

**SOMATIC GROWTH, BODY COMPOSITION  
AND ENERGY EXPENDITURE  
IN INFANTS WITH  
BRONCHOPULMONARY DYSPLASIA**

**By**

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**A Thesis**

**Submitted to the School of Graduate Studies**

**in Partial Fulfilment of the Requirements**

**for the Degree**

**Doctor of Philosophy**

**McMaster University**

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**GROWTH AND BODY COMPOSITION  
IN PREMATURE INFANTS WITH BPD**

DOCTOR OF PHILOSOPHY (1997)  
(Medical Sciences)

McMaster University  
Hamilton, Ontario

TITLE: Somatic growth, body composition and energy expenditure in  
infants with bronchopulmonary dysplasia

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NUMBER  
OF PAGES: xv, 225

## ABSTRACT

Extremely low birth weight infants who develop severe chronic lung disease, known as bronchopulmonary dysplasia (BPD), commonly experience long term growth failure. A randomized, blinded study was designed with the objective to enhance growth in this population using aggressive nutritional intervention. Growth and body composition were assessed during four months of formula feeding that was either: 1) enriched in energy, protein and minerals (EF) or 2) enriched in energy (isoenergetic to EF) but with standard concentrations of protein and minerals (SF). We hypothesized that between randomization (37 weeks post-menstrual age) and 3 months corrected age (CA) the EF group would demonstrate a faster rate of growth with greater lean (versus fat) mass deposition, and greater bone mineral accretion. In addition, higher energy expenditure (EE) as determined by the doubly labelled water method (DLW) would accompany the enhanced rate of growth. The measurements continued after the intervention ended to determine if any growth benefits derived from the enhanced nutrition up to 3 mo CA would be sustained to 12 mo CA.

Body composition of the infants was measured with dual energy x-ray absorptiometry (DXA), which was validated against the reference method of chemical analysis. Repeated measures and carcass analysis of infant pigs identified that DXA was accurate and precise in determining bone and lean masses of piglets weighing 6 kg; thus, validating the methodology for use in infants of post-term age.



At the end of the nutrition intervention (3 mo CA) infants in the EF group were longer, with greater absolute amounts of lean and bone mass compared to the SF group. The highest velocity of growth occurred between 37 wk PMA and 1 mo CA. No differences in EE were detected, but the high variability within treatment groups (for undetermined reasons) likely precluded our ability to detect significant results.. Thus, the DLW method requires further validation for use in BPD infants.

Nine months after the intervention ended (12 mo CA), EF and SF groups were similar in weight, length and body composition. When plotted on standard reference growth curves, both groups became significantly more negative in weight-for-length between 3 and 12 mo CA, despite energy and protein intakes deemed adequate by current recommendations for term infants

A longer nutritional intervention study would determine whether growth benefits provided by EF are sustainable, such that catch-up is inducible. Alternatively, catch-up growth may not be possible in infants of such extremely low birth weights under the influence of nutrition alone, and investigations of other factors such as the disease process may be warranted.

## Acknowledgements

I would like to thank Dr. Stephanie Atkinson for providing me with the opportunity to obtain a graduate degree by studying a population of infants with whom I had become enamoured. The inspiration to conduct this study came from watching the struggle which premature infants and their families endure every day. I feel honoured to have been able to participate in this research. I would also like to thank Dr. Atkinson for allowing me to present this research to colleagues at many major scientific meetings. These experiences were invaluable to me.

Thank you to Dr. Jack Sinclair, Dr. David Pengelly, and Dr. Saroj Saigal for generously sharing your wisdom and experience with me throughout the process. It was much appreciated. Also, this research would not have been possible without the assistance of Dr. Bosco Paes, Dr. Jay Shah, and the nursing staff of the NICUs at St. Joseph's and McMaster. Thank you for your help, and for tolerating our frequent disruptions.

My heart felt thanks goes out to my friend Michelle Whelan, whose warm and caring nature was the secret to our successful follow-up of so many infants. My gratitude also goes to Martin Knyf, for technical assistance and for company during more long and boring hours of mass spec work than I care to count.

I have made some lasting friendships during graduate school which were the key to my sanity. No problem ever seemed too great when discussed over beer with friends (or ice cream with Ine). Thank you for your camaraderie.

To my family, my parents and brothers, my nieces and nephew and my in-laws, I am happy to say the end has finally arrived. Thank you so much for your enduring support and for providing that ever-so-important balance in my life. I love you all very much.

I dedicate this thesis to my best friend, Robert F.P. Bertolo. His integrity is a model which I strive to live by, his generosity of spirit is limitless, his wisdom seems too vast for his young age, and his place by my side is the source of my smile.

## List of Abbreviations

<b>APE -</b>	<b>atom percent enrichment</b>
<b>BPD -</b>	<b>bronchopulmonary dysplasia</b>
<b>CA -</b>	<b>corrected age (age adjusted for prematurity)</b>
<b><math>\delta</math> -</b>	<b>the ratio of sample enrichment compared to a reference gas</b>
<b>DLW -</b>	<b>doubly labelled water</b>
<b>DXA -</b>	<b>dual energy x-ray absorptiometry</b>
<b>EF -</b>	<b>enriched formula</b>
<b>EI -</b>	<b>energy intake</b>
<b><math>E_p</math> -</b>	<b>peak enrichment</b>
<b>FFM -</b>	<b>fat free mass</b>
<b>FQ -</b>	<b>food quotient</b>
<b><math>^2\text{H}</math> -</b>	<b><math>^2</math>hydrogen</b>
<b><math>k_{\text{H}}</math>-</b>	<b>water turnover constant determined from <math>^2</math>hydrogen</b>
<b><math>k_{\text{O}}</math>-</b>	<b>water turnover constant determined from <math>^{18}</math>oxygen</b>
<b><math>^{18}\text{O}</math> -</b>	<b><math>^{18}</math>oxygen</b>
<b>MW-</b>	<b>molecular weight</b>
<b>PMA -</b>	<b>post-menstrual age</b>
<b>P-RNI -</b>	<b>Canadian recommended nutrient intakes for premature infants</b>
<b><math>r\text{CO}_2</math>-</b>	<b>rate of carbon dioxide production</b>
<b>RQ-</b>	<b>respiratory quotient</b>
<b>SF -</b>	<b>standard formula</b>
<b>SLAP -</b>	<b>standard light arctic precipitation</b>
<b>SMOW -</b>	<b>standard mean ocean water</b>
<b>SPA -</b>	<b>single photon absorptiometry</b>
<b>TBW -</b>	<b>total body water</b>

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## **CHAPTER 1 RATIONALE, HYPOTHESES AND OBJECTIVES**

### **1.1 Validation of dual energy x-ray absorptiometry for the measurement of body composition in infants**

#### **1.1.1 Rationale for the validation of dual energy x-ray absorptiometry for the assessment of total body bone mineral mass, fat mass and lean tissue mass in growing Infants.**

Quantifying lean, fat and bone mineral masses in response to nutritional interventions in growing infants is critical to the establishment of optimal nutrient prescriptions. A new technique of assessing body composition based on x-ray imaging principles, called dual energy x-ray absorptiometry (DXA), has recently been investigated for use in small infants. Although some investigators have published infant body composition data as determined by DXA (Venkataraman and Winters 1991, Chan 1992), neither the precision nor accuracy of this methodology has been established for small infants. Venkataraman and Winters (1991) reported that coefficients of variation were less than 2.5% using duplicate measures in newborn term infants, but no such work has been done in a group of infants less than 3 kg body weight. The body composition of preterm infants compared to term infants differs not only in absolute body mass, but in the ratio of total body lean:fat which is much greater in the preterm infant (Widdowson 1981). Bone mineral mass is accrued exponentially in the third trimester of gestation; thus, a prematurely born infant may

have only 30% of the mineral mass of a term infant (Greer and McCormick, 1986). The differences in size and body composition of term born compared to preterm infants dictate that the precision of this technology must be established for use with tiny infants. To accurately detect longitudinal changes in measures of body composition, little variability in measures (ie high precision) is essential.

