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EVALUATION OF AIR-DISPLACEMENT PLETHYSMOGRAPHY FOR ASSESSING BODY COMPOSITION IN CHILDREN AGES 6-48 MONTHS

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ABSTRACT

Body composition assessment in children provides insight into the body tissue changes associated with both normal growth and the development of childhood obesity. Most standard body composition assessment methods for adult populations are neither appropriate nor accurate for use in children. Air-displacement plethysmography (ADP) is an age-appropriate method for assessing body composition in children; however, the method has not been proven accurate in children younger than 6 years of age. This study examined the accuracy of ADP as a body composition assessment method for children 6-48 months of age by comparing relative fat mass (%FM) results from ADP versus those obtained from total body water (TBW) by deuterium dilution (reference method). Mean %FM measured by ADP (19.23% ± 9.66%) and by TBW (21.89% ± 6.32%) were significantly different (p=0.009). Regression analysis of %FM by ADP vs. TBW provided a line of best fit with a slope of 0.12, r^2 = 0.04 and total error = 10.48% FM. These data suggest that ADP is currently not an accurate method for the assessment of body composition in this age cohort. Further investigation of the sources of variability will provide insight into ways of improving the accuracy of this technology for body composition assessment of young children.

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CHAPTER 1

LITERATURE REVIEW

Childhood Obesity in the United States

Childhood obesity rates in the United States are alarmingly high. The most recent National Health and Nutrition Examination Survey (NHANES), conducted in 2007-2008, estimated that 21.2% of American children ages 2-5 years were overweight based on body mass index (BMI)[1]. Additionally, 9.5% of U.S. infants and toddlers (aged 12-36 months)were at or above the 97th percentile for weight-for-recumbent length[1]. Although childhood obesity is now widespread, the national prevalence has stabilized since the late 1990's[2]. Previously, the prevalence of childhood overweight and obesity had increased with each NHANES survey since the early 1970's[3].

Obesity in childhood is an important public health concern as it is associated with negative health and psychological outcomes. Obesity is associated with hyperlipidemia, hypertension, impaired glucose tolerance, Type 2 diabetes mellitus, and sleep apnea in children[4-6]. In addition, obesity during childhood can negatively affect emotional health and self-esteem, particularly in young girls[4, 7]. Finally, obesity during childhood increases the risk of being obese during adulthood[8].

The specific mechanisms of the development of obesity during childhood remain unclear. Evidence suggests that there may be important periods during childhood growth and development when children are at an increased risk for the development of obesity[9-11]. The first two years of life has been identified as one of the potentially critical periods for the development of obesity. Studies examining elevated rates of weight gain in infants, referred to as "rapid growth" in the literature, suggest an association between weight gain in infancy and adult weight status. Specifically, infants who accrued body weight at the highest rates from birth until 24 months were more likely to be overweight or obese during later childhood or in adulthood[9-12].

Research examining change in body composition during the first two years of life will help researchers and clinicians understand how childhood obesity develops. Although researchers have studied the timing, rate, and amount of weight gain during early childhood, the contribution of these factors to the development of childhood obesity is unclear. Additionally, contribution of the composition of the weigh gained (lean body mass vs. adipose tissue) to the development of obesity is not well understood, and needs to be explored. The lack of a safe, non-invasive, and practical method of assessing body composition in infants and childhood has limited opportunities to explore the association between infant growth and later obesity.

Current Methods for Assessing Child Growth

The current method for evaluating the amount of weight gain in infants and toddlers in the United States is the use of growth charts produced by the Centers for Disease Control (CDC). In 2000, the CDC released updated growth charts for children from birth to twenty years of age. The new CDC growth charts are revised versions of the growth charts developed by the National Center for Health Statistics (NCHS) in 1977. The reference population sampled for the development of the 2000 CDC growth charts was racially and ethnically diverse, and includes both formula- and breast-fed infants[13]. Therefore, CDC growth charts describe a distribution of how children grow across social, economic, and ethnic conditions.

To assess child growth, infants between birth and 24 months of age are plotted on sex-specific growth charts tracking recumbent length-for-age, weight-for-age, weight-for-length, and head circumference-for-age. A sample growth chart which evaluates length-for-age and weight-for-age in boys is presented in Appendix A. Growth in children aged 2 to 20 years is evaluated using sex-specific growth charts that compare weight-for-age, stature-for-age, and since 2000, BMI-for-age.

For children older than 2 years, the CDC definitions for overweight and obesity are based on BMI-for-age as compared to the CDC growth charts[14, 15]. The CDC considers children with a BMI at or above the 85th percentile as "at risk for overweight," while those at or above the 95th percentile in BMI-for-age "overweight." Conversely, the American Medical Association's Expert Committee on the Assessment, Prevention, and Treatment of Childhood Overweight and Obesity recommends that children at or above the 85th percentile in BMI-for-age be described as "overweight," and those falling between the 95th and 99th percentiles be described as "obese" [16]. For the purposes of this review, the AMA terminology is used when referring to childhood overweight and obesity.

There are two important limitations of growth charts for the purpose of evaluating weight status in children. First, the CDC growth charts represent a distribution of cross-sectional data from samples of American children; they were not created using longitudinal data on the growth of these children. Thus, they do not describe the longitudinal growth of children. However, clinicians misuse the growth charts to plot the longitudinal growth of individual children, as it is a quick and inexpensive way to make inferences about child growth. Second, growth charts describe parameters of weight in children but do not make inferences as to adiposity. As such, growth charts are useful as a screening tool for describing approximately how American children grow over time, but the charts cannot measure obesity in children[17].

BMI in Children

Obesity is a condition of excess adiposity commonly described by elevated body mass index (BMI). BMI is routinely used as a proxy measure for obesity in adults[18]. Unlike in children, weight change in adults is primarily due to change in adipose tissue amount[19]. Thus, BMI is a reasonable proxy measure for adiposity in adults. BMI has been used as an indicator of obesity in children and may useful for screening purposes; however it is often not suitable for defining obesity in children because of several limitations[17]. The accuracy of BMI in children is limited by the difficulty of collecting growth measures

such as length, height, and weight [20]. Appropriate equipment, protocol, and training are necessary to accurately measure growth in children[20, 21]. Furthermore, patterns of weight gain and increase in stature are saltatory in nature, especially in infants, which may result in misclassification of weight status in some children [22]. As with growth charts, BMI may describe weight status in children but cannot be used as a tool to measure obesity in children.

In conclusion, the use of BMI to assess weight status in children does not provide adequate information about body fatness. Growth charts and BMI are useful for obesity screening and epidemiological surveillance; however, alternative methods to directly assess body composition in children are necessary for the study of potential mechanisms by which the composition of the weight gained in early childhood influences weight status later in life. Standard methods used in adults are either not accurate for use in children or unsuitable for use in children. Improvement of available methods for assessing body composition in children is necessary to move the field of childhood obesity research forward.

Body Composition Assessment in Children

The study of the composition of body weight gain in children has been limited due to the lack of suitable body composition methods. Body composition methods developed and validated for use in adult populations are not suitable for use in children for various reasons. To be considered a suitable body composition method for young children, a method must meet these criteria:

- *Behaviorally and cognitively appropriate* – The method should be compatible with the behavior and cognitive development of young children. The technique must require minimal need for subject compliance and understanding.

- *Ethical* – The method must be safe, non-invasive, and pose no greater than minimal risk to the child for clinical application.

- *Practical* – The method needs to be quick and easy to perform by test operator, cost-effective, and widely available if is to be translated into clinical use. Body composition results should be available quickly after the assessment procedure.

- *Accurate* – Above all, the method must provide accurate results and be scientifically validated for the population of interest. For a body composition method to be considered accurate, it must have both trueness and precision. The terms trueness, precision, and accuracy are defined by the International Organization for Standardization (ISO)[23]:

    - Trueness refers to "the closeness of agreement between the average value obtained from a large series of test results and an accepted reference." Trueness can be used to make inferences about the bias associated with a measurement or method.
    - Precision is the closeness of agreement between independent test results obtained under stipulated conditions, computed as a standard deviation or coefficient of variation of the test results. The term describes only the distribution of random errors, and does not describe the test results relate to the reference method.
    - Accuracy, therefore, is "the closeness of agreement between both repeated test results and the accepted reference value." Accurate test results, therefore, have both trueness and precision.

Suitability of adult body composition techniques for use in young children.

Method	Accurate	Practical	Behaviorally App.	Ethical
Skinfold calipers		☑	☑	☑
Hydrodensitometry				
Bioimpedance analysis		☑	☑	☑
DXA	?			
Dilution Techniques	☑		☑	☑
Air-Displacement Plethysmography	?	☑	☑	☑

Adult body composition methods have been used in numerous child studies with mixed results. The following sections will outline the strengths and weaknesses of each method for use in children, and assess their validity in children.

Skinfolds

Skinfold (SF) calipers are used to measure the thickness of a double layer of skin and related subcutaneous adipose tissue at defined points on the body. This method relies on the assumption that subcutaneous fat relates closely to total body fat and that body fat distribution does not markedly change during normal growth in young children[24, 25]. SF measurements can be used in equations specific to a subject's age, sex, and race to arrive at an estimation of relative fat mass.

