetisch veld van het TOBEC apparaat gedragen conform de natuurkundige wetten van ion-geleidbaarheid. Dit is van groot belang voor de TOBEC technologie. Het laat namelijk zien dat de wetten omtrent de gedragingen van elektrolyten, cellen en weefsels in een elektromagnetisch veld, zoals beschreven in de natuurkunde, ook gelden voor TOBEC. Hierdoor is de methode in fysische zin toegankelijker en beter voorspelbaar geworden.

## 11.3. HOOFDSTUK 3

In hoofdstuk 3 werden de precisie, de dag-tot-dag variatie en het profiel van het elektromagnetisch veld van drie TOBEC-HP2 instrumenten onderling vergeleken. Er werd een nieuwe ijklijn berekend door TOBEC bepalingen van lichaamsvet en vetvrije massa, gemeten bij een diermodel (levende minibiggen van 7-99 dagen oud), te relateren aan de uitkomsten van chemische analyse van de karkassen na opoffering (de 'gouden standaard'). Deze nieuwe ijklijn werd vergeleken met de ijklijn die door de fabrikant wordt verstrekt en die vervaardigd is op een prototype TOBEC HP-1 apparaat. De prestaties van de apparaten waren over het algemeen gelijk, op een significant verschil in een klein onderdeel van het magnetisch veld van één van de instrumenten na. Een strategie om deze eventuele ongelijkheden ten opzichte van de hier onderzochte apparaten te ontdekken werd beschreven in het hoofdstuk. De variatie coëfficiënt van een reeks van metingen van een door de fabrikant verstrekte referentie-cilinder (fantoom) met een vaste TOBEC waarde varieerde van 0.2% tot 0.5% . De dag-tot-dag variatie lag in de zelfde orde van grootte. Door deze geringen fout zal de invloed van de meetvariatie pas significant worden bij het meten van kinderen met een zeer laag lichaamsgewicht (< 3-4 kg). De nieuwe ijklijn bleek niet significant te verschillen van de door de fabrikant verstrekte ijklijn (welke een bereik had tot ongeveer 5 kg). De data van de twee verschillende ijklijnen konden worden samengevoegd, en een nieuwe, meer nauwkeurige ijklijn met een lineair bereik tot 10 kg werd berekend.

Er werd geconcludeerd dat het TOBEC-HP2 apparaat geschikt is om op een gemakkelijke, snelle en veilige manier de vetvrije massa te meten van zuigelingen tot de leeftijd van ≈1 jaar. De methode blijkt zeer betrouwbaar en stabiel te zijn en behoort momenteel tot de nauwkeurigste methoden voor bepaling van lichaamssamenstelling bij zuigelingen. Het 95% betrouwbaarheidsinterval (d.i. tweemaal de standaard meetfout van de ijklijn) is ± 150 gram. De resultaten verkregen met verschillende instrumenten kunnen onderling met elkaar worden vergeleken, mits de profielen van de magnetisch velden vergelijkbaar met elkaar zijn.

## 11.4. HOOFDSTUK 4

De isotoop-verdunningstechniek met $^{18}O$ of $^{2}H$ wordt vaak gebruikt voor de bepaling van het totaal lichaamswater. Hieruit kan de vetvrije massa worden berekend, waarbij echter, met name bij kleine kinderen, wel wordt ingeboet in nauwkeurigheid. Deze methode kan daarom

eigenlijk alleen worden toegepast om de gemiddelde vetvrije massa van een groep zuigelingen te bepalen. Bij de berekening van het lichaamswater uit de ruwe $^{2}H$ of $^{18}O$ uitwas-curves zijn twee invalshoeken mogelijk: de 'extrapolatie-tot-t=0 berekening', die een mogelijke onderschatting van het lichaamswater geeft, en de 'plateau berekening' die een mogelijke overschatting van het lichaamswater geeft.

In hoofdstuk 4 werd beschreven hoe de vetvrije massa van zuigelingen werd gemeten met twee methoden die beiden gebaseerd zijn op zeer uiteenlopende onderliggende technische principes: de total-body electrical conductivity (TOBEC) techniek en de genoemde methode van isotoopverdunning. Het eerste doel van de studie was om de grootte van de fout te schatten in de 'extrapolatie-tot-t=0 berekening' met de TOBEC als referentiemethode. De tweede hypothese die werd getest was of de op minibiggen gebaseerde TOBEC ijklijn (zie hoofdstuk 3), welke door anderen reeds is gevalideerd voor de eerste levensmaanden van zuigelingen, ook van toepassing zou blijken te zijn tijdens de tweede helft van het eerste levensjaar. De studiegroep bestond uit 50 gezonde zuigelingen tussen de 1 en 12 maanden oud waarbij in totaal 149 metingen (76 bij jongens en 73 bij meisjes) werden uitgevoerd.

De hoeveelheid vetmassa en vetvrije massa bleken zeer goed overeen te komen met oude referentiewaarden gepubliceerd in 1982 door Fomon e.a. Tussen vetvrije massa bepaald met TOBEC en vetvrije massa bepaald met $^{18}O$-isotoopverdunning werd een strikte lineaire relatie gevonden met een helling die niet significant verschilde van 1 ($r$=0.98, SD=0.29 kg voor jongens en $r$=0.98, SD=.0.32 kg voor meisjes). Gemiddeld bleek dus geen significant verschil te bestaan in de vetvrije massa zoals bepaald met de TOBEC techniek of met de techniek van isotoop verdunning. Berekend met behulp van een paired $t$-test bleek de vetvrije massa bepaald met $^{18}O$-isotoop verdunning significant iets lager te zijn dan de vetvrije massa bepaald met de TOBEC methode. Het verschil was echter klein en bedroeg 0.18 (±24) kg voor meisjes en 0.08 (±0.21) kg voor jongens, wat respectievelijk overeenkomt met 4% (SD=4.5, $p$<0.0001) voor meisjes en 1.5% (SD=3.9, $p$=0.004) voor jongens.

Er werd geconcludeerd 1) Dat de TOBEC ijklijn nauwkeurig en geschikt is voor het gehele eerste levensjaar, 2) Dat de isotoop verdunning methode met behulp van de 'extrapolatie-tot-t=0 berekening' bruikbaar is om lichaamswater en vetvrije massa te bepalen bij groepen zuigelingen.

## 11.5. HOOFDSTUK 5

Onder antropometrie wordt verstaan het uitwendig bepalen van lichaamsproporties zoals gewicht, lichaamslengten, lichaamsomtrekken en huidplooidikten. In hoofdstuk 5 werd bij 435 gezonde, à terme zuigelingen van 21-365 dagen bepaald in hoeverre twee van de belangrijkste op antropometrie gebaseerde methoden om lichaamsvet te meten (volgens Dauncey e.a. [1977] en Weststrate en Deurenberg [1989]) overeenkwamen met TOBEC bepalingen van vet en vetvrije massa. De TOBEC bepalingen golden hierbij als referentie. Onder de leeftijd van 4 maanden *over*schatte de Dauncey methode de hoeveelheid lichaamsvet gemiddeld met 0.135 kg (SD=0.254). Boven de leeftijd van 4 maanden *onder*schatte de

Dauncey methode de hoeveelheid lichaamsvet gemiddeld met 0.202 kg (SD=0.471). Slechts een matige correlatie werd gevonden tussen lichaamsvet bepaald met Dauncey's methode en met TOBEC ($r^2$=0.61). Op anatomische gronden werd de methode volgens Dauncey aangepast door huidplooimetingen van de dijbenen aan de formule toe te voegen, en de waarden van alle huidplooimetingen te halveren. Dit verbeterde de correlatie tot $r^2$=0.75, maar de precisie voor individuele metingen bleef matig. De absolute hoeveelheid vetmassa bepaald met de methode van Weststrate correleerde beter met de TOBEC uitkomsten dan de door ons verbeterde methode van Dauncey ($r^2$=0.85 voor jongens, $r^2$=0.90 voor meisjes). Onafhankelijk van de leeftijd overschatte de methode van Weststrate het lichaamsvet echter met ongeveer 0.5 kg! Deze studie laat de beperkte bruikbaarheid zien van op antropometrie (met name huidplooidikten) gebaseerde methoden om de lichaamssamenstelling te meten van zuigelingen. De resultaten houden niet in dat huidplooimetingen en (andere!) antropometrische bepalingen of combinaties van antropometrische bepalingen hun waarde verloren hebben. De grote in dit hoofdstuk beschreven onnauwkeurigheden welke momenteel nog worden gevonden worden, houden echter in dat het trekken van conclusies uit waarnemingen van de hoeveelheid lichaamsvet per *individu* met de huidige antropometrische technieken met de nodige voorzichtigheid moet plaatsvinden.

## 11.6. HOOFDSTUK 6

Er is weinig bekend over de betrouwbaarheid bij zuigelingen van de meeste antropometrische *indices* (zoals bijvoorbeeld de Quetelet Index) die worden gebruikt als *relatieve* maat voor de hoeveelheid vetmassa of vetvrije massa. In hoofdstuk 6 werd bij 435 gezonde, à term geboren zuigelingen tussen de 21-365 dagen oud het lichaamsvet en de vetvrije massa (bepaald met de TOBEC methode) vergeleken met de bekendste antropometrische indices. De hoeveelheid lichaamsvet correleerde het best met 'gewicht gedeeld door lengte' en, verrassend (!), zeer consistent ook met de kuitomtrek. Vetvrije massa correleerde het beste met lichaamsgewicht. Allerlei vormen van bovenarm antropometrie, maar ook huidplooidikten op 5 verschillende plaatsen, de Quetelet Index en de Ponderal Index bleken slecht te correleren met de totale hoeveelheid vetmassa en vetvrije massa. Nieuwe antropometrische formules en eenvoudige indices om vetmassa en vetvrije massa te voorspellen werden berekend, speciaal voor de leeftijdsgroep van zuigelingen. Voor lichaamsvet werd (gewicht · kuitomtrek / lengte) als beste index gevonden, voor vetvrije massa $\sqrt{}$(gewicht · lengte). De nieuwe formules en indices werden op hun betrouwbaarheid getest in een tweede populatie met een tweede onderzoeker (cross-validation). Interobserver variatie tussen de eerste en de tweede onderzoeker bleek het grootst te zijn voor die vergelijkingen waar huidplooimetingen in verwerkt waren. Geconcludeerd werd dat antropometrie bruikbaar is voor grove schattingen van de lichaamssamenstelling. Voor zuigelingen blijken echter andere formules en indices geschikt te zijn dan voor oudere kinderen en volwassenen.