The accuracy of DXA for use in infants has been investigated by in vitro methods only. Specifically, tissue composition of meat and bone phantoms estimated by DXA were compared to tissue weights determined by dissection and bone ashing (Chan 1992). In another study, small human bones which were excised post mortem were subsequently measured by DXA and compared to bone ash ( Braillon et al 1992). These in vitro studies have reported conflicting results. Therefore, a study of repeated whole body measures using a suitable animal model with subsequent comparison to chemical carcass analysis is necessary to demonstrate both the accuracy and precision of this technology in an intact, living animal.

The piglet is a good model to mimic the absolute soft tissue composition of the small infant. The newborn preterm infant is reported to have total body fat of well under 10% (Widdowson 1981). Compared to preterm infants, newborn piglets have similarly low body fat stores, and comparable body water content (Widdowson, 1981; Manners and McCrea, 1963). This makes the infant piglet a good model to establish the sensitivity of DXA-estimates of soft tissue for use in small infants. The newborn

piglet has greater total body ash (3.9%) (Manners and McCrea, 1963) than the preterm infant of similar birth weight (2.6%) (Zeigler et al 1976) or the term born infant (3.2%) (Fomon et al 1982). This difference between newborn piglets and humans must be considered when extrapolating results from validation studies with piglets to the use of DXA for small infants. However, as both piglets and human infants approach 6 kg, the proportion of body weight contributed by ash is very similar (2.9% versus 3.0%, respectively) (Manner and McCrea 1963, Fomon et al 1982). Thus, the piglet is an appropriate model to assess DXA for use in growing preterm infants after term corrected age.

#### **1.1.1.2 Hypothesis (validation of DXA for use in small infants)**

DXA is a precise and accurate method for quantitative estimation of total body fat, lean and bone mineral mass in piglets of 1.6 kg and 6 kg bodyweight.

#### **1.1.1.3 Objectives (validation of DXA for use in small infants)**

1. To assess the precision of DXA by conducting repeated whole body scans on individual piglets and determining the variability between measures.
2. To assess the accuracy of DXA by comparing the DXA-determined total body fat mass, lean mass, bone mineral mass and total body weight of piglets to values determined from carcass chemical analysis.

## **1.2 Randomized controlled nutritional intervention trial to enhance growth in infants with bronchopulmonary dysplasia (BPD).**

### **1.2.1 Rationale for a randomized, controlled nutrition intervention trial to influence somatic growth, body composition and energy expenditure in infants recovering from BPD**

Bronchopulmonary dysplasia (BPD) is a chronic obstructive lung disease which afflicts a high proportion of very low birth weight infants (Saigal and O'Brodovich 1988). It occurs in infants who are treated for respiratory distress syndrome and/or hyaline membrane disease, with the etiology related to tissue damage by oxygen toxicity and barotrauma secondary to mechanical ventilation (Northway 1990). The disease has residual consequences to health long beyond the perinatal period, including frequent re-hospitalization (Sauve and Singhal 1985) and increased incidence of respiratory sequelae (Yu et al 1983).

Growth failure in infants with BPD begins in the perinatal period (Bozynski et al 1990), and may continue into childhood (Robertson et al 1992). Aggressive nutritional intervention during the early stages of respiratory disease is important for maintaining growth at a desirable rate (ie intrauterine growth rate). Optimal nutritional management may also be important to impede the progression of respiratory distress syndrome into BPD. Impaired diaphragm function with quicker fatiguability was induced in rats by acute undernutrition (Lewis and Sieck 1991). If



this effect of undernutrition occurs in mechanically ventilated infants, it may intensify ventilator dependence which would increase the risk for developing BPD (Bancalari and Gerhardt 1986). Adequate antioxidant nutrient availability may be very important when a hyperoxic state is induced to treat respiratory distress syndrome; otherwise oxidative tissue damage could contribute to the development of BPD (Frank 1992). Furthermore, adequate nutrition is essential because the growth of new healthy lung tissue (following trauma from respiratory therapy) is key to the recovery from BPD (Frank 1992). Growth failure in BPD infants at term corrected age (Markestad and Fitzharding 1981, Davidson et al 1990, Brunton and Atkinson 1991) is a gross indicator that nutrient requirements may not have been met with current aggressive nutritional care during the neonatal period. Therefore, nutritional care after hospital discharge is likely an important factor in facilitating recovery from the disease.

Reports of high morbidity in infants with BPD after term corrected age, particularly in slow growing infants, suggests there may be a relationship between inadequate nutrition and recurrent illness (Markestad and Fitzhardinge 1981, Marks et al 1979). Protein/energy malnutrition, sub-optimal vitamin status, and inadequate zinc intake have been associated with impaired immune function (Beisel 1991). Infants with BPD are highly susceptible to respiratory infections prior to and after hospital discharge (Yu et al 1983). However, the relationship between long term nutrient intake, growth and morbidity has not been investigated.

The nutritional care provided after hospital discharge is highly variable, and there are no evidence based guidelines describing the optimal nutritional intervention for infants recovering from growth failure secondary to BPD. Common practice is to add energy to a standard infant formula, in the form of carbohydrate or fat, since elevated energy expenditure has been identified in BPD infants with growth failure (Kurzner et al 1988). An alternative practice is to increase total nutrient density by concentrating infant formula (Reimers et al 1992). While both of the above mentioned nutritional interventions are common, they have not been compared in a randomized controlled trial to determine whether they facilitate different rates or composition of growth. Rapid weight gain can occur in malnourished children with adequate energy intake, but may consist of disproportionately high fat deposition (Hansen-Smith et al 1979, MacLean and Graham 1980). Therefore, assessment of weight gain alone will not identify the dietary requirement of protein and/or minerals which will optimize muscle function and immunocompetence in infants recovering from BPD.

No harmful effects of feeding energy dense formulas to infants with BPD post-term age have been reported. In theory, the provision of excessive energy would result in increased demands for oxygen, elevated energy expenditure and carbon dioxide production; ultimately this could increase the risk for respiratory failure. During the neonatal period, the source of metabolic fuel also has an impact on respiratory gas exchange in infants with BPD. Replacement of carbohydrate with fat

as an energy source results in a lower respiratory quotient and lower carbon dioxide production (de Gamarra 1992, Baumgart et al 1991). The importance of this relationship in infants who are no longer receiving respiratory support after hospital discharge is not known, and will not be addressed in this clinical trial.

The question asked in the randomized controlled trial was whether a balance of nutrients (protein and minerals) provided in an energy dense formula, would support a rate and composition of growth in infants which was superior (ie faster with a greater lean and bone mass) to growth of infants fed a formula with comparable energy, but with protein and mineral concentrations similar to standard term infant formulas.

## **1.2.2 Growth and body composition**

### **1.2.2.1 Hypotheses**

1. Infants recovering from BPD who receive formula with higher concentrations of minerals (particularly calcium, phosphorus and zinc) until 3 months CA, will exhibit greater bone mineral mass over the entire first year of corrected age, when compared to similar infants receiving formula with minerals at concentrations present in standard term formula.
2. Infants recovering from BPD who receive formula containing a higher concentration of protein until 3 months CA will experience a faster rate of growth with a more optimal composition of growth when compared to similar infants receiving a

standard, isoenergetic formula. Specifically, we will observe a greater lean:fat deposition ratio, and improved linear growth.