SF methods are attractive because the calipers are relatively inexpensive and portable, and the procedure is fast and non-invasive making them an acceptable field method for body composition assessment in select populations. However, the SF methods are highly sensitive to variability between measurements (imprecise) and among trained individuals, compromising both accuracy and reliability[21, 25]. Equally important, the result is only as valid as the equation used in the calculation, and equations for estimating relative fat mass in children are especially sensitive to age and population which limits their generalization and applicability to other study cohorts.

Only one study examined the applicability of SF measurements in American infants. The author found that while SF measurements can be used to make inferences about regional body fatness, the method is generally not appropriate for total body composition assessment [24]. The ultimate weaknesses of SF methods lies in the assumptions regarding subcutaneous body fat distribution, lack of standardized measurement sites, and the lack of accurate equations to calculate body composition in young children.

Hydrodensitometry

Hydrodensitometry has historically been considered the "gold standard" body composition assessment technique to which other methods are compared [26]. Better known as underwater weighing, it is a method that uses body density to estimate body composition. Densitometric techniques assume that the body can be divided into 2 types of tissue: fat mass (FM) and fat-free body mass (FFM). Combined, these 2 components are equal to the total weight of the body. During underwater weighing,

the participant is submerged in a small tank of water and exhales as much air as possible from the lungs. The mass of the participant is measured while submerged in the tank. Body volume is equal to the volume of water displaced by the subject and is calculated by subtracting body mass measured underwater from total body mass. Body density is calculated by dividing total body mass (in kg) by body volume (liters). Body density is then used in age- and sex-specific equations developed by Siri, Brozek, and others to calculate fat mass and fat-free body mass [26].

Hydrodensitometry is not suitable for use in infants and toddlers as it requires a high degree of compliance and understanding by the participant. Additionally, it requires a degree of water competency not found in young children. Accordingly, no studies have examined its accuracy in young children.

Bioelectric Impedance Analysis

Bioelectric impedance analysis (BIA) is a method used to estimate body composition based on the conductive properties of biological tissues [27]. In BIA, an electrical current is passed longitudinally through the body, and the resistance to the current's flow is measured. Tissues high in water content, such as blood and muscle are highly conductive, while those high in fat or air (bone, adipose, and lung) are resistive to current flow. Basic BIA units consist of skin surface electrodes which attach to the dorsal surfaces of the right hand and right foot of the subject being measured, and the unit that generates and measures alternating (generally 50kHz) electrical current. Using population-specific equations and impedance results, total body water, extracellular fluid, FM, and FFM can be estimated.

While the method is behaviorally appropriate, practical, and ethical in children, it is not an accurate method for this population. BIA has been studied as a body composition assessment method in children[28-32]. Eisenkolbl (2001) found BIA to under-predict % FM in children ages 6-18 years old, while studies by Elberg (2004) and Goran (1996) found BIA to significantly over-predict %FM in children ages

7-18 and 5-8 years[29-31]. BIA is another method that relies on age- and sex-specific equations to estimate body composition, and accurate equations for use in young children are lacking.

Dual Energy X-ray Absorptiometry

Dual energy x-ray absorptiometry (DXA) is a method by which two x-ray beams are passed through the body in the anterior-posterior direction and the attenuation of radiation caused by body tissues is measured. Computer software uses these data to generate a digital image of the body. Each pixel of the image is analyzed and classified as bone tissue, fat-free lean tissue, fat, or a mixture of fat and lean mass[33]. The absolute and relative amounts of bone, fat, and fat-free mass can then be determined, allowing DXA to estimate body composition.

DXA is an accurate and widely-used method of body composition assessment in adults and children. Specialized training and state licensure, as well as the relatively high-cost of DXA units are additional factors limiting their applicability for use in clinical body composition assessment. DXA is generally not well-suited for use in young children as it requires the subject being scanned to lie still for several minutes, and most are unable or unwilling to lie still enough for high quality scans until age 3 or later. Our research group has observed that only ~25% of normally awake children aged 6-48 months are able to lie still enough for a DXA scan (pilot data, 2010).

The accuracy of DXA in young children has been previously explored. Fields and Goran (2000) found DXA body composition estimates to be highly correlated to the body composition results of a 4-component model in children 10-13 years of age [34]. Conversely, Wells et al. (2010) found DXA to significantly overestimate FM and underestimate FFM in children aged 5-18 years, with these biases increasing in children of elevated BMI status[35]. Williams et al. found DXA to significantly underestimate FM in non-obese boys and overestimate relative fat mass in obese children of both sexes aged 5-21 years[36]. The use of DXA as an accurate body composition assessment method in children needs further investigation.

Dilution Techniques

Dilution methods utilize biological tracers, most commonly deuterium oxide (^2H_2O), to estimate the total body water (TBW) content of the body. An oral dose of the biological tracer is administered to the subject after collection of a baseline body fluid sample, and the tracer is allowed to equilibrate in all body water compartments. Subsequent body fluid samples are collected 3-10 hours after isotope administration[37, 38]. Sample analysis is performed via isotope ratio mass spectrometry, and enrichment values for body water before dosing and after isotope equilibration are used to calculate TBW via the dilution principle: $C_1V_1 = C_2V_2$.

TBW is a two-component method; only fat mass and fat-free mass can be estimated using this technique. Fat mass is assumed to be water-free, and thus TBW is entirely contained by the fat-free mass (FFM) of the body. Using the assumption that FFM is ~73% water in adults, the FFM and FM can be calculated. The hydration of FFM is age and sex-dependent; infants average ~81% water content in their FFM at birth, and toddlers between 77-78%[39, 40]. The hydration of FFM must be adjusted when using the TBW technique to estimate body composition in young children.

TBW has been used as a reference standard or part of multiple-component models in many body composition assessment studies in both adults and children. Deuterium oxide, an isotope of water, is safe for use in humans. However, it has significant drawbacks that limit its widespread applicability. It is costly, time consuming, and requires specialized equipment for sample analysis. In children, obtaining urine samples at the appropriate time and in sufficient volume is important but difficult. TBW is a reasonable choice for research settings, but due to its drawbacks it is not suitable for widespread clinical use.

Air-Displacement Plethysmography

Air-Displacement Plethysmography (ADP) is a 2-component whole-body densitometry technique that utilizes the same basic principles as hydrodensitometry. Instead of water, the test subject displaces

air during body composition testing. This difference makes body composition assessment in infants and toddlers feasible in principle; the method only requires the subject to sit in the unit while measurements of air pressure are made by the system. ADP systems are manufactured by Life Measurement Inc. (Concord, CA) under the trade names BOD POD, which is intended for body composition assessment in adults and children over 6 years of age, and PEA POD for body composition assessment in infants up to 8kg (~6 months of age).

ADP has many strengths as a body composition assessment method. It is behaviorally and cognitively appropriate for use in young children. It is a quick, safe, and non-invasive method for body composition assessment. It is cost-effective and practical for use in both the research and clinical settings. The major limitation of ADP for body composition assessment lies in its unknown accuracy in children aged 6-48 months. To date there have been no published studies examining the accuracy of ADP as a body composition method in this age range.

Body Composition Assessment by ADP

Detailed descriptions of the operating principles of the BOD POD have been described previously [41]. In brief, the unit is comprised of two chambers: a reference chamber (~300L) and a testing chamber (~450L) (see Appendix B). The chambers are separated by a partition which also acts as the seat for the unit. During the body composition test, an oscillating diaphragm located between the two chambers creates small sinusoidal pressure waves in each chamber. The changes in pressure are measured by the system sensors and gas laws relating pressure and volume are used to determine the change in volume of the test chamber (450L minus the body volume of the test subject).

Prior to testing, a test subject changes into clothing that fits tightly on the body; garments made of spandex are recommended. Additionally, a spandex swim cap is worn on the head to compress the hair. Clothing that traps air against the body will provide inaccurate results. The body mass of the

subject is measured using an integrated high-precision scale. Before the subject enters the test chamber, the unit is calibrated by measuring the volume of the test chamber while empty and with a ~50L calibration volume. During a body composition test, the subject sits on the bench-like partition between the two chambers while the diaphragm oscillates causing minute pressure changes in the chambers. The volume of air displaced ("missing" volume from the test chamber) by the subject sitting in the unit is equal to the subject's body volume. As described for hydrodensitometry, body density is calculated as body mass divided by body volume and population-specific equations are used to determine relative amounts of fat mass and fat-free body mass.

Precision of ADP

Precision as it applies to body composition assessment methods refers to the repeatability/agreement of two or more measurements [42]. Precision is usually represented by statistically by coefficient of variation (CV), the standard deviation of a group of measurements divided by its mean. ADP shows good precision when measuring inanimate objects. In a study published by the manufacturer, twenty consecutive measurements of a 50.039L aluminum cylinder by the BOD POD on two separate days yielded mean volumes of 50.027L and 50.030L, with CV's of 0.025 and 0.027% respectively [41]. Indeed, we have observed excellent precision when measuring the volumes of adult-sized calibration cylinders in our own laboratory (pilot data, see Appendix D). ADP does, however, appear to have poorer precision when measuring the volumes of smaller objects (less than 40L) [43](RPR pilot data, 2010). This is an important consideration for body composition assessment in children less than 4 years of age, whose body volumes are frequently less than 20L.