## 11.7. HOOFDSTUK 7

Gegevens over de lichaamssamenstelling van zuigelingen kunnen een bruikbaar instrument zijn om de ernst van voedingsstoornissen en groeiafwijkingen op te sporen, zeker wanneer zij gerelateerd kunnen worden aan percentiel curven. Ze zijn ook nodig om het effect van bepaalde voedingstherapieën goed te kunnen evalueren. Voor lichaamsvet en vetvrije massa bij zuigelingen waren tot op heden geen groeicurves (percentielen) voorhanden. In hoofdstuk 7 wordt beschreven hoe groeicurven werden berekend voor lichaamsvet en vetvrije massa bij jongetjes en meisjes van ongeveer 1 tot 12 maanden oud. De percentiel-curven zijn berekend ten opzichte van leeftijd, gewicht en lengte. De vetmassa en vetvrije massa gegevens werden verkregen met behulp van de TOBEC methode bij 423 gezonde, blanke zuigelingen in de leeftijd tussen 14 en 379 dagen. De percentielcurven werden berekend met behulp van de methode volgens Altman, welke gebaseerd is op polynomiale regressie van de ruwe gegevens (voor bepaling van de vijftigste percentiel) en daarna regressie van de absolute waarden van de residuele standaarddeviatie, om het verloop van de mate van spreiding rond de P50 vast te leggen.

Het percentage lichaamsvet nam snel toe met de leeftijd, lengte en het gewicht in de eerste helft van het eerste levensjaar en nam daarna langzaam af. Met de regressie vergelijkingen van de P50 (het gemiddelde) en van de residuele standaarddeviatie kan een ieder de groeicurven zelf geconstrueren en kunnen standaard-deviatie-scores (SDS) worden berekend.

## 11.8. HOOFDSTUK 8

Hoofdstuk 8 is een prospectieve *pilot* studie beschreven waar bij borst gevoede (BV) en fles gevoede (FF) zuigelingen een empirische schatting werd gemaakt van de energiebehoeften zoals berekend uit metingen van energie verbruik en energie opslag. Met behulp van de TOBEC techniek kon nu voor het eerst met redelijke nauwkeurigheid de gemiddelde hoeveelheid opgeslagen energie worden berekend uit de toename van de vetvrije massa en het lichaamsvet. Tevens werd in deze pilot studie de hypothese getest of het geven van borstvoeding (BV) of flesvoeding (FV) veranderingen te zien geeft in de groei, de dagelijkse energieconsumptie, het dagelijks energieverbruik of in de opslag van energie in de vorm van vet en vetvrije massa. Het onderzoek vond plaats bij 46 gezonde, à term geboren zuigelingen. Metingen werden verricht op de leeftijden van 1, 2, 4, 8 en 12 maanden. De baby's werden tot een leeftijd van 4 maanden alleen gevoed met òf BV òf FV. Energieverbruik werd gemeten met behulp van de $^{2}H_{2}{}^{18}O$ (dubbel-gelabeld water) methode. Lichaamssamenstelling werd gemeten met behulp van de TOBEC techniek. De energieconsumptie werd bepaald via testwegen (bij BV in combinatie met het afkolven van moedermelk). Daarnaast werd de energieconsumptie berekend door het energieverbruik op te tellen bij de opslagen hoeveelheid energie in de vorm van vet en eiwit. De opgeslagen hoeveelheid energie werd bepaald met

behulp van met de TOBEC verkregen gegevens over de toename van de hoeveelheid vetmassa en vetvrije massa in de tijd. In de periode tussen 1-2, 2-4, 4-8 en 8-12 maanden werd een netto energieconsumptie berekend van respectievelijk 431±38, 393±33, 372±33 en 355±21 kilojoule/kg/dag voor jongens, en 401±59, 376±25, 334±33 en 326±17 kilojoule/kg/dag voor meisjes. Op de verschillende leeftijden werd geen verschil gevonden tussen BV en FV zuigelingen in gewicht, lengte, hoofdomtrek, lichaamsvet, vetvrije massa en energieverbruik, uitgezonderd een klein maar significant verschil in gewicht, lichaamsvet en vetvrije massa bij *meisjes* van 4 maanden oud. Tussen 1 en 4 maanden was de energieconsumptie gemeten met behulp van testwegen significant verschillend tussen de BV en de FV groep (FV>BF, $P<0.005$). Dit verschil werd echter niet teruggevonden bij de energieconsumptie berekend vanuit energieopslag en energieverbruik! Een verschil in energieconsumptie, gemeten met testwegen *versus* berekend uit energieopslag en energieverbruik, werd alleen in de BV groep gevonden, en wel tussen 1 en 4 maanden. Het aantal milliliters geconsumeerde melk van BV en FV zuigelingen bepaald via testwegen was niet significant verschillend met de hoeveelheid zoals berekend met gegevens uit de dubbel-gelabeld water methode. Het vinden van BV-FV verschillen in energieconsumptie met testwegen en niet via de berekende waarden uit energieverbruik en energieopslag zou dus een artifact door het afkolven kunnen zijn.

De gegevens uit deze studie lieten zien dat de energiebehoefte van zuigelingen gemiddeld lager ligt dan in de huidige richtlijnen is aangegeven.

## 11.9. CONCLUSIES UIT DE IN DIT PROEFSCHRIFT BESCHREVEN STUDIES.

De TOBEC techniek behoort momenteel tot de meest bruikbare methoden voor snelle, veilige, en reproduceerbare bepaling van de lichaamssamenstelling bij zuigelingen tijdens het eerste levensjaar. Daar chemische analyse van karkassen de gouden standaard van lichaamssamenstelling onderzoek is kon de validiteit van de TOBEC techniek niet direct worden vastgesteld. De validiteit van de TOBEC methode werd op verschillende indirecte manieren aannemelijk gemaakt. Middels lineaire regressie bleek vetvrije massa bepaald door $^{2}H_{2}{}^{18}O$ en door TOBEC (methoden gebaseerd op wijd uiteeenlopende meetprincipes) niet verschillend en bleek het verband strikt lineair gedurende het gehele eerste levensjaar. Gemiddelde waarden van vetmassa en vervrije massa kwamen goed overeen met de bekende referentiewaarden van Fomon e.a. uit 1982.

Traditionele antropometrische indices en voorspellingsformules werden gevalideerd ten opzichte van de TOBEC techniek. Op "gewicht gedeeld door lengte" en kuitomtrek voor lichaamsvet, en lengte en gewicht voor vetvrije massa na, bleken de traditionele antropometrische indices en voorspellingsformules niet geschikt voor gebruik bij zuigelingen. Kuitomtrek blijkt een 'nieuwe', veelbelovende antropometrische parameter te zijn voor de hoeveelheid lichaamsvet en zou het gebruik van bovenarm antropometrie bij zuigelingen moeten vervangen als screeningsinstrument voor de lichaamssamenstelling en de voedingstoestand.

Voor het eerst werden percentielcurven voor zuigelingen geconstrueerd voor lichaamsvet en vetvrije massa, ten opzichte van leeftijd, lengte en lichaamsgewicht. Ook werden nieuwe

indices en voorspellingsformules voor lichaamsvet en vetvrije massa bij zuigelingen berekend, die in combinatie met de nieuwe groeicurven voor lichaamsvet en vetvrije massa kunnen worden gebruikt.

De prospectieve pilot studie bij borst en fles gevoede zuigelingen gaf aanwijzingen dat de energiedichtheid van borstvoeding mogelijk niet goed kan worden bepaald via afgekolfde moedermelk. De manier van meten van de hoeveelheid geconsumeerde energie bij borstgevoede kinderen via afgekolfde moedermelk en testwegen staat hiermee ter discussie.

De energie consumptie per zuigeling werd tevens berekend met behulp van metingen van het energieverbruik en de lichaamssamenstelling (energieopslag). Deze berekeningen kwamen overeen met eerder gepubliceerde berekeningen van Prentice et al. [1988]. Deze gegevens lieten zien dat de gemiddelde energiebehoefte van zuigelingen lager ligt dan wordt aanbevolen volgens de huidige richtlijnen.

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*Spreuken 15:22*

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En lest best dank ik Margo! Dit boekje is ook jouw werk geweest! Dank je dat je steeds weer de juiste prioriteiten voor ons wist te stellen in alle drukte. Bij twee bevallingen heb ik *jou* proberen te steunen, nu heb je *mij* er geweldig doorheen geholpen bij deze *partus promoven-di.*

# Curriculum Vitae

Niels de Bruin was born in Rotterdam, the Netherlands, on 25 July 1963. He passed his secundary school exam (VWO) in 1981 at the Rijksscholengemeenschap at Brielle. He started his medical training in 1983 at the Medical Faculty of the Erasmus University Rotterdam (after one year of in-service bible college and secundary school youth evangelism training at the Jong & Vrij Bible College te Rockanje). During his medical training he worked as a junior research assistent at the Department of Pediatric Infectious disease (Head: Prof. Dr. H.J. Neijens) and at the Pediatric Research Laboratory (head: Prof. Dr. H.J. Degenhart). In December 1990 he obtained his medical degree. He worked part-time as a physician for mentally retarded and disabled children from 1989 to February 1991 (KDV "Myosotis", Keerkring / Pameijer Foundation. Head: J. Alblas). From January 1991 to July 1995 he worked as a research fellow at the Department of Pediatrics of the University Hospital Rotterdam / the Sophia Children's Hospital (Head: Prof. Dr. H.K.A. Visser). The research bundled in this thesis was carried out under supervision of Prof. Dr. H.K.A. Visser and Prof. Dr. H.J. Degenhart (Head of the Pediatric Research Laboratory of the Sophia Children's Hospital, Rotterdam). From August to December 1995 he worked as a resident in Pediatrics at the Sophia Children's Hospital Rotterdam. In december 1995 he switched to the geriatric side of medicine and started as a Dutch nursing home physician in nursing home "Slingedael" (head: K.G.A. Nieuwenhuis). In September 1996 he started his specialist training as a Dutch nursing home physician at the "Vrije Universiteit" at Amsterdam (Head: Prof. Dr. M.W. Ribbe). He is now working in nursing home "Pniël" and the psycho-geriatric nursing home "Slingedael" of the "Protestant Christelijke Zorgfederatie Rijnmond". He is married to Margo van der Waal, and they have two children: Marlies and Richelle.