#### **1.2.2.2 Objectives**

1. To serially measure rate of growth during the nutrition intervention (from randomization to 3 months CA), and at 6 and 12 months CA including: i) rate of weight, length and head circumference growth, and ii) rate of accretion of lean and fat tissue, and bone mineral mass.
2. To measure apparent retention of nitrogen (protein), energy, calcium, phosphorus, and zinc by classic metabolic (mass) balance while in hospital, in order to substantiate the hypothesis that provision of additional nutrients is effective in altering growth and body composition.
3. To compare measurements of growth and body composition between treatment groups, and to compare these measures to reference values of growth and body composition in normal term infants.

#### **1.2.3 Energy expenditure**

##### **1.2.3.1 Hypothesis (energy expenditure in infants with BPD)**

Provision of greater amounts of protein and minerals, but comparable amounts of energy, to infants recovering from BPD, will result in greater energy expended,

secondary to an increased rate of tissue synthesis, when measured prior to hospital discharge, at term and 3 months CA.

**1.2.3.2. Objectives (energy expenditure in infants with BPD)**

1. To quantify energy expenditure by doubly labelled water (DLW) in hospital and post hospital discharge at term and 3 months CA.
2. To measure energy stored (i.e. new tissue synthesis) and the composition of growth while in hospital, using DLW and metabolic (mass) balance methods.
3. To compare serial measures of energy expended, rate of growth, and whole body composition between treatment groups.
4. To compare measures of energy expended at term and 3 months CA to reference (literature) values determined from normal healthy term infants.

## CHAPTER 2 LITERATURE REVIEW

### 2.0 Literature search strategy

Current nutritional management strategies for infants with BPD in hospital and after discharge are based largely on clinician's preference, as opposed to being supported by research (Riemers et al 1992). Repeated attempts to locate nutritional intervention studies of preterm infants with BPD were unrewarded. Since one of our goals was to facilitate improved growth post-hospital discharge, nutrition intervention studies in non-BPD preterm infants were also sought.

Using the Medline data base at the McMaster Health Sciences library, an initial search was conducted for the period 1990 to 1992. The search was conducted as follows:

MeSH terms: Infants, premature, diseases

bronchopulmonary dysplasia

and diet therapy

or rehabilitation

or prevention and control

limited to randomized controlled trials

The second phase of the search encompassed the publication years 1984 to 1990; for this data base, the search term "diet therapy" was not presented as an option, therefore "metabolism" was included. Only four nutrition-related articles were identified for the

whole period from 1984 to 1992. None were intervention studies to improve longitudinal growth. The same search was conducted without the term BPD, and these studies are included in this literature review to rationalize the nutrient composition of the intervention. Other descriptive, and case control studies are included in the literature review. The limitations of these studies must be acknowledged, but they are important to describe the growth failure and to suggest possible etiologies. The literature cited in this chapter is limited to reports published up to and including 1992, at which time the thesis proposal and protocols for the research projects were finalized. Reports which are relevant to this thesis which were published after 1992 are incorporated into the discussion sections of the following chapters which describe the research.

## **2.1 Introduction to bronchopulmonary dysplasia**

BPD is a chronic obstructive airway disease, which is generally unique to prematurely born infants. The injury to the airways occurs secondary to prolonged mechanical ventilation, and while the actual etiology of the tissue damage is unknown, barotrauma and oxygen toxicity have been implicated (Northway, 1990) The true incidence of BPD is difficult to determine, with an increasing number of very low birth weight (VLBW) infants (<1000 g) developing the disease (Northway, 1990). Aggressive medical support leading to improved survival of this population is partially

responsible for the rising incidence (Parker et al, 1992). Saigal and O'Brodovich (1988) reported that 50% of survivors <1000 g birth weight, and virtually all infants <750 g developed BPD. Residual effects of the disease are evident well beyond the initial hospital discharge, and frequent re-hospitalizations in the first two years post-discharge (Yu et al 1983, Sauve and Singhal 1985) have a significant impact on health care resources.

## **2.2 The impact of BPD on nutritional status and growth in premature infants**

Infants born of a shortened gestation are at increased risk for nutrient deficiencies, as the third trimester is a critical period of accretion of nutrient stores (Widdowson et al 1974). An early birth is commonly accompanied by high morbidity during the neonatal period, particularly from respiratory distress syndrome. The perceived metabolic and functional immaturity of extremely low birth weight newborns in terms of nutrient absorption or utilization obstructs the provision of adequate nutritional support during the acute catabolic phase of respiratory distress (Frank and Sosenko, 1988). This can quickly lead to the depletion of sparse nutrient stores (Georgieff et al 1989, Thureen and Hay 1992). Thus, premature infants are highly susceptible to malnutrition early in life. Evidence from animal studies suggests that undernutrition contributes to the progression of respiratory distress into the development of BPD (Frank and Sosenko, 1988). Recovery from BPD should occur



when the rate of repair of injured lung tissue and new tissue growth exceeds the rate of tissue damage caused by mechanical ventilation. Thus, the provision of adequate nutrition in both the early and late phases of the disease is likely essential for recovery.

### **2.2.1 Long term growth of infants with BPD**

Sub-optimal long term growth performance has been documented by a number of studies of infants with BPD (Davidson et al 1990, Yu et al 1983, Vohr et al 1982, Markestad and Fitzhardinge 1981, Sauve and Singhal 1985). Descriptive studies of BPD infants at two years CA collectively report mean body weights falling below the 10th percentile, and mean lengths falling below the 25th percentile on standard growth curves, indicating the body weight is generally more compromised (Markestad and Fitzhardinge 1981, Yu et al 1983, Meisels et al 1986). Davidson et al (1990) prospectively followed a cohort of 30 very low birth weight infants with BPD to 21 months CA (mean birth weight = 936 g) , and compared their growth to 41 similar non-BPD infants (mean birth weight = 1017 g). Mean weight, length and head circumference of the BPD infants remained below the 10th percentile of the Gairdner growth curves (Gairdner and Pearson 1971) at all time points. The final growth centiles of the non-BPD infants were not significantly different from the BPD infants at the end of the study, suggesting that birth weight, rather than the disease state, was

responsible for sub-optimal growth. However, Markestad and Fitzhardinge (1981) observed a positive correlation between improving respiratory function and growth in infants with BPD after hospital discharge, indirectly supporting a relationship between nutritional status, growth and rehabilitation from lung disease.

There are no reports demonstrating complete catch-up growth in very low birth weight infants with BPD. A prospective follow-up study of children who were diagnosed with BPD in infancy was conducted in Alberta (Robertson et al 1992). At eight years of age, children who had BPD as infants were shorter and lighter than term born peer controls. High morbidity has been identified in slow growing infants with BPD post-hospital discharge (Markestad and Fitzhardinge 1981, Marks et al 1979), suggesting that inadequate nutrient intake and morbidity are both impediments to catch-up growth. None of the above studies investigated the influence of nutrient intake on the outcomes of growth or morbidity. Therefore, we planned to conduct a nutrition intervention trial to determine if catch-up growth was an attainable goal in early life, such that infants with BPD ultimately reached their genetic potential for growth.

### **2.2.2 Body composition in infants with BPD**

The body composition and changes in composition during growth of infants with BPD is largely unknown. A group of 30 very low birth weight infants (<1500 g), eight of whom had BPD, were followed to one year CA to assess catch-up growth.

Muscle and fat accretion were also assessed using the imprecise method of mid-arm muscle circumference determined from skin fold measurements (Georgieff et al 1989). The infants did not exhibit catch-up in weight or length growth up to one year, and the presence of BPD was a significant negative influence on catch-up growth. The infants were light for their length at one year CA, with the weight deficit being attributed to fat as opposed to muscle mass.