In precision studies of body volume measurement of adult humans, precision appears to be good. Wells and Fuller (2001) report body volume precision to be between 0.16 and 0.44% of the mean when measuring adults repeatedly on the same day [44]. Similarly, Anderson found the CVs to be between 0.27 and 0.95% when measuring adults on subsequent days [45]. ADP is capable of precise body

volume measurements in adults; however the precision of body volume measurements has not been previously investigated in children.

Accuracy of ADP for Body Composition Assessment

ADP as a body composition method for adults is both accurate and reliable [42]. Regression analysis comparing ADP to underwater-weighing, the "gold-standard" in body composition assessment, was performed in 4 studies. The standard error of the estimate (SEE), the summed squared residuals from the regression line, were found to be less than 2.3 in all cases[46-49]. A SEE of 3.0 is considered "very good" when comparing body composition assessment methods in adults [50]. In the same studies, the difference in relative fat mass as measured by ADP and underwater weighing was found to be within ±2.0%, suggesting ADP to be accurate for adult body composition assessment.

The accuracy of ADP in children has been previously investigated in children older than 5 years of age. Most studies to date have focused on the validity of ADP in adolescents, while few have included younger children. Wells *et al*. (2000) compared ADP to underwater weighing in children aged 7-14 years, finding agreement of the methods within 0.6% FM for the sample (n=10) [51]. Similarly, Dewit and colleagues (2000) found a mean difference of 0.8% FM in children aged 8-13 years [52]. Data on the accuracy of ADP in younger children is limited. Wells *et al*.(2003) examined the accuracy of ADP in children aged 5-7 years versus $^{2}H_2O$ dilution [53]. The authors found that the difference in %FM between ADP and $^{2}H_2O$ dilution was less than 0.5% for the study group, but the 95% limits of agreement were ±4.2% FM for individuals suggesting poorer accuracy of ADP at the individual level in this age range. No studies have been published investigating the accuracy of the BOD POD for body composition assessment in children younger than 5 years.

The Case for Investigating ADP as a Body Composition Assessment Method in Young Children

Obesity is a condition of excess body fatness. As such, it is not necessarily captured by growth charts or BMI values. Evidence suggests that obesity during childhood and later life is linked to weight gain during the first years of life. Without data about the composition of weight gain during this period, the field of childhood obesity research is limited in its ability to explore the quality of this growth. Knowledge of the body composition changes associated with both "normal" child growth and growth resulting in obesity is critical in designing future studies that seek to elucidate the potential mechanisms underlying childhood obesity. Without this knowledge, we are limited in our ability to understand and ultimately combat the childhood obesity epidemic.

There is a need for accurate methods to measure body composition in infants and toddlers. Presently, the options for assessing body composition in young children are limited and none are ideal. ADP may be a good method for assessing body composition in infants and toddlers, but the accuracy of ADP in young children needs to be investigated. The following study will examine the accuracy of ADP as a body composition method in children aged 6-48 months. To our knowledge it is the first study to investigate ADP in this age range and will fill a significant gap in knowledge in child body composition research.

CHAPTER 2

JOURNAL MANUSCRIPT

Introduction

Childhood obesity is a public health concern in the United States and evidence suggests that infancy and early childhood are critical periods of growth that may affect risk for overweight and obesity later in life [9, 12, 54]. Epidemiological studies have illustrated that weight gain during infancy is a strong predictor of later obesity[9-11]. However, the composition of weight gain during infancy and early childhood is less well-understood. Unlike adults, for whom weight change reflects mostly change in adiposity, children accrue bone mineral, fat-free body mass, and fat mass during normal growth and development[40, 55]. Determination of the composition of weight gain during infancy and early childhood may help to elucidate the mechanism by which rapid weight gain during this period affects weight status in later life. Assessment of body composition during infancy and early childhood is likely to become an emerging field of study as researchers and clinicians seek to understand and combat the increasing rates of obesity in children.

Methods for assessing body composition in adult populations are often unsuitable for use in infants and toddlers. Body composition assessment methods for young children should be behaviorally and physically appropriate for their age and cognitive development [56]. Standard techniques commonly used in adults, including DXA, skinfold thicknesses, and underwater-weighing are not appropriate for young children due to the behavioral and physical demands needed for accurate testing [28, 34, 57]. The advancement of the field of child body composition research depends on developing and/or identifying methods that are accurate, safe, non-invasive, and practical for use in children[30, 34, 58].

Air-displacement plethysmography (ADP) is a whole-body densitometric technique in which the density of the body is calculated and related to relative body fatness. During ADP testing, body volume is calculated by the unit through measurement of the change in air pressure in the chamber caused by

the presence of the subject in the testing chamber. Gas laws allow the ADP unit to use pressure changes during the test sequence to calculate a body volume. Body volume is divided by known body mass to calculate a whole-body density value. Body density is equated to relative body fat by the use of the standard 2-compartment densitometric formula [41]. In children, age- and sex-specific constants for whole-body density and hydration of fat-free mass are provided by Butte and Fomon [39, 40].

ADP is an accurate body composition assessment method in adult populations[42, 46]. Units for measuring body composition in adults are commercially produced under the trade name BOD POD (Life Measurement Inc., Concord CA). A separate unit, the PEA POD (Life Measurement Inc. Concord CA), has been developed and validated for measuring body composition in infants weighing less than 8kgs[59, 60]. Several researchers have examined the accuracy of ADP in children aged 5 years and older and reported mixed results when comparing the method to multi-component models [34, 52, 61-67]. One study has examined the use of ADP in children aged 5 to 7 years and reported high accuracy in this age range [53]. There have been no published studies examining the accuracy of ADP in children younger than 4 years using the BOD POD system.

In the present study, we investigated the accuracy of ADP as a body composition method in children aged 6-48 months. The Bod Pod body composition system was used to measure %FM in healthy infants and toddlers with deuterium dilution as the reference method. Based on ADP validation studies in children older than 5 years of age, we hypothesize that ADP will be accurate to within ±2.0% FM for body composition assessment in younger children.

Methods

Subjects and Protocol

A convenience sample of 72 healthy children aged 6 to 48 months was recruited for this study. Parents and children from State College, Pennsylvania and surrounding communities were recruited

using fliers and news advertisements. The study protocol was approved by the Institutional Review Board at The Pennsylvania State University and all parents gave written informed consent for the participation of their child.

Participation in the study protocol required one 5-hour visit to the Human Growth and Body Composition Laboratory at the Pennsylvania State University. Upon arrival, children were screened for study protocol eligibility. Children who were in poor health, known to be claustrophobic, recently ill, or dehydrated were excluded from testing. A study visit consisted of anthropometric measurements, body composition assessment by air-displacement plethysmography, and measurement of total body water (TBW) by deuterium dilution.

Anthropometry

For children 6-23 months, recumbent length was measured to the nearest 1mm on an infantometer (Seca Model 416, Hamburg, Germany) and weight was measured using an infant scale (Seca Model 374, Hamburg, Germany). For children 24-48 months, standing height was measured to the nearest 1 mm using a wall-mounted stadiometer (Seca Model 240, Hamburg, Germany) and weight was measured using the scale provided with the BOD POD system (Tanita Corp., Model BWB-627-A, Japan). Length, height, and weight measurements were performed in duplicate as detailed in the *Anthropometric Standardization Reference Manual* [21].

Air-Displacement Plethysmography

Body density in children was measured using the BOD POD body composition measurement system (Life Measurement Instruments, Concord, CA) according to the manufacturer's instructions as previously detailed by other authors [41]. Manufacturer recommendations for testing attire were followed: children wore a tight-fitting swimsuit or were nude and all children wore a spandex swim cap. Lung volume (V_{TG}) was estimated for each subject by BOD POD software according to age, sex, and height as described by Fields[68, 69].

Several modifications to the BOD POD system were made to enable the testing of young children. First, a child feeding-type seat with a removable tray (Life Measurement Inc., Concord, CA) was secured in the BOD POD testing chamber to prevent children from falling from the bench seat during testing. In addition, a portable DVD player or test-compatible toys were placed on the tray of the child seat to entertain children during the testing procedure. These items were added to the testing chamber during system calibration, and were effectively "zeroed" from the system so as to not affect subject body volume measurement. Second, prior to subject testing, the BOD POD system was calibrated using a child-sized (~20L) NIST-traceable volume phantom. The smaller calibration volume was used as it more closely matched the approximate body volumes of young children. Third, several proprietary software modifications to the computer system were made by Life Measurement Inc. to facilitate body composition testing of small children.