# List of Publications

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1. **De Bruin NC, De Groot R, Den Hollander JC, Ágoston SI, Van Dongen JJM, Neijens HJ.** Small-cell undifferentiated (neuroendocrine) carcinoma of the cecum in a child with common variable immunodeficiency. *Am. J. Pediatr. Hematol. Oncol.* **15**:258-261, 1993

2. **De Bruin NC, Luijendijk IHT, Visser HKA, Degenhart HJ.** Effect of alterations in physical and chemical characteristics on TOBEC-derived body composition estimates: validation with non-human models. *Phys. Med. Biol.* **39**:1143-1156, 1994

3. **Fiorotto ML, De Bruin NC, Brans YW, Degenhart HJ, Visser HKA.** Total body electrical conductivity measurements: an evaluation of current instrumentation for infants. *Pediatr. Res.*, 37:94-100, 1995

4. **De Bruin NC, Van Velthoven CAM, Stijnen T, Juttmann RE, Degenhart HJ, Visser HKA.** Quantitative assessment of infant body fat by anthropometry and total-body electrical conductivity. *Am. J. Clin. Nutr.* **61**:279-86, 1995

5. **De Bruin NC, Van Velthoven CAM, Stijnen T, Juttmann RE, Degenhart HJ, Visser HKA.** Body fat and fat-free mass in infants: new and classic anthropometric parameters and prediction equations compared with total- body electrical conductivity. *Am J Clin Nutr* 1995; **61**:1195-205

6. **De Bruin NC, Westerterp KR, Degenhart HJ, Visser HKA.** Measurement of fat-free mass in infants. *Pediatr Res* 1995; **38**:411-417

7. **De Bruin NC, Van Velthoven CAM, Stijnen T, Juttmann RE, Degenhart HJ, Visser HKA.** Standards for infant body fat and fat-free mass. *Arch Dis Child* 1996; **74**:386-399

8. **De Bruin NC, Degenhart HJ, Gàl S, Stijnen T, Westerterp KR, Visser HKA.** Energy utilization and growth in breast-fed and formula-fed infants measured prospectively during the first year of life: the Sophia study. *Am. J. Clin. Nutr.* (submitted)

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1. **De Bruin NC, Degenhart HJ, Visser HKA.** Body composition in the first year of life. In: Bindels JG, Goedhardt AC, Visser HKA (Eds.), *Recent Developments In Infant Nutrition. 10th Nutricia Symposium.* Kluwer Academic Publishers BV, Lancaster UK, 1996, pp 81-97

2. **De Bruin NC.** Nieuwe methoden ter evaluatie van de voedingsstatus. In: Van Suijlekom-Smit LWA (Ed), *Therapeutisch Handelen in Kindergeneeskunde en Kinderverpleegkunde - Cursusboek 24ste kinderartsenweek 1996.* Rotterdam: Post Academisch Onderwijs Kindergeneeskunde, 1996, pp 65-69

ABSTRACTS

1. **De Bruin NC, Van den Berg R, Degenhart HJ, Visser HKA** (Abstract). TOBEC, a good predictor of fat free mass and body fat: instrument calibration with minipigs by carcass analysis and D2O dilution. In: *Proc 33rd Dutch Federation Meeting, Rotterdam, April 1992* (Nijmegen: Federation of Medical Scientific Societies), p.54 ,1992

2. **De Bruin NC, Luijendijk IHT, Degenhart HJ, Visser HKA** (Abstract). Effect of several object characteristics on TOBEC. In: *Proc 33rd Dutch Federation Meeting, Rotterdam, April 1992* (Nijmegen: Federation of Medical Scientific Societies), p.54 ,1992

3. **De Bruin NC, Van Velthoven CAM, Brugman R, Degenhart HJ, Visser HKA** (Abstract). Measuring body fat in infancy: anthropometry versus total body electrical conductivity (TOBEC). *Pediatr Res* 35:268, 1993

4. **De Bruin NC, Van Velthoven CAM, Brugman T, Degenhart HJ, Visser HKA** (Abstract) Bepaling van totaal lichaamsvet bij zuigelingen. In: *15th Annual meeting of the Dutch Society for Pediatrics*, Velthoven, 1993

5. **De Bruin NC, Van Velthoven CAM, Degenhart HJ, Visser HKA** (Abstract) Nutritional assessment in infants by TOBEC and anthropometry. *Pediatr Res* 36:8A, 1994

6. **De Bruin NC, Degenhart HJ, Visser HKA** (Abstract) Fat-free mass (FFM) estimations in infants by total body electrical conductivity (TOBEC) and 18-oxygen dilution. *Pediatr Res* 36:8A, 1994

7. **De Bruin NC, Degenhart HJ, Visser HKA** (Abstract) Body composition in breast-fed (BF) and formula-fed (FF) infants: different effects of feeding mode in boys and girls - a 12 months follow-up. *Pediatr Res* 38:430, 1995

8. **De Bruin NC, Gàl S, Degenhart HJ, Visser HKA** (Abstract) Energy and macronutrient intake in breast-fed and formula-fed infants: a 12 months follow-up. *Pediatr Res* 38:430, 1995

# Appendix I

*Results of "all possible subsets regression"*
*(chapter 6)*

***Appendix 1:*** **Table 1.** Results of all possible subsets regression. [a]

| Eq. | $r^2$ | SD | Independent variables | regression coefficients $A_0$ | $A_1$ | $A_2$ | $A_3$ |
|---|---|---|---|---|---|---|---|
| ***Dependent variable: ln [body fat (kg)]*** | | | | | | | |
| 2a | 0.86 | 0.153 | ln(calf circumference), ln(weight) | -6.180 | 1.800 | 0.8140 | |
| 2b | 0.86 | 0.158 | ln(calf circumference), ln(sum of 5 skinfolds) | -8.9995 | 2.7389 | 0.4623 | |
| 2c | 0.85 | 0.159 | ln(calf circumference), ln(sum of 3 skinfolds) | -8.8219 | 2.7431 | 0.4511 | |
| 2d | 0.84 | 0.164 | ln(calf circumference), ln(age ) | -7.0276 | 2.7139 | 0.1520 | |
| 3a | 0.90 | 0.131 | ln(calf circumference), ln(weight), ln(sum of 5 | -6.3032 | 1.1052 | 0.8876 | 0.5149 |
| 3b | 0.90 | 0.134 | skinfolds) | -6.1506 | 1.1453 | 0.8722 | 0.4951 |
| 3c | 0.89 | 0.136 | ln(calf circumference), ln(weight), ln(sum of 3 | -5.4117 | 1.2223 | 1.0348 | 0.2587 |
| 3d | 0.88 | 0.143 | skinfolds) | -6.9185 | 1.9585 | 0.5430 | 0.1949 |
| | | | ln(calf circumference), ln(weight), ln(suprailiac skinfold) | | | | |
| | | | ln(calf circumference), ln(sum of 5 skinfolds), ln(age) | | | | |
| ***Dependent variable: body fat (%)*** | | | | | | | |
| 2a | 0.65 | 2.93 | calf circumference , sum of 5 skinfolds | -6.8645 | 1.1384 | 0.2586 | |
| 2b | 0.62 | 3.04 | calf circumference , suprailiac skinfold | -8.9557 | 1.6423 | 0.8990 | |
| 2c | 0.62 | 3.07 | thigh circumference , sum of 5 skinfolds | -3.6903 | 0.6664 | 0.2908 | |
| 2d | 0.55 | 3.31 | calf circumference , gender [b] | -10.779 | 1.9030 | 1.8624 | |
| 3a | 0.67 | 2.84 | calf circumference , sum of 5 skinfolds, chest circ. | 0.4664 | 1.6515 | 0.2576 | -0.3757 |
| 3b | 0.67 | 2.86 | calf circumference , sum of 5 skinfolds, gender [b] | -9.4029 | 1.2128 | 0.2435 | 1.3216 |
| 3c | 0.65 | 2.94 | calf circumference , triceps skinfold, suprailiac skinfold | -9.4356 | 1.4948 | 0.7818 | 0.3671 |
| 3d | 0.65 | 2.95 | calf circumference , suprailiac skinfold, gender [b] | -11.821 | 1.6912 | 0.8542 | 1.5495 |
| ***Dependent variable: ln [fat-free mass (kg)]*** | | | | | | | |
| 2a | 0.95 | 0.045 | weight, total length | 0.3571 | 0.080 | 0.0110 | |
| 2b | 0.95 | 0.045 | weight, sum of 5 skinfolds | 0.8987 | 0.1252 | -0.0029 | |
| 2c | 0.95 | 0.045 | weight, head circ. | 0.2185 | 0.0904 | 0.0187 | |
| 2d | 0.91 | 0.058 | chest circumference, head circumference | -1.1872 | 0.0313 | 0.0355 | |
| 3a | 0.95 | 0.042 | weight, total length, gender [b] | 0.3969 | 0.0757 | 0.0116 | -0.0337 |
| 3b | 0.95 | 0.042 | weight, total length, sum of 5 skinfolds | 0.5309 | 0.0948 | 0.0083 | -0.0022 |
| 3c | 0.95 | 0.042 | weight, total length, head circ. | 0.0291 | 0.0689 | 0.0084 | 0.0136 |
| 3d | 0.92 | 0.056 | head circ., chest circ., thigh circ. | -1.0989 | 0.0333 | 0.0264 | 0.0097 |

a) In case of the "all possible subsets regressions" we used the adjusted $r^2$ instead of the normally used $r^2$ as selection criterium: $r^2_{adjusted} = r^2 - \{ [k(1-r^2)] / (n-k-1) \}$, where k is the number of independent variables in the equation. Use of adjusted $r^2$ allows comparison between two subsets with different numbers of predictors.