Nutritional rehabilitation to induce catch-up growth may result in a variable composition of growth. Studies of rapid catch-up growth in infants and children after a period of malnutrition have demonstrated that the desirable weight was often achieved with a disproportionately high deposition of fat tissue (Jackson 1990). In our clinical trial, as we attempted to induce catch-up growth of infants with BPD, we assessed the composition of growth in response to varying nutritional regimens. The study facilitated both qualitative and quantitative assessments of growth.

### **2.2.3. The etiology of growth failure in infants with BPD**

The growth failure identified in infants with BPD is likely multifactorial, and possible etiologies include: 1) medical management in conflict with optimal nutritional management, 2) inadequate nutrient intake or nutrient malabsorption, and 3) altered energy utilization and expenditure. None of these factors has been investigated

in controlled studies, but the existing evidence is discussed in the following sections.

***The impact of medical management on growth and nutrient status***

The malnutrition and growth delay which occur during the acute phase of BPD may be an indirect consequence of necessary therapeutic management. Severe respiratory disease may preclude oral feeding, thus prolonged parenteral nutrition is often necessary. The provision of adequate energy and protein in early life may be limited by metabolic immaturity, with excessive intakes leading to symptoms of metabolic acidosis and hyperglycemia. If energy needs of the newborn infant are not met exogenously, then the meagre stores of glycogen and fat are quickly depleted, and protein catabolism occurs within the first few days of life (Frank 1992). The concentration of calcium and phosphorus necessary to maintain bone mineralization at intrauterine rates cannot be provided parenterally due to solubility limitations (Hanning et al 1991), thus long term parenteral nutrition will result in osteopenia.

Another factor in the development of malnutrition is the potential for drug/nutrient interactions. Diuretics are used chronically and intermittently to treat pulmonary edema which commonly accompanies BPD. This has been demonstrated to cause electrolyte imbalances and urinary mineral losses, further contributing to inadequate intakes (Atkinson et al 1988, Albersheer et al 1989). Fluid restriction is often prescribed along with diuretics, limiting nutrient delivery.

Recently, the corticosteroid dexamethasone has been used chronically to treat

BPD (Cummings et al 1989). The doses used in the treatment of BPD exceed the physiologic secretory rate of hydrocortisone, thus profound metabolic effects may occur such as hyperglycemia with glucosuria (Alkalay et al 1990; Kazzi et al 1990). The common therapeutic response of decreasing the carbohydrate load (Alkalay et al 1990) likely compromises the nutritional status of infants, since energy intake is concomitantly decreased. The gluconeogenic action of glucocorticoids is supported by the catabolism of skeletal muscle, and a decrease in non-hepatic protein synthesis also occurs (Guyton, 1986). Elevated plasma amino acids (Williams and Jones 1992) and evidence of lean tissue catabolism (Brownlee et al 1992) have been identified in preterm infants during treatment with dexamethasone.

The long term implications of corticosteroid therapy on growth and nutritional status in the premature infant was identified by Silverman et al (1951), who described the inhibition of weight gain and linear growth in preterm infants during treatment with tapering doses of ACTH for retrolental fibroplasia. Clinical trials investigating growth in infants treated with chronic dexamethasone have reported conflicting findings with respect to growth. Yeh et al (1990) reported significantly greater weight loss and delayed weight gain in newborn infants treated with dexamethasone compared to controls, but no difference in length or head circumference was detected. Kazzi et al (1990) found no differences between dexamethasone treated and control infants in rates of weight, length or head circumference growth when measured for a

prolonged period from entry into study until hospital discharge. Growth during the seven day treatment period was not reported. However, infants in both groups appeared to have sub-optimal weight gain from entry into study until discharge, since the mean daily increase was only 17 g/d (compared to fetal weight gain of approximately 22 g/d) (Ziegler et al 1976). Differing drug treatment protocols used in these randomized trials limits generalizability of results for all infants treated with steroids, since it has not been determined whether peak dose, cumulative dose or length of treatment has the greatest impact on growth.

It is well known that chronic glucocorticoid therapy in adults and children is a risk factor for the development of osteopenia (Hahn 1990). Infants born of a shortened gestation are at high risk for osteopenia and metabolic bone disease, and fractures due to a deficit in skeletal mineral mass at birth (Koo et al 1988). The additional insult of chronic dexamethasone treatment may have serious implications on bone mineralization. Co et al (1991) used a newborn rat model to demonstrate that 7 days of dexamethasone therapy, at treatment levels similar to or less than infant protocols, impaired bone growth and bone mineralization, compared to placebo treated controls.

To date, no long term studies of growth and skeletal status have been conducted on infants who received exogenous glucocorticoids in early life. Growth delays previously identified in infants with BPD may not be representative of the current state

of the problem, since chronic dexamethasone therapy has only recently become routine clinical practice. Glucocorticoids appear to have significant impact on the rate and composition of growth, and therefore must be controlled for in studies of nutrition and growth in infants with BPD.

### ***Nutrient intake and malabsorption***

Chronic hypoxia and/or pharmacotherapy for the treatment of BPD could alter the nutrient absorption capacity of infants with lung disease. Preliminary evidence suggests that nutrient malabsorption is not implicated in the etiology of growth failure. Yeh et al (1989) measured energy intake and absorption in five infants with BPD and five controls matched for birth weight and gestational age, prior to hospital discharge. Intake was significantly lower in BPD infants than controls, but energy absorption was not impaired. Kurzner et al (1988) conducted a one-time measure of energy and protein intake at six months CA using food records completed by parents of infants with BPD. Infants were divided into two groups (n = 5 per group) based on the presence or absence of growth failure defined as weight and length less than the tenth percentile. No significant differences in energy or protein intakes were noted between groups, but nutrient intakes in both groups were highly variable, thus the study likely did not have the power to demonstrate a difference. No evidence of fat malabsorption was identified from 72 hour fecal fat collections compared to normative standards. However this is an insensitive measure which does not provide a quantitative estimate

of absorption unless simultaneous chemical analysis of intake is conducted. Protein losing enteropathy was ruled out by the measurement of fecal  $\alpha_1$ -antitrypsin, but this is not a measure of protein absorption or utilization (Kurzner et al 1988). This limited evidence is far from conclusive, thus controlled nutrient balance studies for nitrogen, energy and minerals are necessary to unequivocally demonstrate that malabsorption is not implicated in growth failure.

### ***Energy expenditure***

Prolonged, elevated energy expenditure secondary to chronic lung disease could certainly contribute to growth failure in infants with BPD. A small number of studies have demonstrated elevated oxygen consumption prior to term corrected age (Weinstein and Oh, 1981; Yeh et al, 1989; Billeaud et al 1992) and at 6 months corrected age (Kurzner et al, 1988). Based on this evidence, the concept of elevated energy expenditure has been widely accepted as an etiology of growth failure (Southall and Samuels 1990, Bancalari 1986, Reimers et al 1992). Weinstein and Oh (1981) first identified a 25% elevation in oxygen consumption in a small group of five week old infants with BPD compared to controls, which translated into energy expenditures of 242 and 196 kJ/kg·d<sup>-1</sup>, respectively. Tachypnea was an enrollment criterion, and the authors hypothesized that this additional respiratory effort may be responsible for the increased oxygen consumption. Weight gain was similar between groups measured



in grams per day, but absolute body weight was not reported. If the BPD infants were growth delayed and smaller, then proportionately the weight gain might have been greater. Differing rates of growth could partially explain the differences in energy expenditure between groups. A similar study by Yeh et al (1989) also found that BPD infants had greater oxygen consumption and energy expenditure compared to matched controls. It is interesting to note that the control infants in the study by Yeh et al (1989) had a mean energy expenditure (242 kJ/kg·d<sup>-1</sup>) that was similar to the BPD infants in the study by Weinstein and Oh (1981). The BPD infants in the study by Yeh et al (1989) had a mean energy expenditure (318 kJ/kg·d<sup>-1</sup>) that was 30% greater than the BPD infants in the study by Weinstein and Oh (1981). All BPD infants in both studies were receiving supplemental oxygen at the time of study, which can reduce the accuracy of estimated oxygen consumption (Kalhan and Denne 1990, Shortland et al 1992) (see section 2.5).