A complete body composition test included a sequence of three separate body volume measurements for all subjects. The two body volume measurements closest in agreement were used by the system software to calculate an average body volume for each subject to be used in the densitometry equation to calculate body composition. Relative fat mass (% FM) was calculated using the basic equation from body density (D_b) values provided by the BOD POD:

$$\% FM = [1/D_b * (D_{FFM}*D_F)/(D_{FFM} – D_F) – (D_F/D_{FFM}-D_F)]*100$$

D_{FFM} = age- and sex-specific density of fat-free mass as provided by Butte et al. [39].

D_F = density of fat mass (0.901g/ml)

Deuterium Dilution

^2H_2O dilution was performed as previously described by Schoeller [38]. In brief, a baseline urine sample was collected from disposable gel-free diapers (Tushies, Eau Claire, WI) or from a toilet-training seat upon arrival to the laboratory. Subsequently, each subject consumed a dose of 0.06g ^2H_2O/kg body weight diluted with tap water (16.7% ^2H_2O) to increase dose volume (Cambridge Isotope Laboratories

Inc., Andover, MA). Fruit flavoring was added to the dose solution to increase acceptance. Urine samples were collected at 3 and 4 hours post-dosing from either a gel-free diaper or toilet-training seat. Urine samples were frozen and stored at -80C until the time of analysis. $^{2}H_2O$ enrichment values were determined via isotope ratio mass spectrometry using standard procedure [38]. The 4-hour post dose urine sample enrichment value was used to calculate TBW, except in cases where a 4-hour post dose urine sample was not produced and the 3-hour post dose urine sample was used for TBW analysis.

Food and beverage intake during the equilibration period (hrs 0-3) was measured and recorded to the nearest 0.1g. Water intake from food and beverage was determined using Nutrition Data System for Research 2009 software (University of Minnesota, Minneapolis, MN). Water output by urine and feces was also measured and recorded to the nearest 0.1g. Insensible water loss was assumed to be 1ml/kg/hr during the equilibration period [70]. A net water balance during hours 0-4 was calculated and used to adjust the final TBW value for each child. Net positive values were subtracted from TBW results and net negative water balances were added to TBW results.

Fat-free body mass was determined from TBW by using age- and sex-specific hydration coefficients for fat-free mass (Hyd_{ffm}) as described by Butte *et al*. (2002) for children up to 2 years of age, and Fomon (1982) for children ages 2 to 4 years using these equations [39, 40]:

$FFM\ (kg) = (TBW_{Liters}/Hyd_{ffm})$

FM (kg) = Body Wt.(kg) – FFM (kg)

%FM = (FM (kg) / Body Wt. (kg)) *100

Statistical Analysis

Anthropometric data for each child was compared to age- and sex-specific CDC growth chart standards using SAS (Version 9.1.3, Cary, NC). Linear regression analysis between the body composition assessment methods was performed. A line of best fit, regression equation, and R^2 for the data were calculated. Total error was equal to the averaged absolute deviation of individual ADP results from the

line of identity. A paired T-test was used to test for statistical significance between the difference in mean sample %FM as estimated by ADP and deuterium dilution. Statistical significance was set at $p \leq 0.05$. The agreement between methods for individual children was assessed using the methods of Bland and Altman.

Results

Table 1 describes the characteristics of the children in the study sample. We were not able to perform all study procedures on all children. Of the 72 children recruited, anthropometry data are available for 96% of children. TBW and %FM by deuterium dilution were completed by 94% of children in the sample. 76% of children in the sample completed 2 ADP body composition tests, 14% completed 1 test, and 10% did not complete any ADP tests. Each ADP body composition test was analyzed as an independent data point for comparison against deuterium dilution, making a total dataset of 109 ADP body composition tests.

The mean ±SD %FM values for the sample were 19.23% ± 9.66% and 21.89% ± 6.32% as estimated by ADP and ^2H_2O dilution methods, respectively. The mean difference (%FM ADP – %FM ^2H_2O) was 2.66% ± 10.50%FM. The difference in estimated relative fat mass between ADP and ^2H_2O dilution methods was statistically significant (p=0.009). A regression of individual ADP test results, compared to the reference value estimated from ^2H_2O dilution is presented in Figure 1. Total error (TE), the average deviation of individual ADP results from the line of identity, was equal to 10.78%FM.

Figure 2 is a Bland-Altman plot of the difference in %FM between methods (% FM by ADP - % FM by ^2H_2O) versus the average %FM between the methods. The mean difference ± 2 standard deviations establish limits of agreement that are -23.66% to 18.34% FM.

Within-day Precision of ADP body volume measurements was assessed by calculating coefficients of variation (CV) for the 3 raw body volume measurements made as part of each ADP body

composition testing sequence. The CV for the sample was 0.665% ± 0.845 (0.043-6.036%). In addition, the precision between ADP body composition results for each child completing 2 tests was calculated. For the entire sample, mean difference in %FM between ADP tests (ADP test 1 – ADP test 2) was -0.14% ± 6.90%. The difference in mean %FM between ADP tests was not significantly different (p=0.889). The average absolute difference between ADP test 1 and ADP test 2 was 6.83% FM.

Discussion

The purpose of this study was to investigate the accuracy of ADP as a method for assessing body composition in children aged 6-48 months. This study is important, as data on the compositional makeup of body tissues during early childhood growth are necessary to explain the observed relationship between early childhood weight gain and later obesity. The development of accurate body composition assessment methods that can be practically utilized in young children is necessary to collect this data and advance the field of childhood obesity research.

For a body composition method to be considered accurate, it must have both trueness and precision. The terms trueness, precision, and accuracy are defined by the International Organization for Standardization (ISO). Trueness refers to "the closeness of agreement between the average value obtained from a large series of test results and an accepted reference [23]." Trueness can be used to make inferences about the bias associated with a measurement or method. Precision is the closeness of agreement between independent test results obtained under stipulated conditions, computed as a standard deviation or coefficient of variation of the test results. The term describes the only the distribution of random errors, and does not describe the test results relate to the reference method. This term is often referred to as "reliability" in the literature, although precision is a more appropriate term. Accuracy is "the closeness of agreement between both repeated test results and the accepted reference value [23]." Accurate test results, therefore, have both trueness and precision. Relating these

terms to the investigation of the validity of ADP for childhood body composition assessment, an accurate BOD POD body composition test would yield a result in agreement with those obtained by deuterium dilution (trueness), and the results of repeated within-day body composition tests on a child should not deviate significantly (precision).

Accuracy studies often report averaged statistics of trueness for the sample; however it is equally important that the method has trueness on an individual basis as well. For this study sample, the average %FM as estimated by ADP and $^{2}H_{2}O$ were 19.23% ± 9.66% and 21.89% ± 6.32% respectively (mean ± SD). The difference between the means is 2.66% FM suggesting that on a *group level*, ADP slightly underestimates %FM versus $^{2}H_{2}O$ dilution in young children. Conversely, interpretation of the trueness of ADP as it relates to *individual children* provides a total error of 10.78% FM. This is not an acceptable amount of error for valid methods to be used in either the research or clinical settings. To put this in perspective, Lohman rates a method with an SEE of 3.0% FM to be versus the reference standard to be "very good [50]." In this study, the SEE was greater than 50% FM. The regression line equation, R^2 value, and standard error of the estimate are not useful values in interpreting the results of this study, as there is poor correlation between ADP and the reference method.

Precision of ADP measurements was investigated by calculating CVs for body volume measurements and by repeating the ADP body composition test on each child when possible, for a maximum of two ADP tests per child. For the entire sample, the mean CV during body volume measurement was 0.665%, similar to what has been previously described in adults[44, 45]. When using body volume measurements to calculate body composition, the mean difference on the *group level* in %FM between ADP tests (ADP test 1 – ADP test 2) was -0.14% ±6.90% (p=0.889). The average absolute difference between ADP test 1 and ADP test 2 was 6.83% FM, suggesting that precision is poor when repeatedly measuring *individual children*. ADP is neither precise nor does it appear to have acceptable trueness when assessing body composition in infants and toddlers. It is not clear why the observed

error in ADP measurements in infants and toddlers is occurring, as the observed error in ADP measurements is not related to child age, sex, weight, or body size (data not shown).

A limitation to address regarding this study is the use of ^2H_2O as the reference method. ^2H_2O dilution was chosen as the reference standard technique because it is an accurate, safe, and non-invasive method for body composition assessment in infants and toddlers [38]. In body composition studies in children, ^2H_2O dilution is commonly used as the reference technique due to lack of better alternatives, but limitations to the use of ^2H_2O dilution for determination of total body water in young children have been noted. The plateau method of TBW estimation was used; an oral dose of ^2H_2O solution is administered, allowed to equilibrate for 3-4 hours, and samples of a body fluid were collected for isotope enrichment. This method assumes that the ^2H_2O solution dilutes equally in all body water compartments within 4 hours. Wells *et al*. (2005) note that the plateau method may significantly overestimate TBW in children less than 2 years of age, however the assumed overestimation can be accounted for during TBW calculation [71]. The plateau method has been used in previous validation studies of ADP in infants and children and ^2H_2O dilution remains an acceptable method[53, 59, 65, 71].