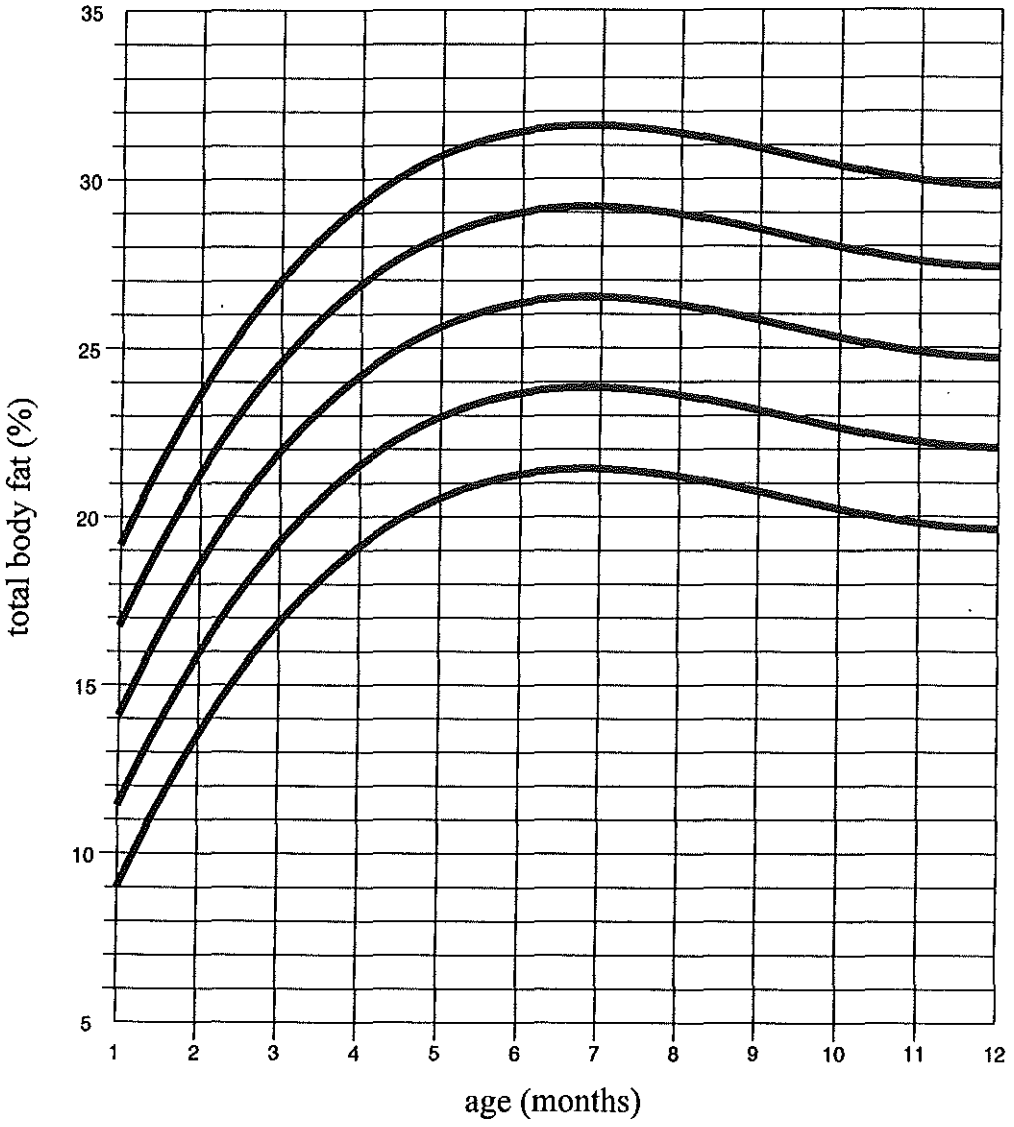
b) Gender: male=1, female=2.

# Appendix 2

*Standard curves for body fat and fat-free mass plotted against age, weight and length (chapter 7)*

# Boys

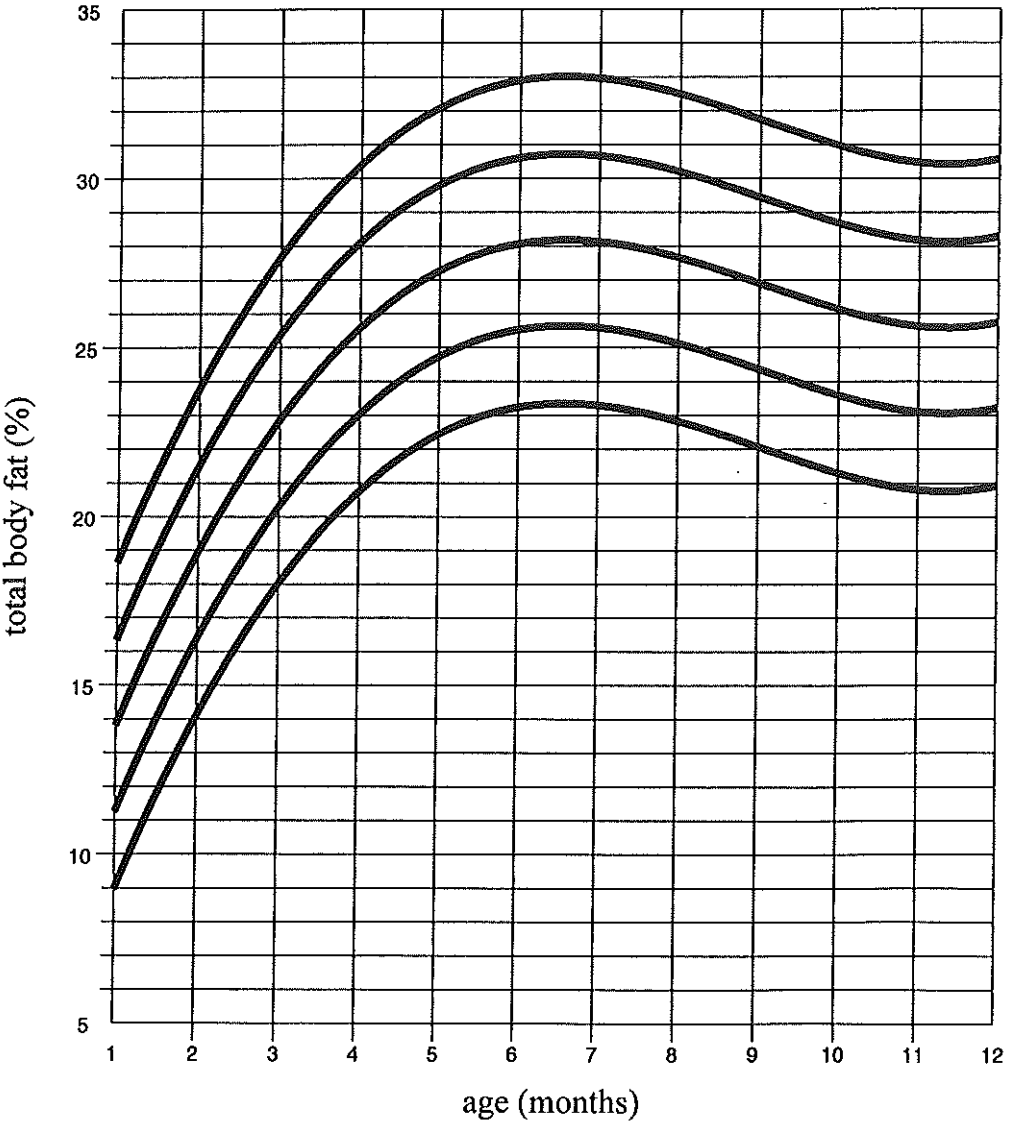
## 90th, 75th, 50th, 25th and 10th centile




*SKZ, Rotterdam, 1995*

# Girls

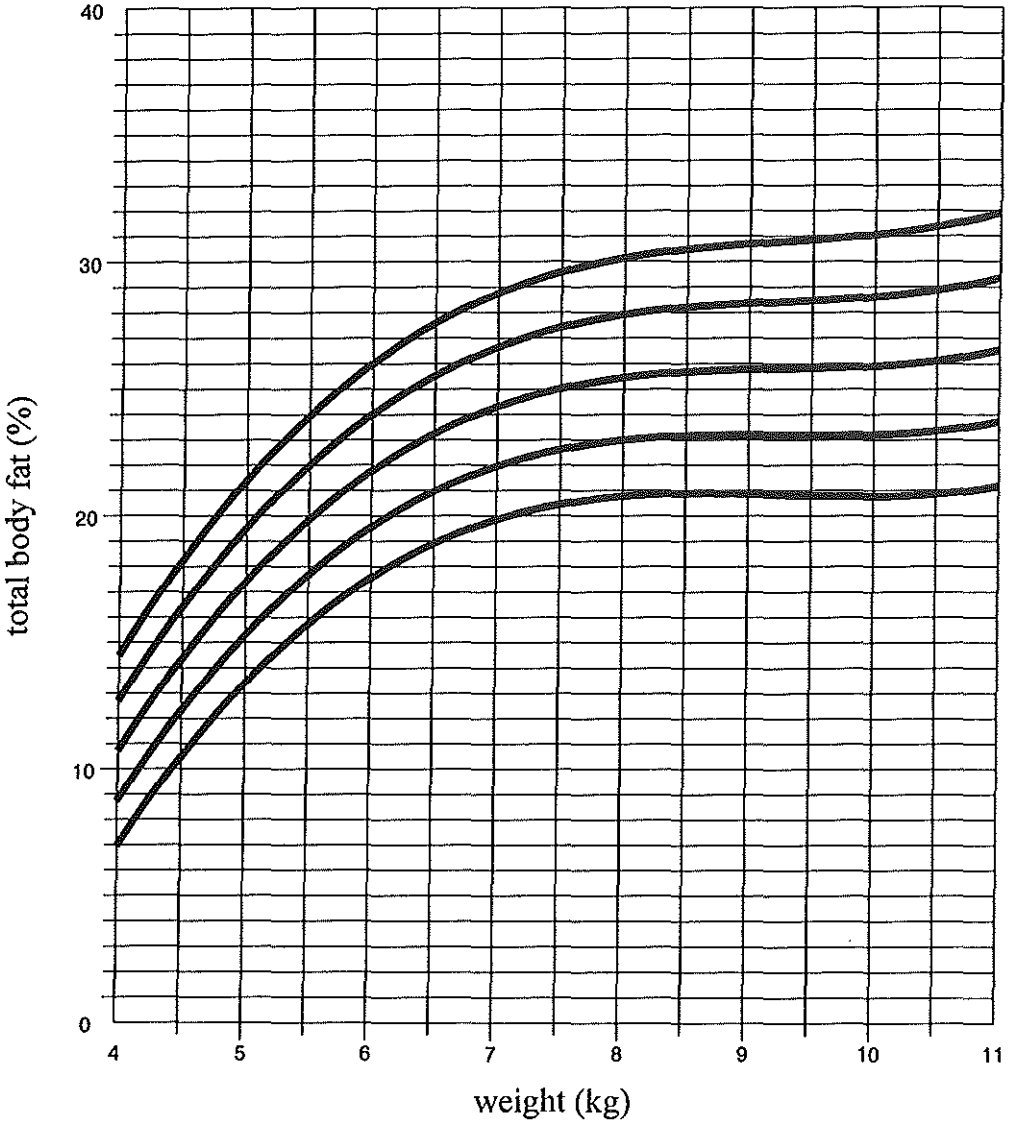
## 90th, 75th, 50th, 25th and 10th centile