Kurzner et al. (1988) conducted the only study of oxygen consumption and energy expenditure in BPD infants after hospital discharge, at six months corrected age. They demonstrated that BPD infants who were experiencing growth failure had a mean resting metabolic expenditure which was greater than 30% higher compared to BPD infants without growth failure, or healthy term-born size matched controls. None of the infants were receiving supplemental oxygen. The size-matched control group was included to eliminate differences in body size as a confounding factor. However,

there is currently no evidence that BPD infants with growth failure and healthy infants of the same weight have equal proportions of metabolically active tissue. If the BPD infants were leaner, the difference between groups would not be so extreme. This study did not report longitudinal growth during this study period or beyond, therefore it cannot be determined whether the elevated energy expenditure was due to the disease state, differing body composition, or perhaps a phase of rapid growth during recovery from BPD.

Evidence that "work of breathing" does not account for the additional oxygen consumption in infants with BPD was presented by Kao et al (1988). In a double blind cross-over study, they determined that oxygen consumption in infants with BPD did not decline with improved pulmonary function, which was induced by pharmacotherapy. Therefore, if elevated oxygen consumption and resting energy expenditure do indeed occur for a prolonged period of time, it is not due to the increased respiratory effort that accompanies BPD.

In order to clearly establish whether elevated energy expenditure is a factor contributing to growth failure in BPD infants, we planned to conduct serial measures of energy expenditure and growth in a carefully controlled study. Demonstration of rapid growth would provide a more plausible hypothesis to explain elevated energy expenditure (compared to pulmonary mechanics) in infants recovering from BPD.

## **2.3 Growth in response to nutrient intake: Evidence from healthy preterm infants**

Development of a nutrition intervention to facilitate catch-up growth during recovery from BPD is impeded by the lack of data demonstrating growth responses to nutrient supplementation by this population. Controlled studies of nutrition and growth have been conducted with healthy preterm infants prior to term age, but only a few studies have investigated growth in the period after hospital discharge. The following section will review what is known for healthy preterm infants, in order to establish the rationale for the nutrient composition of a long term intervention for infants with BPD.

### **2.3.1 The accretion of lean and fat masses**

The dietary protein to energy ratios that will facilitate protein accretion similar to intrauterine rates have been established for healthy growing premature infants (Schulze et al 1987, Kashyap et al 1988, Polberger et al 1989). In a blinded study of formula fed premature infants (Schulze et al 1987), rates of weight, length and head circumference gain were superior in the group of infants receiving 114 kcal and 3.6 g protein versus 115 kcal and 2.24 g protein/kg·d<sup>-1</sup>. An even greater weight gain was noted in a third group receiving 149 kcal and 3.6 g protein/kg·d<sup>-1</sup>. The composition of weight gain varied, depending upon the protein:energy ratio consumed. Infants

receiving low protein and with adequate energy deposited the same ratio of protein:fat as the infants receiving the highest energy and protein intake. A protein intake of  $2.24 \text{ g/kg}\cdot\text{d}^{-1}$  did not support protein deposition at a rate equal to fetal accretion rates (Ziegler et al 1976). With lean tissue synthesis limited by protein availability, energy was diverted to fat synthesis. The highest energy intake group (with adequate protein) had protein accretion that was similar to fetal accretion rates, but so did the group with moderate energy intake and adequate protein. The difference in weight gain was attributed to fat. Therefore, additional energy does not appear to enhance the lean tissue synthesis rate, but instead energy is diverted into fat synthesis. Whether this relationship is valid in infants with BPD remains to be determined. However, the danger in providing excessive energy was demonstrated by elevated energy expenditure in the infants receiving  $149 \text{ kcal/kg}\cdot\text{d}^{-1}$  (Schulze et al 1987). The authors speculated that infants with chronic lung disease may not be able to accommodate the increased demand for gas transport, which they attributed to lipogenesis from carbohydrate. Since the protein intake of  $2.24 \text{ g/kg}\cdot\text{d}^{-1}$  was inadequate, and  $3.6 \text{ g/kg}\cdot\text{d}^{-1}$  was sufficient, the question of critical or minimum protein requirement becomes apparent. Polberger et al (1989) investigated the relationship between varying protein/energy intakes and rate of growth in premature infants, using mothers' unfortified or fortified milk. Chemical analysis indicated a wide biological variability in the nutrient composition of the human milk, and provided a range of protein and energy intakes

from which correlations to growth could be made. They determined that rate of weight gain increased with increasing protein intake up to  $2.8 \text{ g/kg}\cdot\text{d}^{-1}$ , and rate of length growth increased with intakes up to  $3.0 \text{ g protein/kg}\cdot\text{d}^{-1}$ . Energy intake of approximately  $120 \text{ kcal/kg}\cdot\text{d}^{-1}$  supported the highest rate of length gain with weight gain similar to intrauterine rates.

This evidence provides a starting point in terms of protein and energy prescription when developing a nutrition intervention for infants recovering from BPD. It has been argued that energy intake should be increased for infants with lung disease (Koops et al 1984), but the discrepancies previously identified between studies in energy expenditure values makes it difficult to estimate requirements. Therefore, a controlled nutrition study which provides  $2.7$  to  $3.0 \text{ g protein/kg}\cdot\text{d}^{-1}$  and at least  $120 \text{ kcal/kg}\cdot\text{d}^{-1}$  as the intervention is justifiable.

The duration of time that aggressive nutritional support is necessary to facilitate catch-up growth is unknown for healthy preterm infants or those with BPD, since no controlled studies have been published. A group of infants with BPD who were measured at term corrected age had a mean body weight that was less than the 10th percentile on the Tanner growth curves (Brunton and Atkinson 1991), and many were similar in size to the reference fetus at 35 weeks gestation or less (Ziegler et al 1976). Since these infants had not yet achieved the body weight of a term born infant, it would seem reasonable to continue with a nutrition prescription that supported growth

at a rate similar to fetal rates, at least until body size and composition are comparable to healthy infants born at term.

### **2.3.2 Accretion of bone mass in response to mineral intake**

For infants recovering from chronic lung disease, who incur large skeletal deficits due to prematurity and complications of medical management, post-hospital discharge may be the critical period for restoration of mineral deficits.

Longitudinal studies of infants born prematurely have provided evidence for the persistence of osteopenia prior to and beyond 40 weeks post conceptional age. Greer and McCormick (1986) prospectively followed the radial bone mineral content of a group of 38 very low birth weight infants (<1300 g) infants; 15 of whom developed BPD. By term, all infants fell well below the intrauterine bone mineral accretion curve. The infants with BPD consistently had a mean distal one third radial bone mineral content lower than non-BPD infants. The difference was not significant, however the small sample may have resulted in a Type II error (i.e. concluding that the null hypothesis is true, when in fact it is a false negative error). A further study by the same investigators followed premature infants with and without BPD, matched for age and birth weight (Greer and McCormick, 1987). Both groups of infants were similarly growth delayed compared to reference standards, and once again, all infants fell below normal distal radial bone mineral mass growth curves for the first year of

life. The authors speculated that factors associated with extreme prematurity other than BPD were associated with hypomineralization. No effort to relate nutrient intake to bone mineral content was attempted in this study, nor was there any attempt to investigate whether the bone mineral content was normal relative to body weight or body length. The only other study which has reported bone mineral content in infants with BPD used retrospective data of mid-ulnar measures in infants with BPD compared to control infants matched for birth weight and gestational age at birth (Ryan et al 1987). No differences in bone mineral content were detected between groups from a one-time measure taken near 40 weeks post-menstrual age. Importantly, all infants were receiving an average daily calcium intake well below values recommended to maintain mineral accretion at fetal rates (ESPGAN 1987, AAP 1985).