To our knowledge, this is the first published study to examine the accuracy of the Bod Pod ADP system in children aged 6 months to 4 years. ADP measurement is feasible in the majority of young children; 90% of children completed at least one ADP body composition test in this study, which is encouraging for future investigations. While this study did not find ADP to currently be an acceptable body composition method for young children, the results suggest that the error may be a result of the systems inability to accurately measure child body volume. Changes in hardware design may improve the accuracy of body volume measurements. Further investigation into the possible causes of the error observed in ADP results when estimating body composition in infants and toddlers is needed.

Figure Legends and Tables

Table 1. Subject Characteristics.

	Children 6-23m		Children 24-48m		Total Sample
	Male	Female	Male	Female	
Variable	(n = 13)	(n = 23)	(n = 16)	(n = 20)	(n = 72)
Age (months)	12.2 ± 5.5	15.7 ± 6.5	36.5 ± 7.8	36.4 ± 7.7	25.4 ± 13.1
Stature (cm)	73.7 ± 8.0	76.0 ± 8.2	95.1 ± 4.5	94.7 ± 6.4	84.6 ± 12.1
Stature z-score	-0.5 ± 1.1	-0.4 ± 0.9	0.0 ± 0.8	0.2 ± 0.8	-0.2 ± 0.9
Weight (kg)	10.0 ± 1.8	10.0 ± 2.1	14.6 ± 1.2	14.5 ± 2.2	12.2 ± 3.0
Weight z-score	-0.1 ± 0.6	-0.2 ± 1.1	0.0 ± 0.8	0.2 ± 0.8	0.0 ± 0.9
Weight-for-length[1]	73.3 ± 24.5	63.0 ±26.0			60.8 ± 26.5
W-F-L z-score	0.9 ± 1.0	0.4 ± 0.9			0.4 ± 0.9
BMI			16.1 ± 1.0	16.2 ± 1.1	16.1 ± 1.1
BMI z-score			0.0 ± 0.7	0.3 ± 0.9	0.2 ± 0.8

[1]Weight-for-length presented as percentile based on CDC 2000 growth charts.
z-scores for stature, weight, weight-for-length, and BMI calculated from CDC 2000 growth charts.
Data presented as mean ± SD
"Stature" refers to recumbent length in children less than 24 months of age and standing height in children older than 24 months.

Figure 1. Regression of %FM measured by D2O dilution method vs ADP. The solid line is the regression equation (line of best fit); the dashed line is the line of identity.

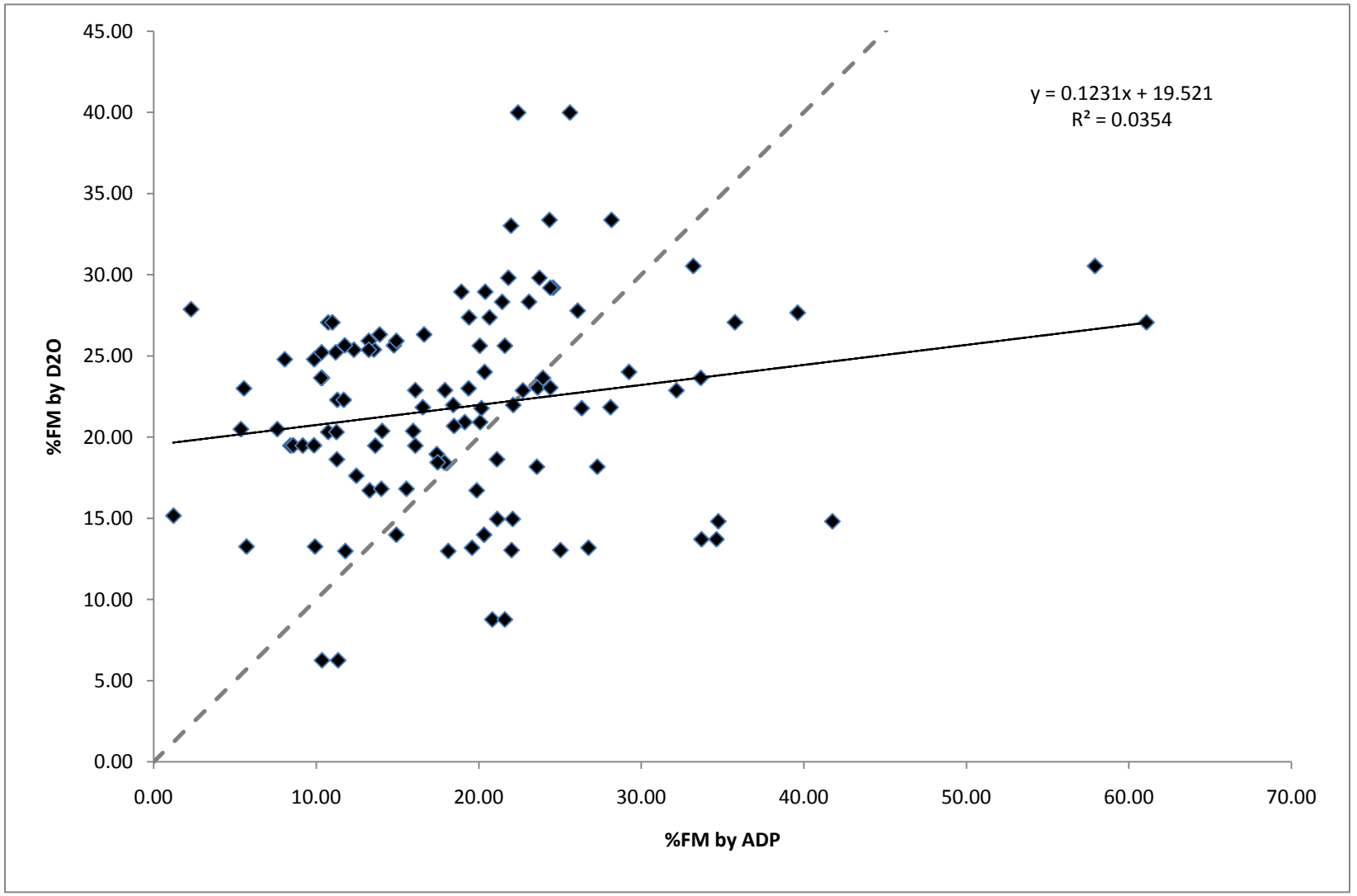
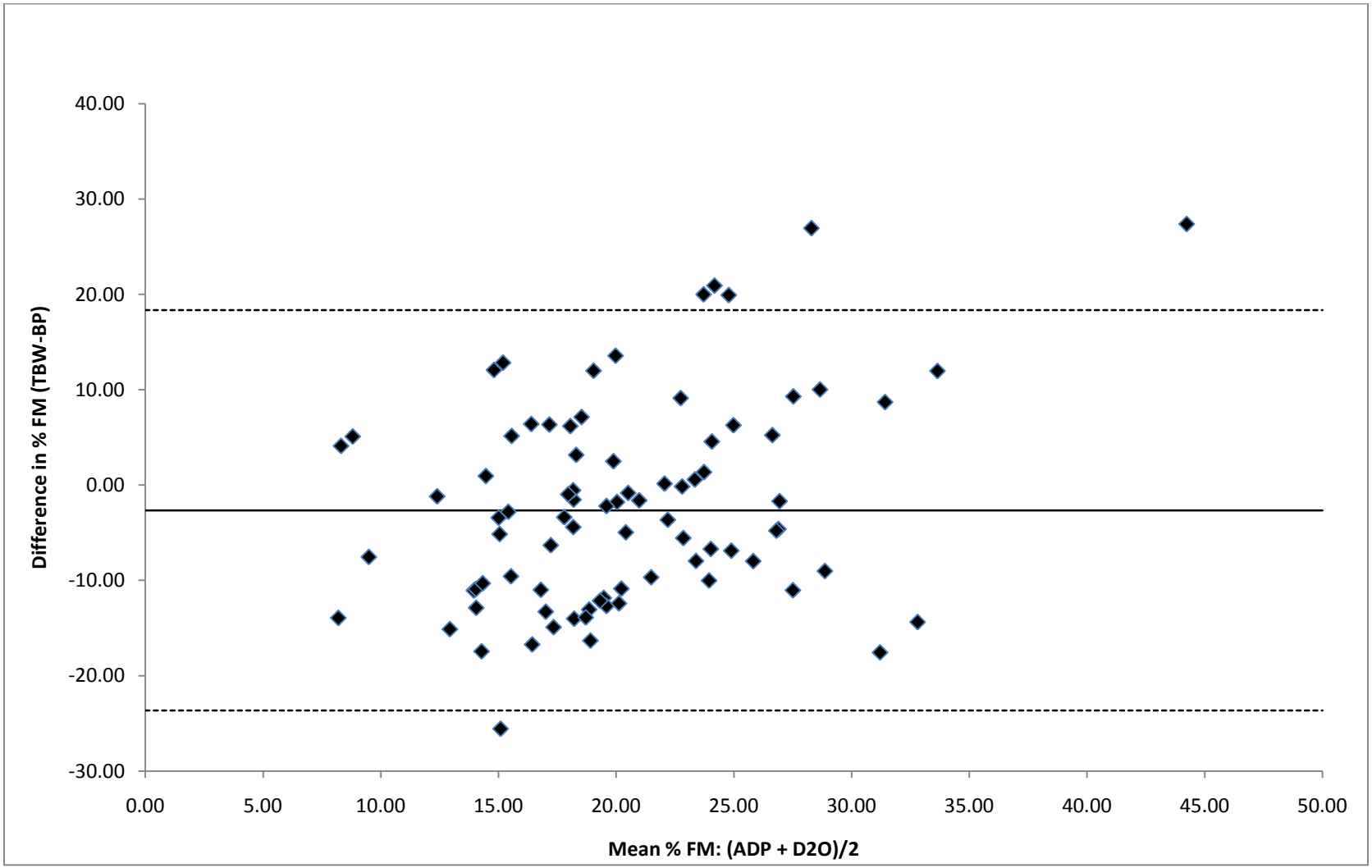


Figure 2. Individual differences in estimated %FM between ADP and D2O dilution compared with the mean %FM between both methods. The solid line represents the mean difference. The dashed lines are ± 2 SD.