*SKZ, Rotterdam, 1995*

# Boys

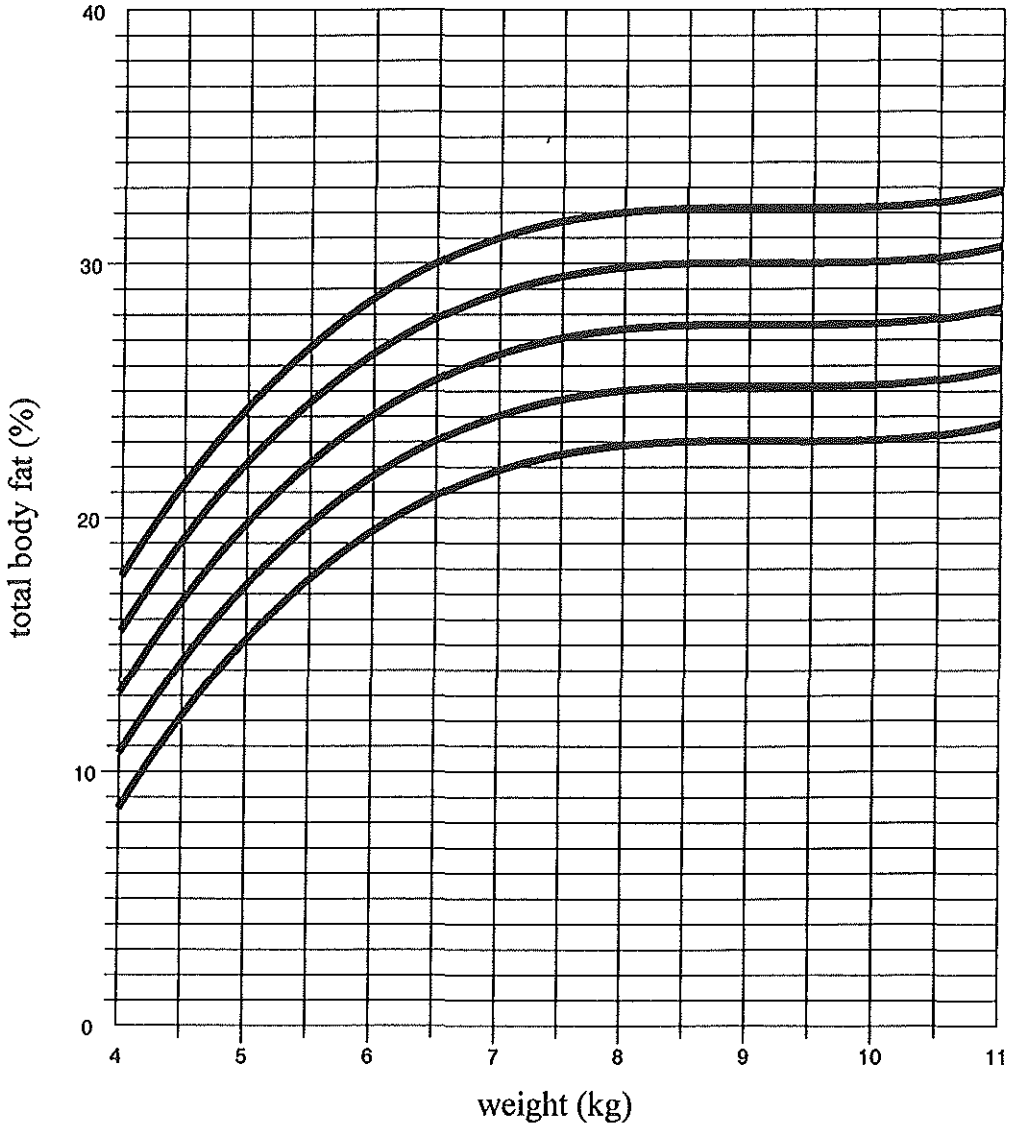
## 90th, 75th, 50th, 25th and 10th centile