No randomized controlled trials of mineral supplementation of infants with BPD who are post-term age have been conducted to determine whether catch-up mineralization is possible, however studies have been done in healthy premature infants (Abrams et al 1988, Venkataraman et al 1990, Chan and Mileur 1985). Provision of a mineral fortified formula for four months post-hospital discharge significantly improved bone mineral mass as measured at the distal radial site in premature infants (Venkataraman 1990). Two studies reported significantly lower radial bone mineral content in breastfed versus standard formula fed infants after hospital charge at approximately 16 weeks CA (Abrams et al 1988, Chan and Mileur

1985). Therefore even the small advantage in mineral content of standard formula over breast milk resulted in significant improvement in bone mass.

The greatest period of fetal mineral accretion and bone mineralization is between 36 and 40 weeks gestation (Ziegler et al 1976, Greer and McCormick 1986). This corresponds with the time when premature infants are discharged from hospital on breast milk or standard infant formulas, both relatively low in minerals. Evidence for a period of rapid accretion in preterm infants around term age has been provided by descriptive studies of healthy preterm infants and term born controls. Formula-fed compared to breast-fed preterm infants had higher mineral intakes and had significantly greater bone mineral content by 25 weeks post-natal age, with the difference in the rate of accretion greatest between 10 and 16 weeks post-natal age (39 to 45 weeks post-menstrual age) (Abrams et al 1988). Comparison of preterm infants to term born infants near 40 weeks post-menstrual age identified significantly lower mid-ulnar bone mineral content in preterm infants; but a second measurement after 65 weeks post-menstrual age indicated that the term and preterm infants were no longer different (Horseman et al 1989). Similar "catch-up" mineralization was observed between 46 and 71 weeks post-menstrual age despite the use of standard term infant formulas (Congdon et al 1990). Mineral intake was not measured, but the authors hypothesized that the preterm infants consumed much greater volumes of formula to account for the differing accretion rates. The remarkable recovery from hypo-



mineralization with complete resolution prior to one year of age is in contrast to the findings of Greer and McCormick (1987) in their non-BPD control infants, who showed no evidence of catch-up. This demonstrates the need for well controlled longitudinal studies.

The only whole body bone mineral data in infants with BPD which was reported at the time of this review were published in abstract form (Venkataraman et al 1991). It is important to note these data were acquired using dual energy x-ray absorptiometry, which had not been appropriately validated for use in small infants at the time of the study (see section 2.4.3). However, since peripheral bone sites such as the radius or ulna may not accurately represent whole body skeletal status, these data warrant mentioning. Infants with BPD were matched by body weight to a group of term born control infants. The BPD infants were more than 10 weeks post-term, thus were growth delayed. Whole body bone mineral density was significantly lower in the infants with BPD, as was lower limb bone mineral content, despite similar body size between groups. These preliminary data suggest that composition of growth of BPD infants may be altered, and assessment of whole body skeletal status during a controlled trial of high versus standard mineral concentrations post-hospital discharge is necessary. This will elucidate whether catch-up mineralization is attainable in BPD infants, and if recovery is spontaneous or facilitated by mineral supplementation.

### 2.3.3 Zinc status and growth

In fetal growth, the accumulation of zinc occurs rapidly during the last trimester of gestation (Widdowson, 1974), a period of time which is unavailable to infants born prematurely. Inadequate zinc availability may impact growth, metabolism, function and maintenance of virtually all human cells (reviewed by Linder 1991). It is essential to the processes of cellular growth at virtually all stages. At a basic level, zinc acts as a co-factor for DNA and RNA polymerases, thus is essential for cell division and protein synthesis. Furthermore, zinc may control gene transcription by altering the binding capacity of transcription factors via the zinc finger elements in the proteins. When zinc is bound, the ensuing structural changes enhance binding of transcription factors to DNA (Linder 1991).

A major deposition site for zinc is in bone, but unlike other major bone minerals, zinc is not readily mobilized during periods of insufficient dietary intake (Linder 1991). Impaired skeletal growth occurs in zinc deficient animals and humans, which may be due to abnormalities in epiphyseal cartilage formation (Cousins and Hempe 1990). Sub-optimal zinc status, short stature and severely retarded bone age has been described in Middle Eastern adolescents and young adults who were consuming diets high in phytate, which impaired zinc absorption (Hurley 1980). The association between "mild" zinc deficiency (ie no obvious clinical signs of zinc deficiency) and impaired linear growth in otherwise "healthy" infants and children has

been identified in middle class populations (Hambidge et al 1972, Gibson et al 1989). Therefore, while overt zinc deficiency is rare, the impact of mild zinc deficiency on bone and linear growth in prematurely born infants with high dietary requirements must be established.

The availability of dietary zinc provided to preterm infants may be limited. Recently, Atkinson et al (1990) determined that preterm infants fed human milk or preterm formula remained in negative zinc balance up to 35 days postnatal age, such that endogenous losses of zinc exceeded dietary zinc retention. This occurred despite the high bioavailability of zinc from human milk (Sandstrom et al 1983) or the high concentration in preterm formulas. Others have reported negative zinc balances in preterm infants (Dauncey et al 1977). In contrast, Erhrenkranz et al (1989) reported positive zinc retention in formula-fed three week old premature infants. The mean ( $\pm$  SD) retention was  $12 \pm 30\%$ , therefore many infants in that study were actually in negative zinc balance. Thus, the deficit incurred by the premature birth, and prolonged negative zinc balance may cause premature infants to be at risk for zinc deficiency in early neonatal life and beyond. While overt signs of zinc deficiency are rare, Friel et al (1985) identified that zinc intake at 3 months corrected age was the only significant dietary factor in predicting length growth velocity in a study of term and preterm infants. Assessment of hair zinc concentration at 6 mo corrected age indicated that 37% of the preterm infants had levels indicative of zinc deficiency,

compared to only 7% of full term infants (Friel et al 1984). This occurred even though zinc intakes were similar (on a per kilogram basis) between groups, indicating that preterm infants may have additional needs.

Evidence of improved growth in response to zinc supplementation was supplied by a randomized, blinded placebo trial with infants (eight to 26 months) who were declining on weight growth centiles (Walravens et al 1989). Supplementation with zinc for 6 months resulted in a significant increase in weight z-scores, but no effect on length was noted.

The availability of dietary zinc may also alter the composition of new tissue synthesis. Inadequate zinc availability will limit the growth of lean mass (Golden and Golden 1981) despite adequate protein and energy intake, likely because zinc serves many key functions in protein synthetic processes. Greater adiposity, and a greater cost of tissue synthesis was observed in Jamaican infants, aged eight to 17 mo, recovering from malnutrition who were not supplemented with zinc, compare to infants that were zinc supplemented (Golden and Golden 1981). Other investigators have determined that after recovery from malnutrition, despite attaining normal weight-for-height indices; proportionately less growth of lean tissue has been observed compared to normal standards (Hansen-Smith et al, 1979; MacLean and Graham, 1980). Therefore, zinc supplementation may be critical for "normal" recovery.