CHAPTER 3

FUTURE DIRECTIONS

This study shows that, as currently designed, ADP is not an accurate method for body composition in our investigated age range. However, it is worthwhile to continue investigating the validity of this method for body composition assessment of young children. ADP has the potential to be a good body composition assessment as it is quick, non-invasive, and safe, and it meets the ethical and behavioral requirements needed for use in young children.

A logical next step is an investigation into the source(s) of the error observed in ADP measurement of infants and toddlers. There are several potential sources of error that could be occurring. Appendix C shows a flowchart of the variables that are used to compute body composition values by ADP. Most of these variables are unlikely to be the primary cause of the observed error in body composition measurement in young children, as they ultimately do not contribute significantly to body composition estimation. The variables marked with (*) will be discussed as possible sources of error to consider.

The most likely cause of the error in child body composition by ADP is the system's inability to accurately measure raw body volume in young children. Comparison of raw body volume measurements by ADP to body volume measurements by another method would be a nice way to investigate this hypothesis, unfortunately there is no alternative method of body volume measurement for this age group at this time. The error in body volume measurement could be a result of the relatively small amount of volume that young children occupy in the ADP test chamber or an issue with the system hardware that our data does not capture. Life Measurement Inc. will not disclose all of the proprietary operating information regarding the Bod Pod, so we are limited in our ability to test some of our hypotheses.

Body Volume

Appendix D is a regression of child body weight versus the difference in body composition results between ADP and $^{2}H_2O$ dilution. Child body weight was selected because it can be used as a proxy indicator for body size and child age. The observed differences between the body composition methods appear to be randomly distributed, suggesting that the error in body volume measurement is equivalent across our age range.

Interestingly, our laboratory has collected data on the ability of ADP to accurately measure the volumes of inanimate objects using NIST-traceable calibration volumes. In general, the system can repeatedly measure the volumes of the calibration tanks with excellent trueness and precision. Appendix E shows histograms plotting coefficients of variation (CV) when measuring calibration volumes of differing sizes. When calibrating the ADP system with the ~20L volume, then measuring the ~20L tank five successive times (figure a), ADP makes volume measurements that are both true and precise.

When child body volume is measured by ADP, their volume occupies only a percentage of the volume that the calibration tank occupied during system setup. A realistic way to replicate this occurrence experimentally is to setup the ADP system to measure a calibration tank of a given volume then place a tank of smaller volume in the test chamber to simulate an infant. Histogram (d) in represents this scenario; the ADP system was calibrated to measure a ~20L tank, but a ~3L tank was experimentally measured. The CV's are ten-fold worse in this scenario than when the machine measures a tank for which it has been calibrated for, suggesting that ADP loses some precision when measuring smaller volumes (~3L), but still retains trueness. Thus, the discrepancy observed when measuring body volume in young children is not fully explained, as ADP can measure small objects with suitable trueness.

Surface Area Artifact

During testing, warm air near the surface of the skin is more compressible than the air in the test chamber. As a result, the volume of air in close proximity to the subject during testing is overestimated by 40%. A correction factor, called the surface area artifact (SAA), is calculated based on subject height and body mass, and is added to the raw body volume measurement to account for this occurrence. In a 12.5kg child, SAA contributes ~200ml (1.6%) to the adjusted body volume measurement of 12.165L.

Thoracic Gas Volume

Similar to SAA, the volume of the warm air in the lungs is overestimated by 40%. Thus 40% of the predicted thoracic gas volume (TGV) is added to the raw body volume measurement. TGV in children is difficult to measure, and the Bod Pod utilizes by Fields *et al.* (2004) to estimate lung volume in children [69]. As with SAA, TGV contributes ~200ml (1.6%) to the adjusted body volume measurement of 12.165L in a child weighing 12.5kg. Collectively, neither SAA nor TGV influence the final body volume measurement enough to cause the error in %FM observed during this study.

Child Behavior

Our lab has previously examined child behavior during ADP testing in relation to precision of body volume measurements. Pilot data of child behavior during ADP testing and its affect on precision of volume measurements has been collected by our research group. Child stillness and agitation during each body volume were subjectively scored on a scale of 1-3 for each. Average behavior scores for each child were calculated and compared to the CV of volume measurements for the testing sequence (5 consecutive body volume measurements). Child behavior (movement, talking, crying) during body volume measurement does not appear to be correlated with precision of volume measurements (data not shown).

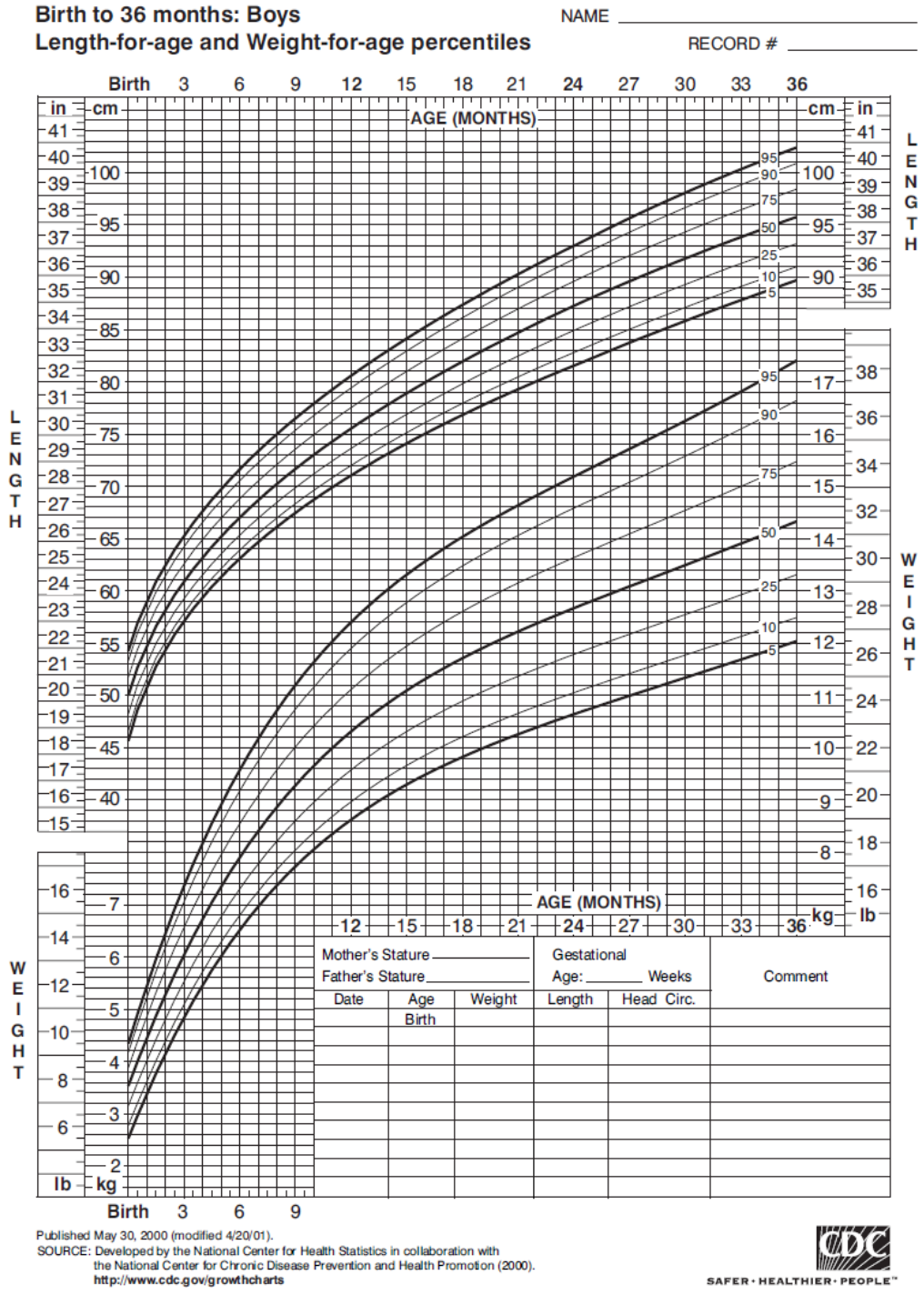
System Hardware

The source of the error in child body volume measurements may be the result of the inability of the ADP system hardware to measure child body volume accurately in the testing chamber. The frequency of diaphragm oscillation in our validation study compared to the frequency used to measure adult body volume was experimentally doubled by Life Measurement Inc., believing that the higher frequency may yield better body volume measurements. The oscillation frequency is linked to respiration rate of the subject. Adults typically take 12-20 breaths per minute while infants breathe at a rate of 40-60 breaths per minute [72]. The oscillation frequency of the diaphragm was changed by Life Measurement Inc. to reflect the breathing rate of toddlers (20-30 breaths per minute) in our study. This suggests that oscillation frequency of the diaphragm may play a key role in body volume measurement in children. Our lab cannot experimentally investigate the contribution of hardware factors at this time, but may be able to collect data on hardware changes in the future.