*SKZ, Rotterdam, 1995*

# Girls

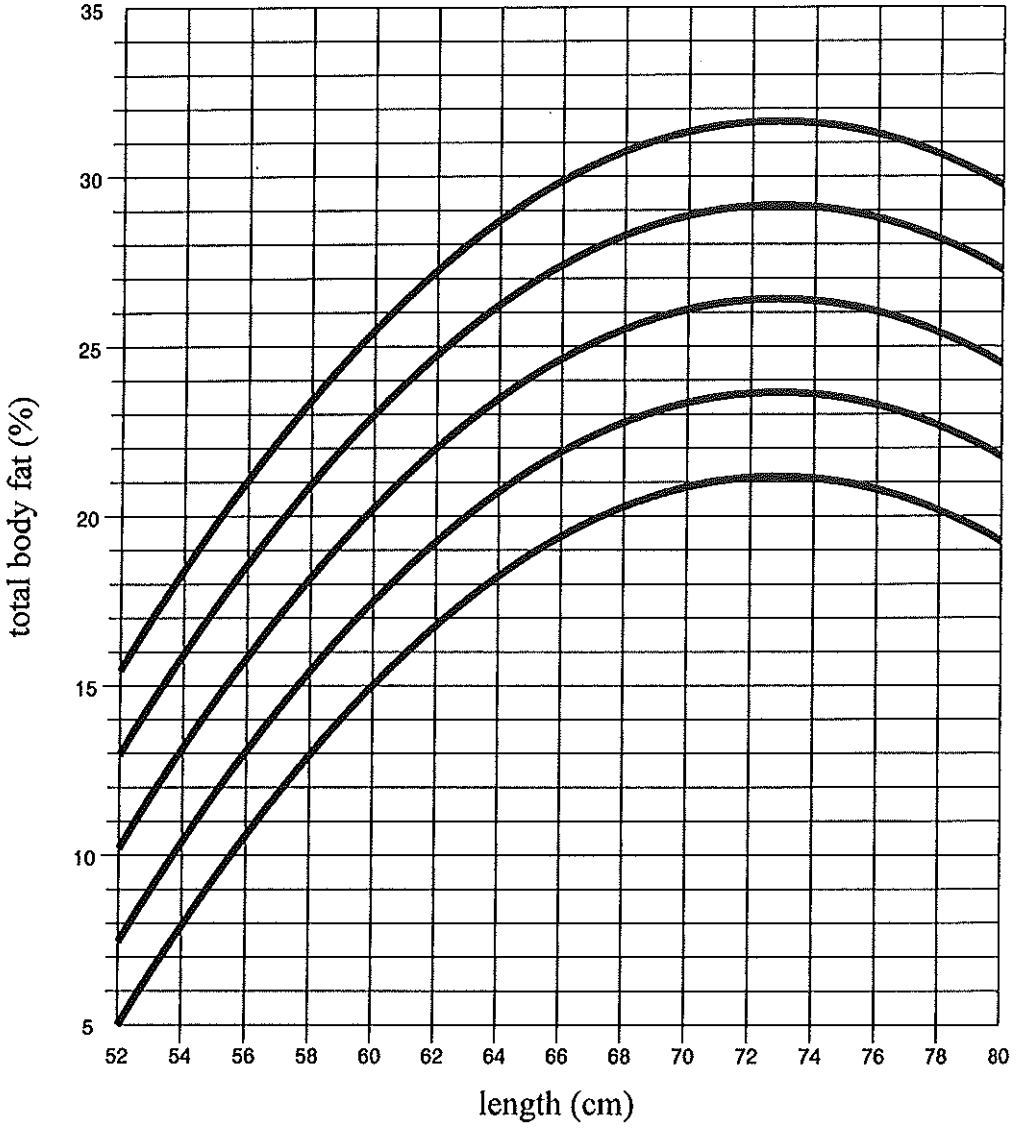
90th, 75th, 50th, 25th and 10th centile




*SKZ, Rotterdam, 1995*

# Boys

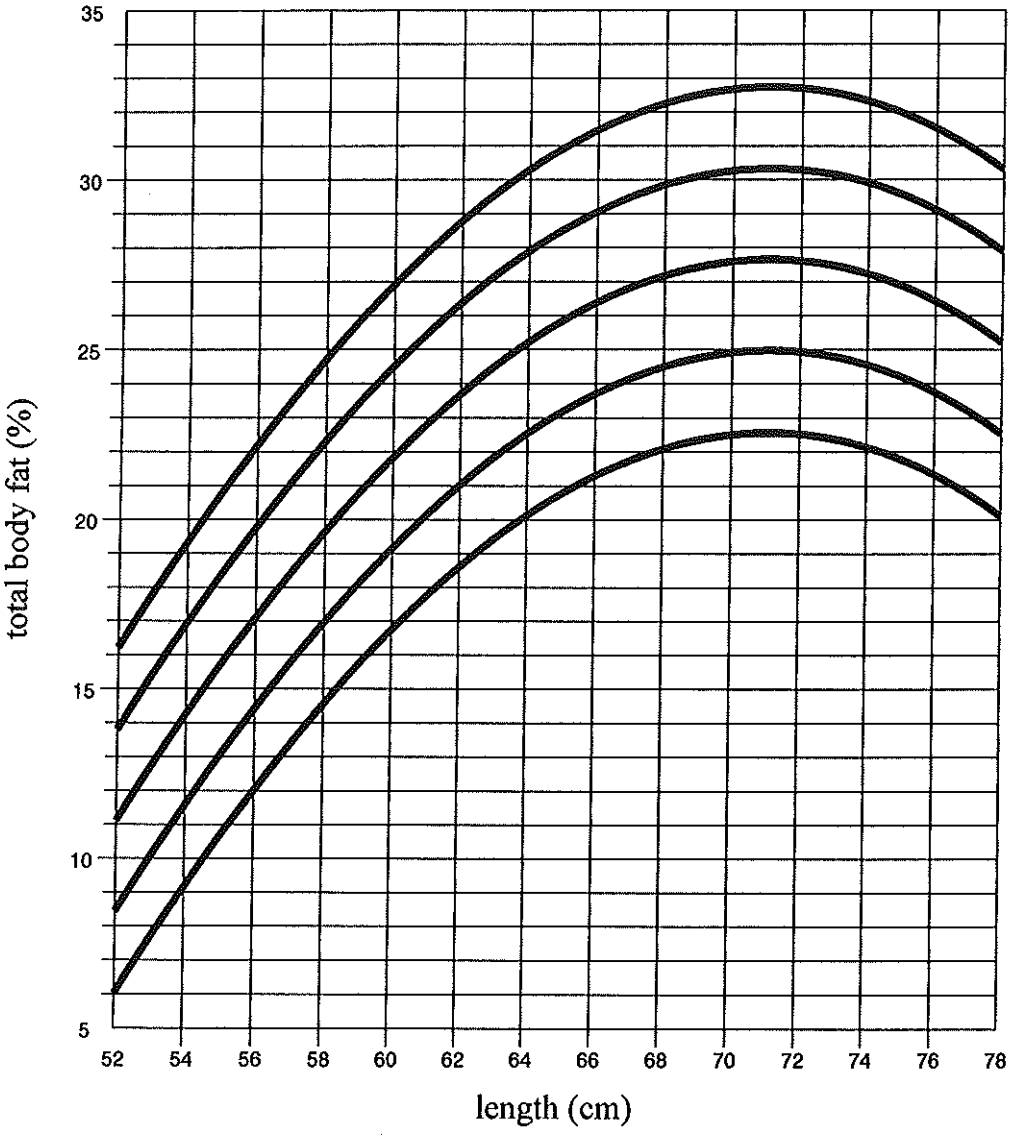
90th, 75th, 50th, 25th and 10th centile




*SKZ, Rotterdam, 1995*

# Girls

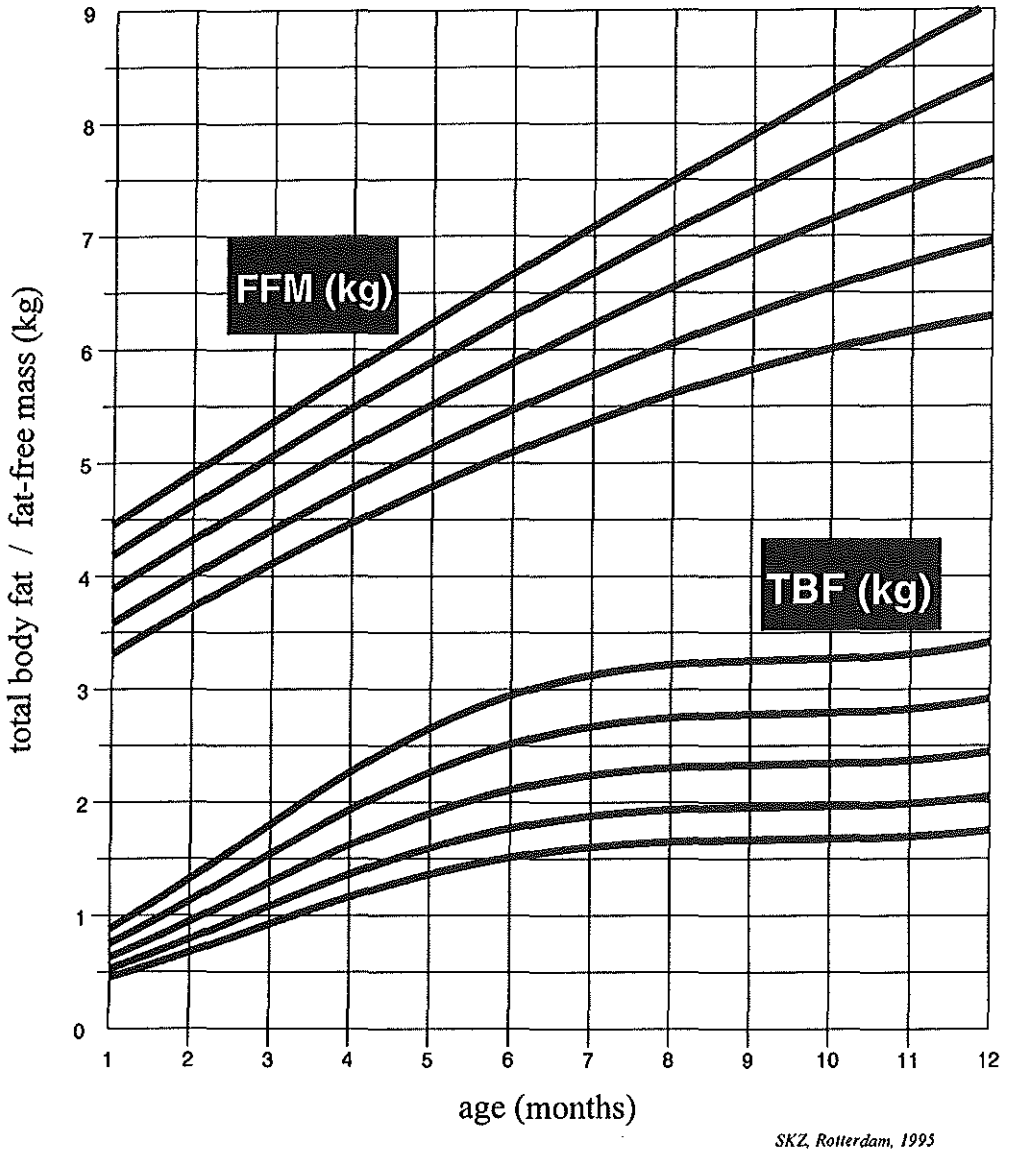
90th, 75th, 50th, 25th and 10th centile




*SKZ, Rotterdam, 1995*

# Boys

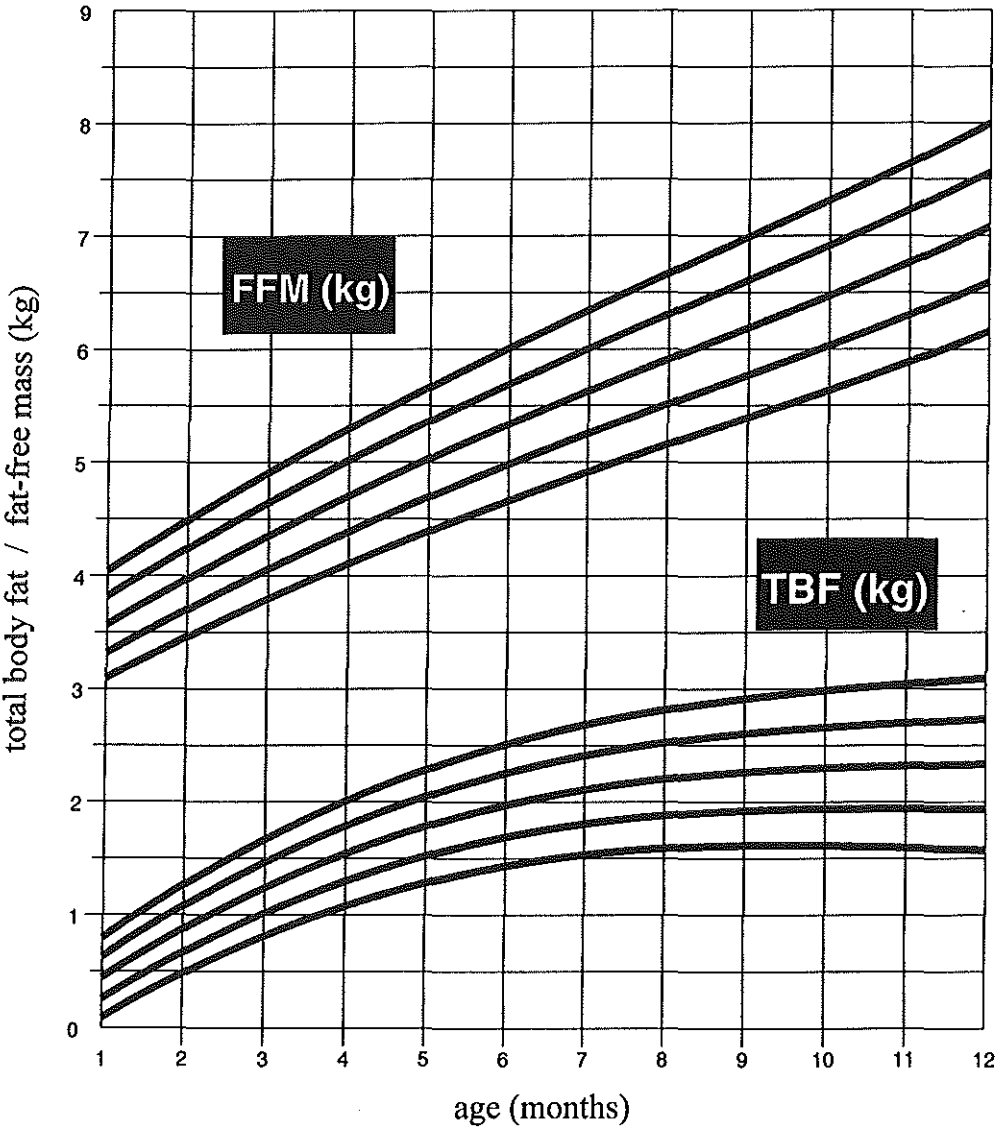
90th, 75th, 50th, 25th and 10th centile




*SKZ, Rotterdam, 1995*

# Girls

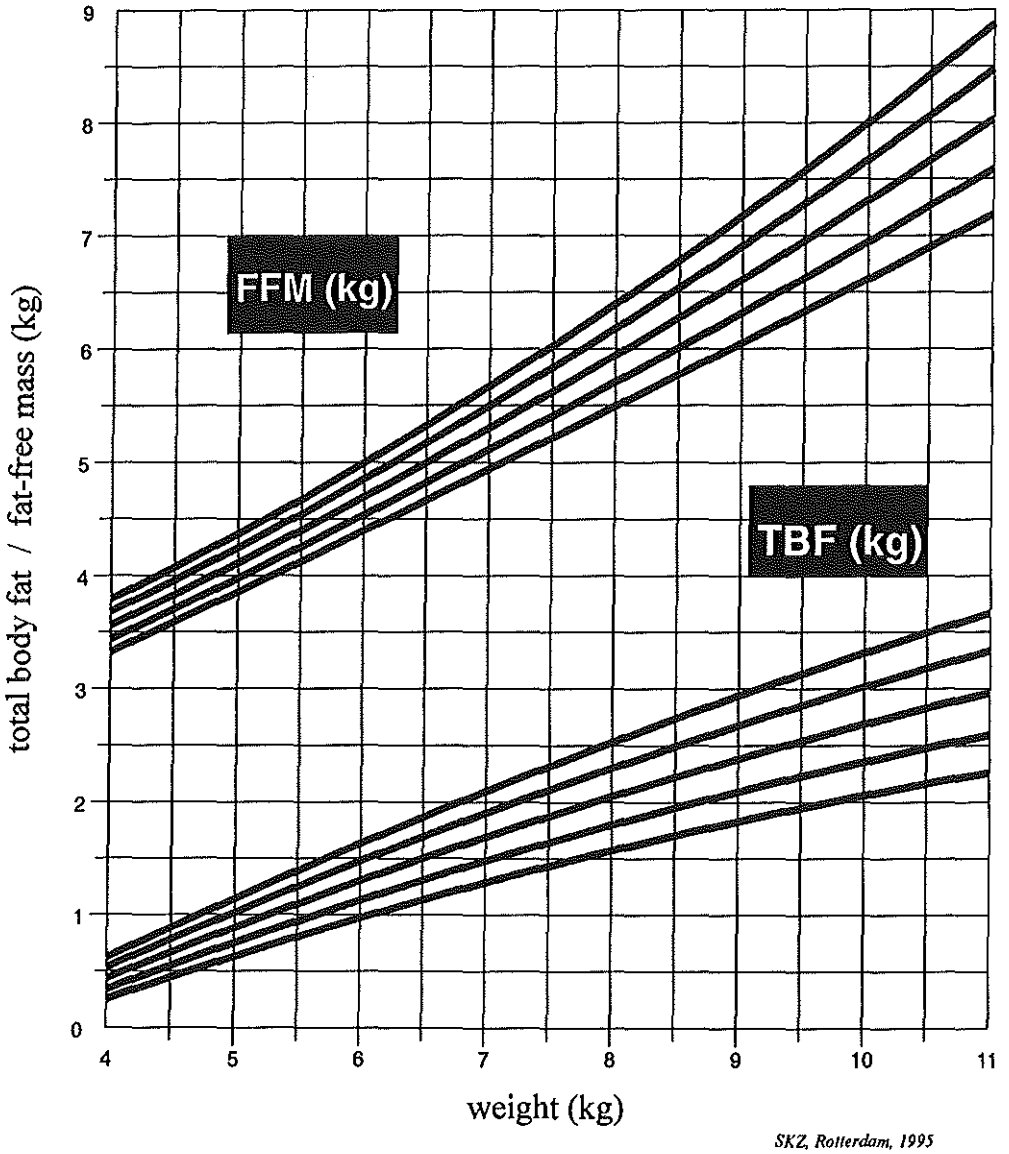
90th, 75th, 50th, 25th and 10th centile




*SKZ, Rotterdam, 1995*

# Boys

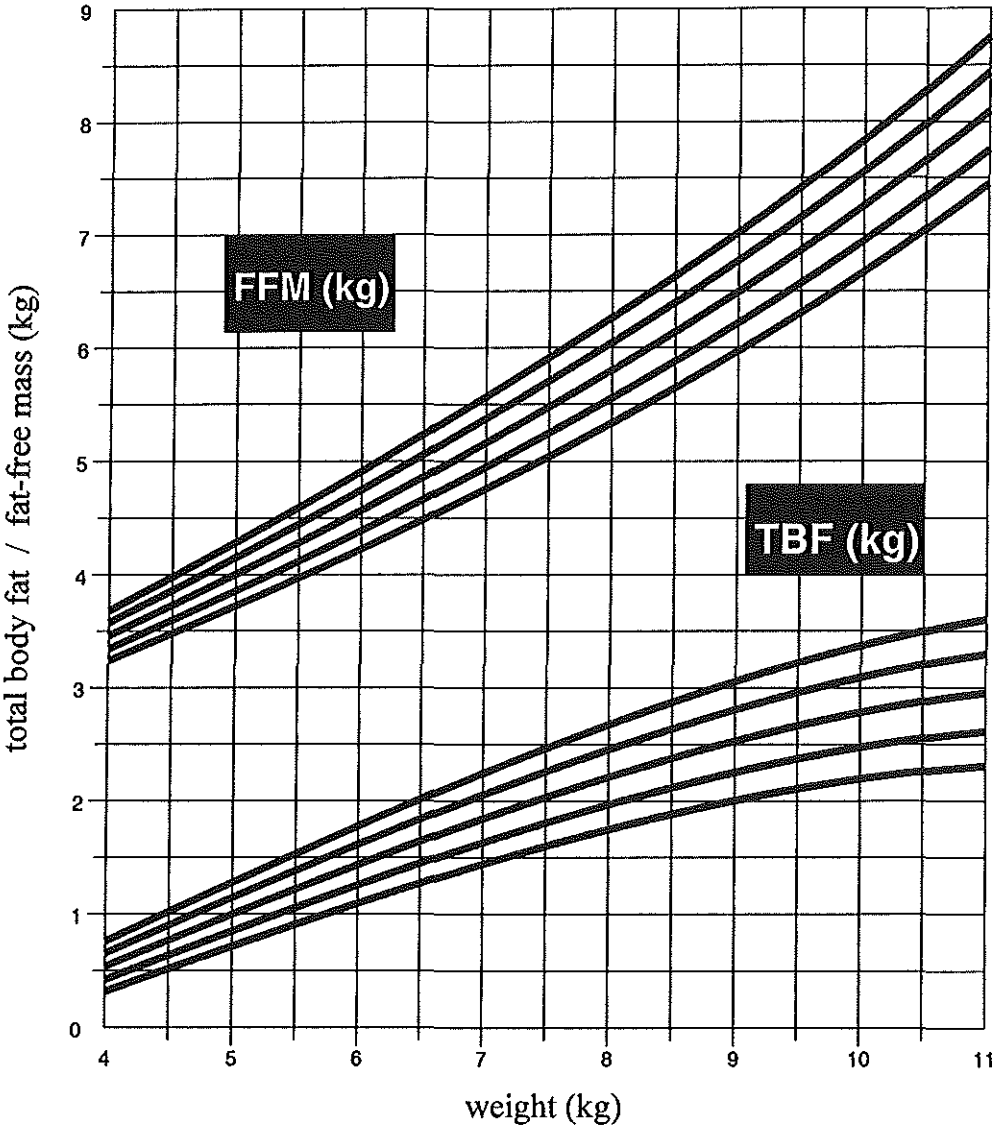
90th, 75th, 50th, 25th and 10th centile




*SKZ, Rotterdam, 1995*

# Girls

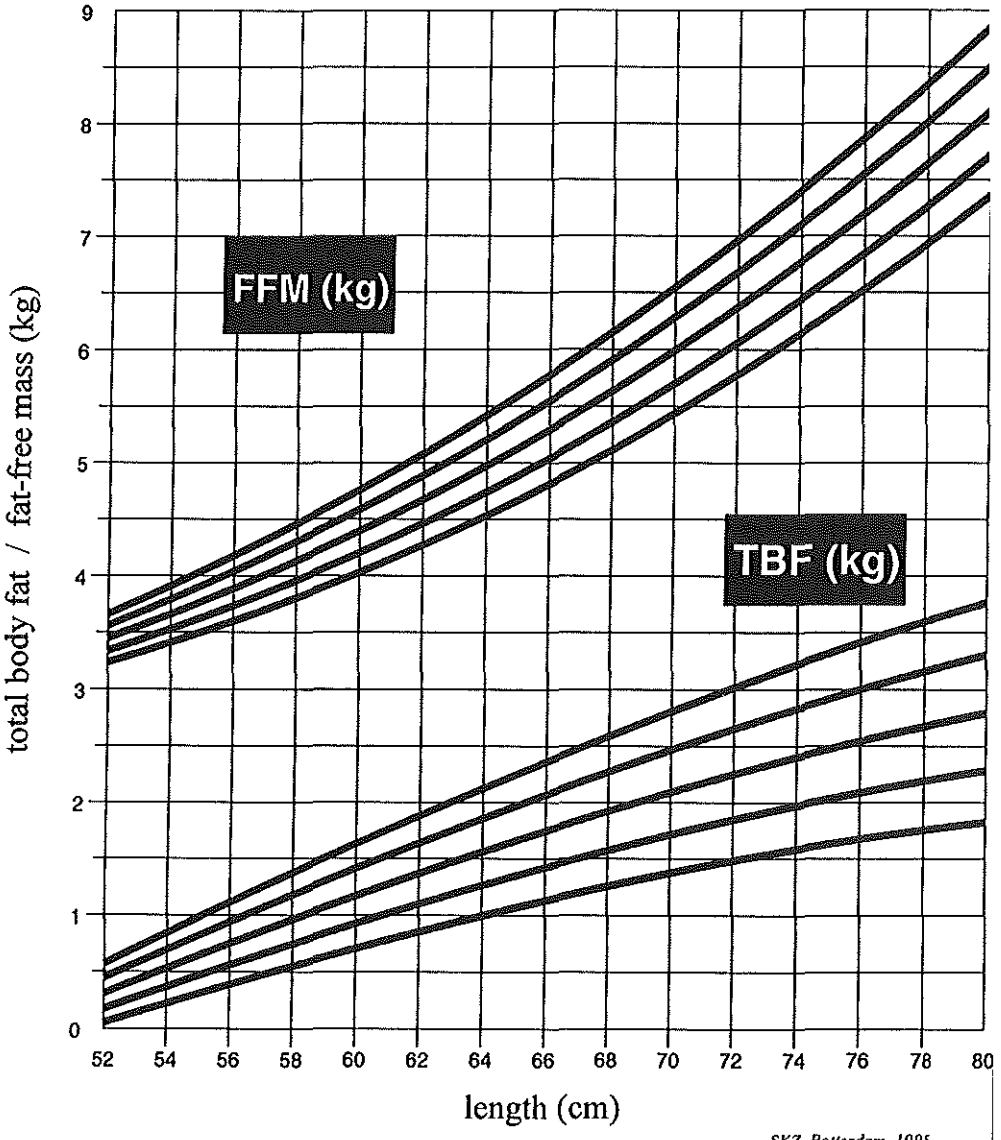
90th, 75th, 50th, 25th and 10th centile




*SKZ, Rotterdam, 1995*

# Boys

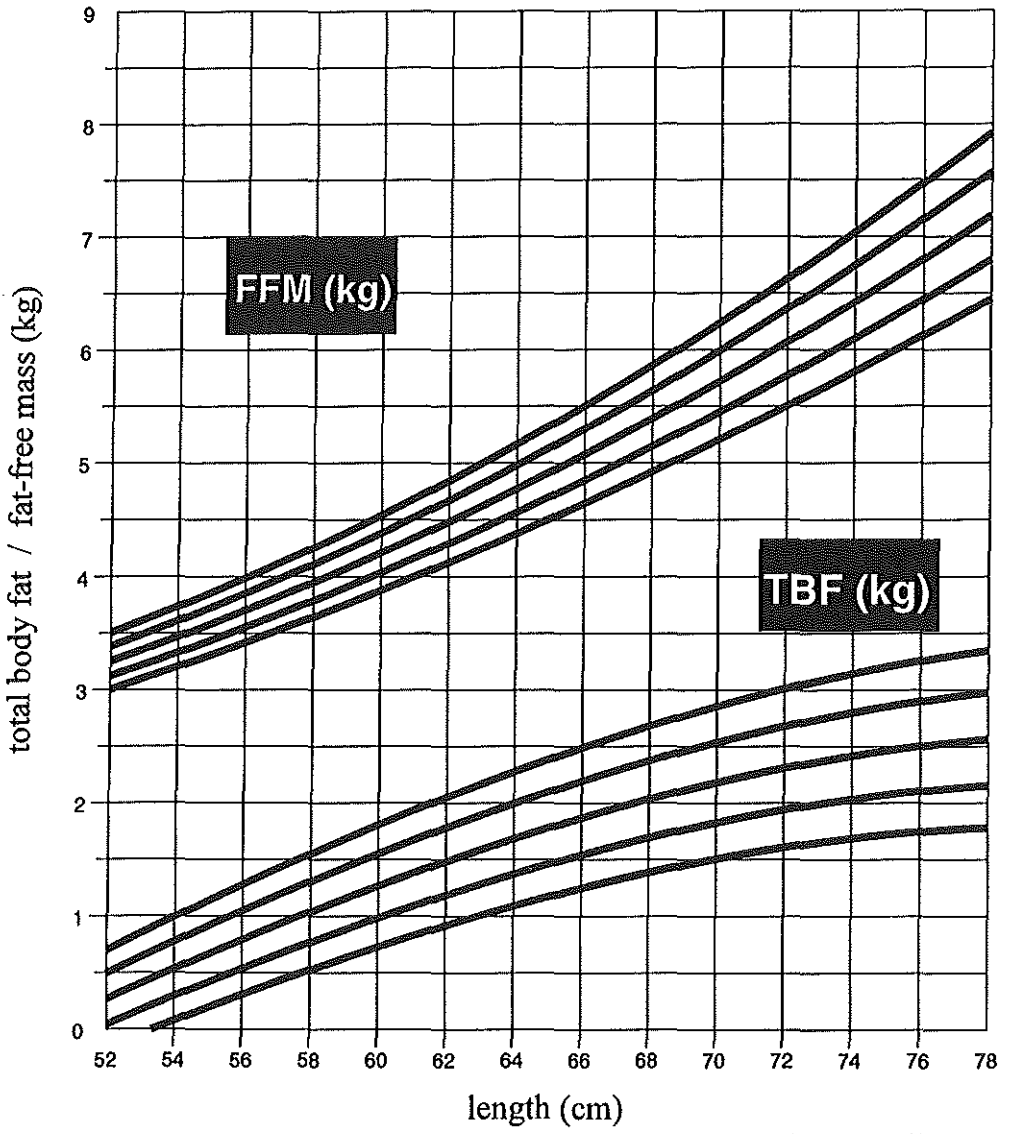
90th, 75th, 50th, 25th and 10th centile




*SKZ, Rotterdam, 1995*

# Girls

90th, 75th, 50th, 25th and 10th centile




*SKZ, Rotterdam, 1995*

# Appendix 3

*Body composition of the "Reference Fetus" and the "Reference Child" (chapter 9)*

*Appendix 3:* **Table 1.** Whole body composition of the reference fetus from 24 weeks to birth.

| Gestational Age (weeks) | Body Weight (g) | Lipid (g) | Water (g) | Per 100 g Body Weight | | | | Per 100 g Fat-Free Mass | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Water (g) | Protein (g) | Lipid (g) | Other (g) | Water (g) | Protein (g) |
| 24 | 690 | 0.69 | 607 | 88.6 | 8.8 | 0.1 | 2.5 | 88.6 | 8.8 |
| 25 | 770 | 5.4 | 669 | 87.8 | 9.0 | 0.7 | 2.5 | 55.4 | 9.1 |
| 26 | 880 | 13.2 | 756 | 86.8 | 9.2 | 1.5 | 2.5 | 88.1 | 9.4 |