It is possible that the ability to absorb zinc occurs with post-natal maturation.

Early zinc supplementation may not guarantee an adequate zinc supply to facilitate rapid growth. Infants with BPD who experience severe acute illness in early life and extreme prematurity may be compromised in zinc status. Supplemental zinc post-term age must be investigated to determine whether zinc deficiency contributes to prolonged growth failure and osteopenia.

## **2.4 Methods of assessment of body composition and longitudinal growth**

### **2.4.1 Nutrient accretion by mass balance and energy balance techniques**

The mass balance technique provides an estimate of the total body net change in nitrogen or minerals, thus indirectly providing an assessment of protein and mineral accretion. When nitrogen retention, energy intake, and energy expended are measured simultaneously, then energy "retained" or stored can be calculated, and the composition of growth (ie. protein and fat mass accretion) can be estimated. The caloric equivalent of the stored protein (determined from nitrogen retention) is subtracted from the known quantity of energy retained (ie energy intake minus energy expended); thus, assuming that the storage of carbohydrate is negligible, the remaining energy is stored as fat (Whyte and Bayley 1991). The mass balance technique has been a useful method for assessing the growth response of premature

infants to varying formula compositions (Atkinson et al 1981, Whyte et al 1983, Schulze et al 1987, Putet et al, 1987), and has provided insight into nutrient needs of premature infants.

Limitations to the balance method are evident in that it is a short term measure of growth and nutrient accretion, therefore may not reflect long term growth responses to a nutrient intervention. Energy retained is divided as protein and fat tissue, while the small amount stored as carbohydrate cannot be determined, thus is disregarded. Within the balance technique itself, there are multiple opportunities for errors, with over-estimation of nutrient retention likely the most predominant problem (Fomon and Owen 1962 , Kopple 1987).

The use of this method in premature infants is likely more successful than in adults or children, since they are in a controlled environment, and have fewer sources of unmeasured losses. More importantly, these infants are growing more rapidly than at any other time during the life cycle (Zeigler et al 1976), and the percentage of nutrient intake that is retained is very high compared to an adult who is close to nutrient balance. Therefore, small errors in the collection procedure do not necessary lead to large errors in the calculated retention, as has been identified in adults (Kopple 1987).

The ideal mass balance technique has not been established. Some investigators have used markers (carmine red or charcoal) fed to infants at the beginning and the

end of the balance period to demarcate stool collection (Fomon et al 1958, Shenai 1980), while others have not (Atkinson et al 1981). The inclusion of the stool containing the second marker may result in overestimation of stool excreted, and an underestimation in nutrient retention (Wirth et al 1990). Collection protocols reported in the literature range from 72 to 96 hours (Fomon et al 1958, Shenai 1980, Atkinson et al 1981, Schanler et al 1988). Longer duration balance periods and stool markers have not been established as more accurate, therefore a 72 hour period was chosen for the clinical study protocol, with a seven day acclimation period prior to the initiation, to be less disruptive to the infants and the bedside care givers.

#### **2.4.2 Single photon absorptiometry as a determinant of bone mineral status**

Single photon absorptiometry is a densitometric technique which provides an indirect assessment of the mineral content of long bones. A collimated (3 mm) photon beam is emitted from a low energy source (<sup>125</sup>Iodine) and transmitted through the bone and soft tissue to be measured by a scintillation detector which moves simultaneously with the photon source. The attenuation of the beam is translated into bone mineral mass. The mineral mass is expressed in milligrams per one centimetre slice, or length of bone, and is given as mg/cm (Barden and Masess, 1988). Bone sites which have been used in studies with infants include the radius, mid-humerus, ulna and femur. The most common site is the distal one third radius (Steichen 1989). The radius at

this point is cylindrical, with a relatively stable bone mineral content over a length of a few centimetres, thus measurement at this site reduces errors due to repositioning (Steichen et al 1988). Therefore the bone shape and the availability of comparative data reported in the literature make the one third distal radius the site of choice for longitudinal assessment of bone in a nutrition intervention trial .

Two separate groups investigated the accuracy of single photon absorptiometry by scanning small infant and animal bones, then ashing the bones to estimate mineral content. Both groups found a high correlation coefficient for the weight of bone ash versus measured bone mineral content (0.99 and 0.98) (Greer et al 1983, Abrams et al 1988). The reproducibility of the measurement has been shown to be excellent. Greer et al (1983) measured 114 preterm infants of varying gestational ages, and found the coefficient of variation for four to six scans (without repositioning the arm) was 3.9%. Abrams et al (1988a) also reported good precision, with a CV less than 4% with and without repositioning the arm.

The single photon absorptiometer is a portable device that can be used at the bedside in a intensive care setting; thus, with proven accuracy and precision, it is a suitable tool for the longitudinal assessment of bone status, beginning in early life.

A major limitation to the use of single photon absorptiometry for the assessment of skeletal status is the fact that it reflects the bone mineralization at a site which is predominantly cortical bone. This may not be indicative of the osteopenia in



metabolically active trabecular bone, which would be affected earlier than cortical bone in metabolic bone disease (ACP 1984). One could extrapolate from this and surmise that for the purpose of estimating bone mineralization in a nutrition intervention trial, any differences detected between treatment groups in radial bone mineral content may actually reflect profound differences in whole body bone mineral content.

### **2.4.3 Dual energy x-ray absorptiometry to determine whole body composition**

Currently, no indirect method of measuring whole body bone and soft tissue composition has been validated for use in infants. While techniques exist to measure individual compartments such as bone, water or lean tissue, most of these have limitations when applied to small infants.

Single photon absorptiometry would likely become obsolete if a safe, accurate and reproducible technique for measuring whole body bone mineral content of infants were available. In adults, dual photon absorptiometry which uses a radionuclide source ( $^{153}\text{Gadolinium}$ ) has been used to determine skeletal status. However, poor sensitivity, a prohibitively long scanning time (Ellis 1989) and excessive radiation exposure (Russell-Aulet et al 1991) are all factors which limit its use in infants.

For obvious reasons, the most widely used method of measuring soft tissue composition, namely hydrodensitometry or underwater weighing, is not appropriate for

use in infants. An alternative method of determining lean tissue mass is the measurement of total body potassium using a whole body counter to estimate the naturally occurring radioisotope  $^{40}\text{K}$ . The assumptions of this method are that a constant fraction of potassium is  $^{40}\text{K}$ , and that the concentration of potassium in lean tissue is equal to a known factor, which may not be the case in rapidly growing infants with bronchopulmonary dysplasia. The method has been further criticized because the ratio of signal to background noise is very high, resulting in a lack of precision (Forbes 1989). A validation study of this method could achieve a precision of only 12% in a 2 kg infant, and 20% in a 1 kg infant, with counting times of greater than 30 minutes (Spady et al 1986). Ellis et al (1992) recently demonstrated improved precision of 5% in a 1 kg infant, but it still required 30 minutes of counting. To obtain optimal results, Ellis et al (1992) use a whole body counter designed specifically for very low birth weight infants, and another for larger preterm infants. Therefore the use of this method for longitudinal growth studies is limited to centres with multiple whole body counters.

Total body electrical conductivity (TOBEC) has recently been investigated for use in infants. This method also measures fat-free mass based on the principle that electrolyte containing water will readily conduct an electrical current (Fiorotto 1989). Estimates of fat free mass and body fat by TOBEC in healthy, term born infants at two, four, eight and 12 weeks of age (Fiorotto et al 1987) were similar to the values

for the reference infant reported by Fomon et al (1982). It must be noted that the data for the reference infant were derived from total body potassium, combined with measuring dilution space, and skin fold thickness, rather than from the gold standard of chemical analysis. TOBEC also requires an instrument designed specifically for small infants, and does not measure bone or fat mass directly.