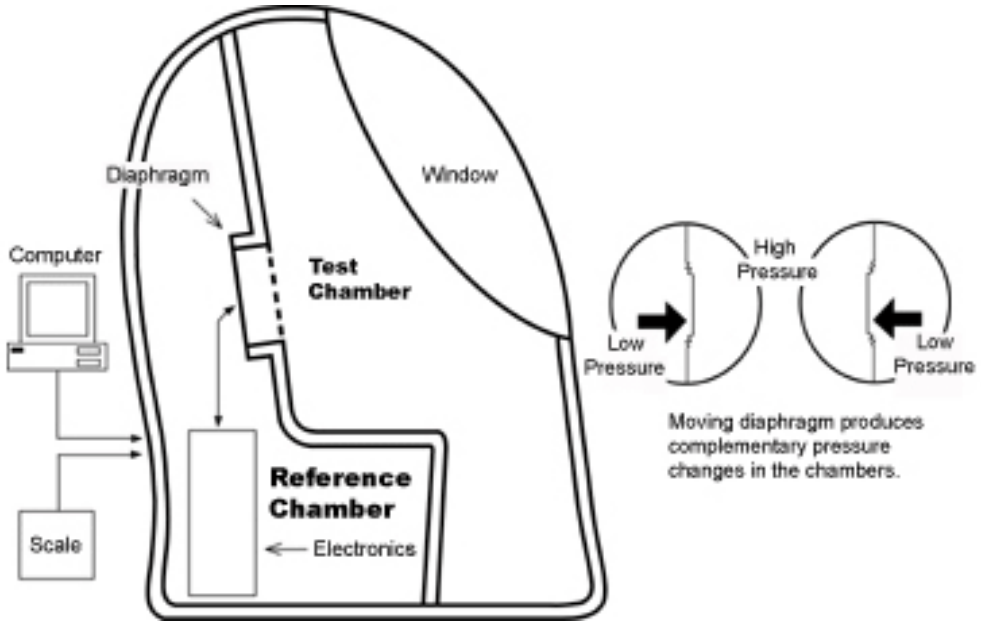
Body composition in larger infants

A study that I plan to begin next semester looks at the performance of ADP in infants weighing between 8 and 10kg. We do see infants weighing in excess 8kg at 6 month of age in the Human Growth and Body Composition Laboratory and have the capability to measure their body composition, but the Pea Pod ADP has only been validated in infants up to 8kg. Our lab has been granted approval to test the accuracy of ADP in infants up to 10kg, a weight which has not been included in previous investigations of the validity of the Pea Pod [59, 60]. We will be using $^{2}H_2O$ dilution as the reference method again; we have learned a lot about dosing and urine collection in young children by trial and error. This study will be a nice addition to the existing literature by assessing the accuracy of ADP in larger infants.

APPENDIX A: CDC (2000) Growth Chart for Boys from birth to 36 months-of-age.

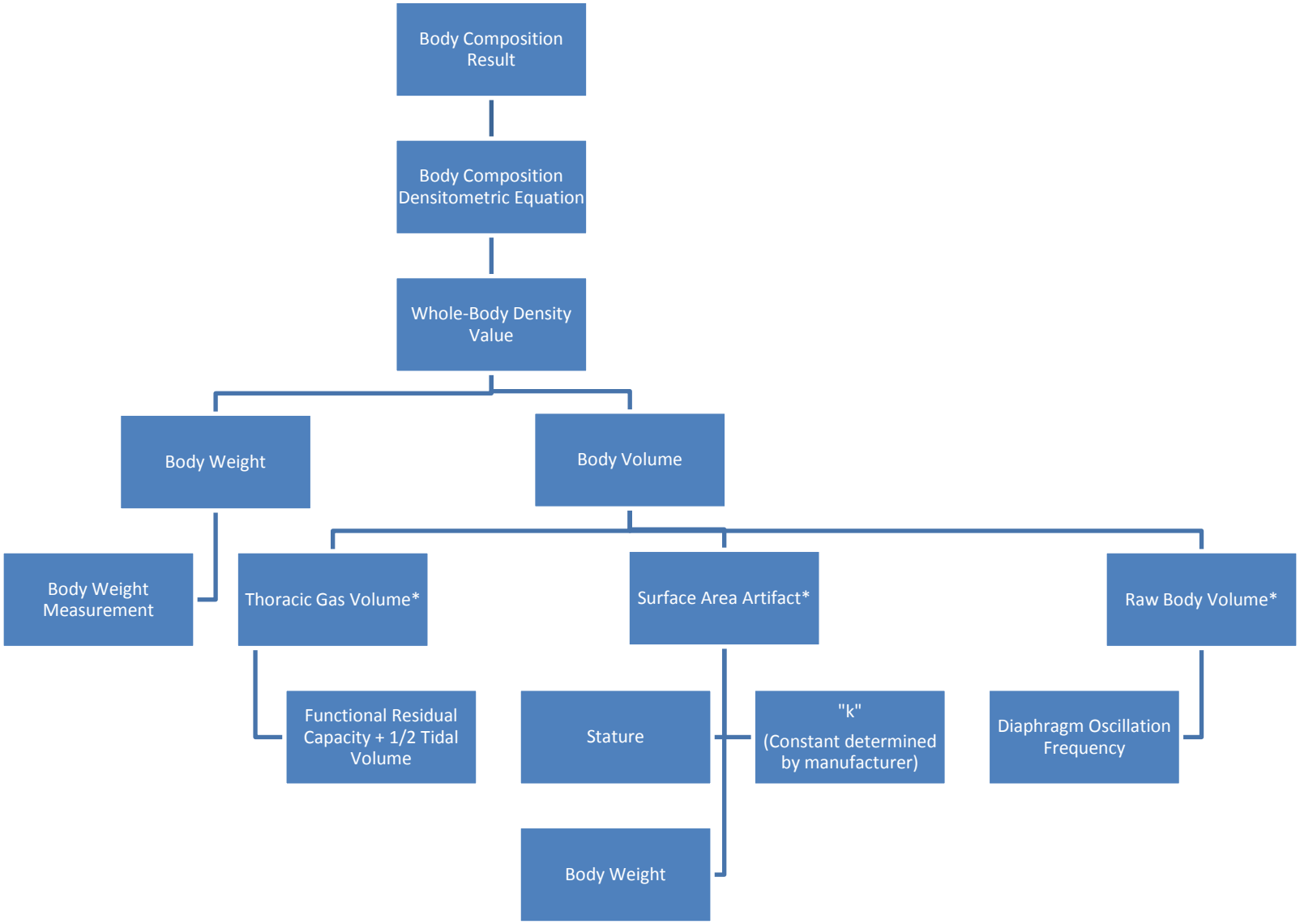


APPENDIX B: Schematic diagram of the BOD POD® system.