| 27 | 1010 | 24.2 | 858 | 85.7 | 9.4 | 2.4 | 2.5 | 87.8 | 9.7 |
| 28 | 1160 | 38.3 | 974 | 84.6 | 9.6 | 3.3 | 2.4 | 87.5 | 10.0 |
| 29 | 1318 | 54.0 | 1093 | 83.6 | 9.9 | 4.1 | 2.4 | 87.2 | 10.3 |
| 30 | 1480 | 72.5 | 1213 | 82.6 | 10.1 | 4.9 | 2.4 | 86.8 | 10.6 |
| 31 | 1650 | 92.4 | 1336 | 81.7 | 10.3 | 5.6 | 2.4 | 86.5 | 10.9 |
| 32 | 1830 | 45.3 | 1464 | 80.7 | 10.6 | 6.3 | 2.4 | 86.1 | 11.3 |
| 33 | 2020 | 139.4 | 1595 | 79.8 | 10.8 | 6.9 | 2.5 | 85.8 | 11.6 |
| 34 | 2230 | 167.3 | 1761 | 79.0 | 11.0 | 7.5 | 2.5 | 85.4 | 11.9 |
| 35 | 2450 | 198.5 | 1911 | 78.1 | 11.2 | 8.1 | 2.6 | 85.0 | 12.2 |
| 36 | 2690 | 234.0 | 2071 | 77.3 | 11.4 | 8.7 | 2.6 | 84.6 | 12.5 |
| 37 | 2940 | 273.4 | 2234 | 76.4 | 11.6 | 9.3 | 2.7 | 84.3 | 12.8 |
| 38 | 3160 | 312.8 | 2370 | 75.6 | 11.8 | 9.9 | 2.7 | 83.9 | 13.1 |
| 39 | 3330 | 349.7 | 2464 | 74.8 | 11.9 | 10.5 | 2.8 | 83.6 | 13.3 |
| 40 | 3450 | 386.4 | 2553 | 74.0 | 12.0 | 11.2 | 2.8 | 83.3 | 13.5 |

Data used from **Ziegler EE, O'Donnell AM, Nelson SE, Fomon SJ.** Body composition of the reference fetus. *Growth* 1976; 40:329-341

*Appendix 3:* **Table 2.** Whole body composition of reference infants from birth to 18 months.

| Age (mo) | Length (cm) | Body Weight (g) | Fat (g) | Fat (%) | Fat-Free Mass (g) | Water (% Body Weight) | Water (% Fat-Free Mass) | Protein (% Body Weight) | Protein (% Body Weight) | Other (% Body Weight)[1)] |
|---|---|---|---|---|---|---|---|---|---|---|
| **Boys** | | | | | | | | | | |
| Birth | 51.6 | 3545 | 486 | 13.7 | 3059 | 69.6 | 80.6 | 12.9 | 15.0 | 3.7 |
| 1 | 54.8 | 4452 | 671 | 15.1 | 3781 | 68.4 | 80.5 | 12.9 | 15.1 | 3.7 |
| 2 | 58.2 | 5509 | 1095 | 19.9 | 4414 | 64.3 | 80.3 | 12.3 | 15.4 | 3.5 |