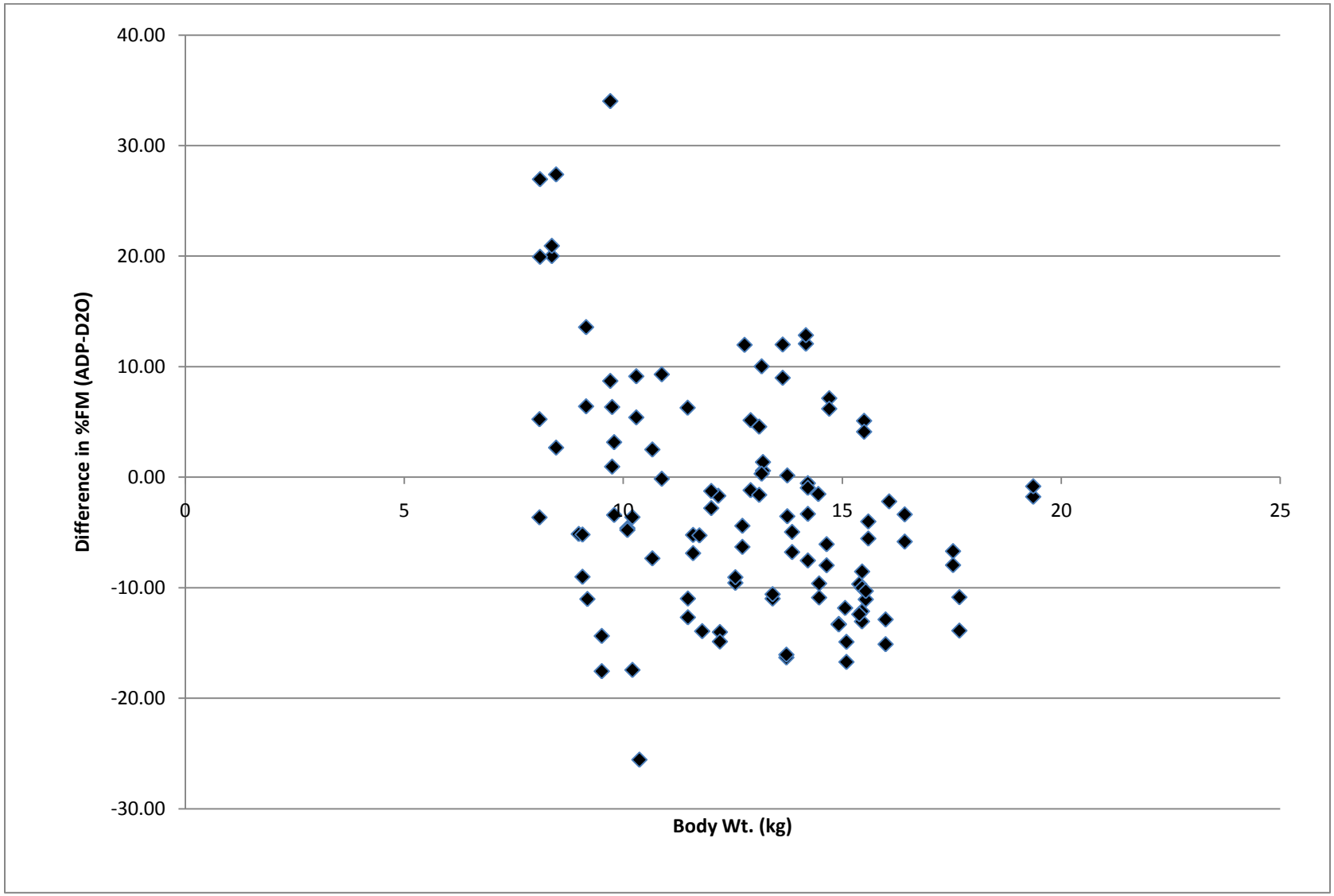


From the manufacturer's website: www.bodpod.com

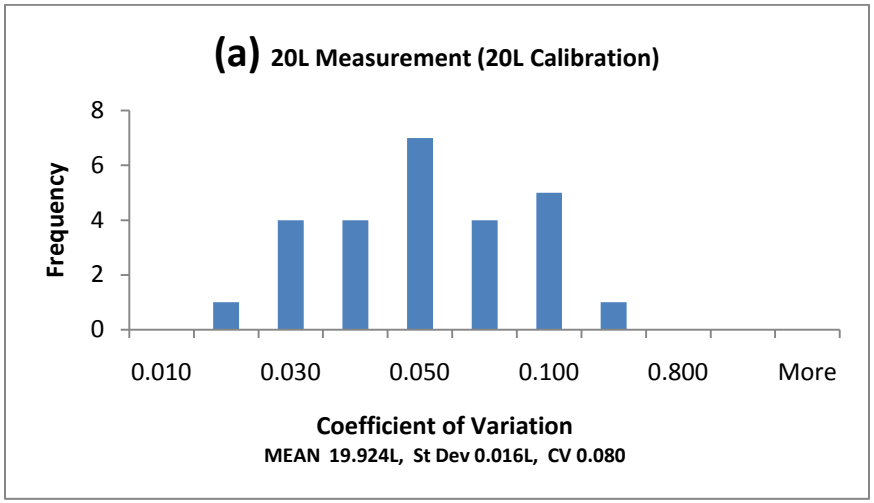
APPENDIX C: Flowchart of variables in ADP Body Composition Assessment.

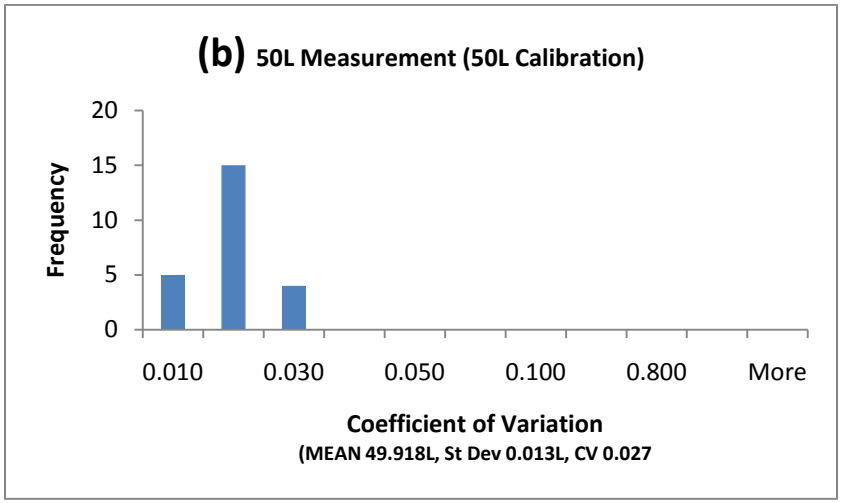


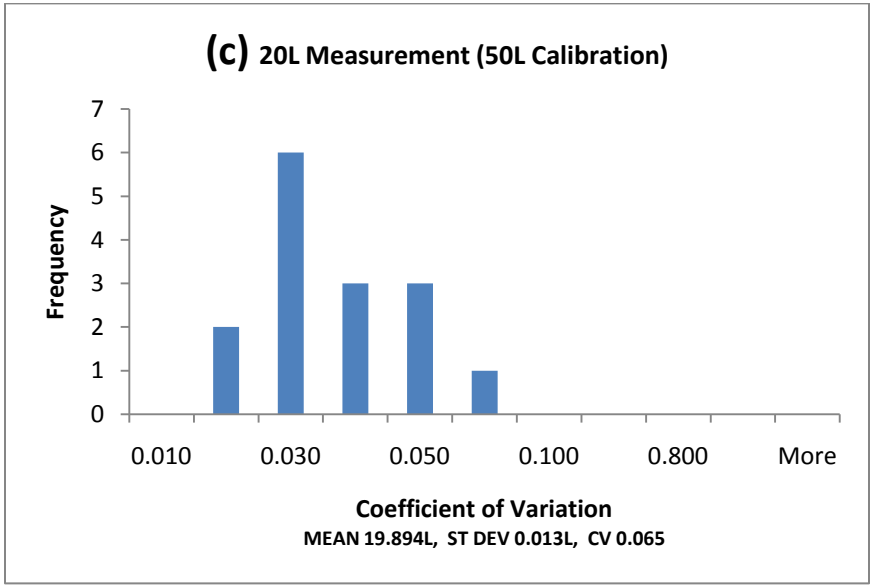
APPENDIX D: Difference in %FM between body composition assessment methods vs. child body weight.

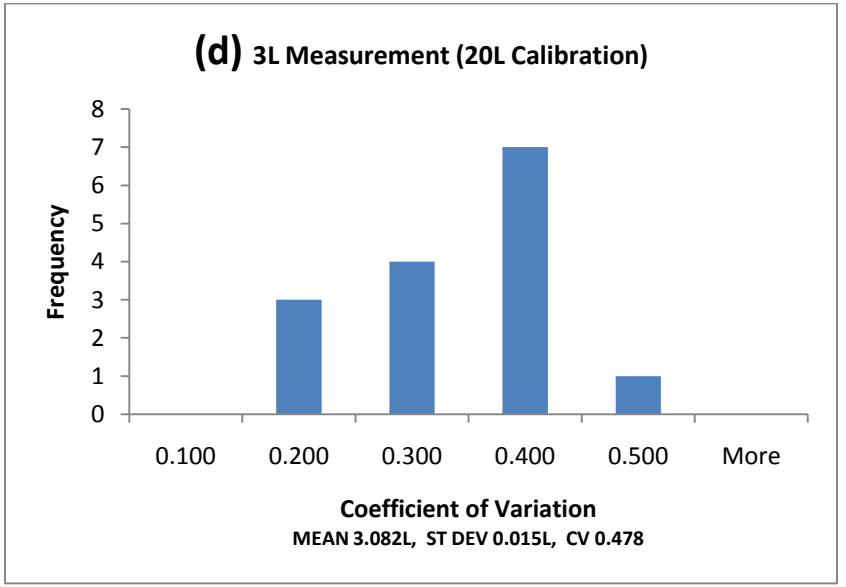


APPENDIX E: Relative frequency histograms of coefficients of variation in precision of measurement of volume cylinders.









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