| 3 | 61.5 | 6435 | 1495 | 23.2 | 4940 | 61.4 | 80.0 | 12.0 | 15.6 | 3.4 |
| 4 | 63.9 | 7060 | 1743 | 24.7 | 5317 | 60.1 | 79.9 | 11.9 | 15.8 | 3.2 |
| 5 | 65.9 | 7575 | 1913 | 25.3 | 5662 | 59.6 | 79.7 | 11.9 | 15.9 | 3.2 |
| 6 | 67.6 | 8030 | 2037 | 25.4 | 5993 | 59.4 | 79.6 | 12.0 | 16.0 | 3.2 |
| 9 | 72.3 | 9180 | 2199 | 24.0 | 6981 | 60.3 | 79.3 | 12.4 | 16.4 | 3.4 |
| 12 | 76.1 | 10 150 | 2287 | 22.5 | 7863 | 61.2 | 79.0 | 12.9 | 16.6 | 3.4 |
| 18 | 82.4 | 11 470 | 2382 | 20.8 | 9088 | 62.2 | 78.5 | 13.5 | 17.1 | 3.6 |
| **Girls** | | | | | | | | | | |
| Birth | 50.5 | 3325 | 495 | 14.9 | 2830 | 68.6 | 80.6 | 12.8 | 15.0 | 3.7 |
| 1 | 53.4 | 4131 | 668 | 16.2 | 3463 | 67.5 | 80.5 | 12.7 | 15.2 | 3.6 |
| 2 | 56.7 | 4989 | 1053 | 21.1 | 3936 | 63.2 | 80.2 | 12.2 | 15.5 | 3.5 |
| 3 | 59.6 | 5743 | 1366 | 23.8 | 4377 | 60.9 | 79.9 | 12.0 | 15.8 | 3.4 |
| 4 | 61.9 | 6300 | 1585 | 25.2 | 4715 | 59.6 | 79.7 | 11.9 | 15.9 | 3.2 |
| 5 | 63.9 | 6800 | 1769 | 26.0 | 5031 | 58.8 | 79.5 | 11.9 | 16.1 | 3.1 |
| 6 | 65.8 | 7250 | 1915 | 26.4 | 5355 | 58.4 | 79.4 | 12.0 | 16.3 | 3.1 |
| 9 | 70.4 | 8270 | 2066 | 25.0 | 6204 | 59.3 | 79.0 | 12.5 | 16.6 | 3.2 |
| 12 | 74.3 | 9180 | 2175 | 23.7 | 7005 | 60.1 | 78.8 | 12.9 | 16.9 | 3.3 |
| 18 | 80.2 | 10 780 | 2346 | 21.8 | 8434 | 61.3 | 78.4 | 13.5 | 17.2 | 3.5 |

Data used from **Fomon SJ.** *Nutrition of Normal Infants.* Mosby-Year Book, Inc St.Louis, 1993, p 60.
[1)] Calculated as the total of osseous minerals, non-osseous minerals and carbohydrates (% body weight).

"En overigens, mijn zoon, wees gewaarschuwd;
er is geen einde aan het maken van veel boeken
en veel doorvorsen is afmatting voor het lichaam."

*Prediker 12:12*