While the indirect methods previously discussed are not validated or easily applied to infants, it remains an important goal to be able to assess the influence of nutrient intake on lean, fat and bone masses in growing infants, particularly low birth weight infants. Recently, a new whole body absorptiometer using dual energy x-ray absorptiometry (DXA) has been investigated for use in small infants (Brailon et al 1992, Chan 1992). Instead of using a radionuclide source as previous generations of densitometers, the DXA system uses an x-ray tube to generate a high and low energy photon beam, which originate below the subject, and are detected by the counter in an arm which moves simultaneously with the beam (Mazess et al 1990). The beam is transmitted in a rectilinear raster throughout the entire body. The relative attenuation of the two photon beams can be related to the mass of either component in a two component system. Consequently, if the body is considered to consist of bone mineral and soft tissue, then total body bone mineral mass can be measured. A second dual photon analysis is performed at sites which contain only soft tissue, and soft tissue mass is divided into lean tissue mass and fat mass.

In vitro validation studies have been conducted to determine the appropriateness of DXA for use in small infants, and have reported conflicting results. High correlation between DXA-estimated bone mineral content and bone ash has been reported in small human (Braillon et al 1992) and animal bones (Chan 1992). The reports of the error of DXA at estimating bone mineral content range from 7% (over-estimated), measured in femurs after excision from still born preterm infants (Braillon et al 1992), to 50% (underestimated) measured in bones prior to dissection from chicken (Chan 1992). The discrepancy in results may be due to instrumentation, however, it may also be due to the differing presentation of bone, such that it is more difficult to accurately measure bones in situ compared to excised.

Validation in human infants has been limited to repeated measurements to attain an estimate of precision. In infants less than 1 year of age, inter-measurement coefficients of variation were 2.5% for bone mineral, and less than 1% for lean and fat masses (Venkataraman and Winters 1991). The number of measures used to calculate precision was not reported.

Standardization of the reporting of body composition measures by DXA will prove to be an important issue for the assessment of growth in infants and children. As the technology is adopted by researchers, studies will soon become available with both normative reference data, as well as results of intervention trials. The interpretation of "normal" will vary, depending upon the method used to present the

data. DXA provides a measure of whole body mineral content, but simultaneously measures whole body bone area. These two values are used to calculate a bone mineral density (BMD). However, this does not truly represent density, as only bone area is measured, and not bone volume. Since bone volume changes with growth, and not necessarily in direct proportion with BMC, the use of BMD does not "normalize" the data for differences in body size (Salle and Glorieux 1993, Prentice et.al 1994). For this reason, total body BMC is the most appropriate way to report bone outcomes; expressing BMC as a function of body length or body weight would serve to facilitate comparisons within growing individuals or groups of infants of different body size.

Despite the lack of validation, DXA has been used to measure body composition of infants. Venkataraman and Ahluwalia (1992) reported body composition data in 28 full term new born infants, and compared the results to body composition data by chemical analysis in the literature. The infants measured by DXA appeared to be similar in body composition to reference data, with the exception of a higher body fat content. The authors suggest that this may be a consequence of better pre-natal nutrition, and that the infant cadavers in the chemical analyses study were likely compromised. While these results are encouraging, validation studies are necessary to determine whether the DXA values were accurate, or whether the high fat values and under-estimation of bone mineral content are a consequence of the measurement technique. For that reason, we undertook a study of repeated in vivo measures of

whole body composition of a suitable animal model followed by chemical carcass analysis.

#### **2.4.4 Reference standards for the assessment of somatic growth**

Assessment of the adequacy of growth implies that there must be a reference standard to which growth can be compared. Numerous sets of reference or "normative" data for infants exist (Tanner 1966, Hamill 1979, Nutrition Canada 1980, Gairdner and Pearson 1971), but to assess longitudinal growth of premature infants born in the Hamilton-Wentworth region, none are ideal. Normative growth data should be collected either longitudinally or cross-sectionally from a large group of healthy infants who represent the genetic and cultural mix of a specific geographical area. Tanner (1986) suggested that it is inappropriate to compare the longitudinal growth of children to cross-sectional reference curves after nine years of age, because differences in the timing of pubertal growth spurt can alter the interpretation of normal growth. In infants and young children, however, it is appropriate since the error introduced is negligible. Therefore, growth curves derived from cross-sectional data may adequately represent longitudinal growth of a group of infants or young children, but it is important to acknowledge that the actual growth pattern of an individual may not be accurately represented (Tanner 1986).

One set of reference growth data is by Tanner et al (1966), which was based on

a longitudinal sample of 160 children in the UK followed from birth to five years of age. Another set commonly used for preterm infants are the Gairdner growth curves (Gairdner and Pearson 1971). These curves start at 28 weeks post-menstrual age, and are a composite of growth data including the Babson (1970) size-at-birth data for infants of 28 - 40 weeks gestation, and the longitudinal data of Tanner et al (1966) from 40 weeks to 2 years. Thus, this curve facilitates the use of one reference standard prior to, and after term age. More commonly used are the American National Center for Health Statistics (NCHS) reference growth data (Hamill et al 1979). The data were collected cross-sectionally between the early 1960's and mid 1970's, and most infants included were from middle class families, and fed proprietary formula. The World Health Organization has adopted the NCHS reference data as an international standard (Gibson 1990). Comparisons between growth monitoring studies among varying populations, including prematurely born infants, are facilitated with a common reference growth standard.

The ideal growth curves for use with prematurely born infants, particularly with BPD, currently have not been established. This may be due to the lack of data regarding long term growth potential of premature infants under optimal conditions, including nutritional management, and infant stimulation for development. Reasonable expectations for catch-up growth in extremely low birth infants have not been established, and remain a controversial issue (Casey et al 1991, Georgieff et al 1989,

Hack et al 1984, Kimble et al 1984).

Recently, Casey et al (1991) described the longitudinal growth of nearly 1000 infants, categorized by birth weight, from 40 weeks post-conceptual age to 36 months corrected age. The infants were monitored through the multi-centred Infant Health and Development Program (IHDP), and no intervention was provided. There was very little evidence for catch-up growth identified for any birth weight category. The authors state that these descriptive data should be used to develop reference growth standards to monitor growth of low birth weight infants. This may be the most comprehensive set of descriptive follow-up growth data, however, the infants studied were predominantly black and hispanic, thus limiting the generalizability. Furthermore, no data were provided to confirm that the infants in the study received adequate nutrition to achieve their full growth potential, which is a prerequisite for establishing a reference population (IUNS 1972). The use of the NCHS growth reference standards for the assessment of longitudinal growth of infants with BPD may not be ideal, but unlike the IHDP curves derived from preterm infants, they likely are closer to representing the genetic potential for growth. For this reason the NCHS data are the most appropriate currently available reference growth standard for use in a nutrition intervention study with an objective of establishing catch-up growth.



## **2.5 The measurement of energy expenditure in infants with bronchopulmonary dysplasia**

It remains important to establish whether BPD infants have elevated energy expenditure prior to and after hospital discharge, to help elucidate the etiology of growth failure. Studies which have identified increased oxygen consumption in BPD infants on supplemental oxygen must be interpreted with caution (Kalhan and Denne 1990). Accurate energy expenditure measures rely upon a steady concentration of ambient oxygen. In low birth weight infants with small respiratory volumes, the change in concentration of oxygen entering and leaving the infant hood may be as low as 0.05% (Shortland et al 1992). Theoretically, a deviation in the concentration of delivered oxygen as small as 0.05% could translate into an error in oxygen consumption of 100%. Calibration of such a system is essential, but can be dangerous in conditions with oxygen concentrations greater than room air, since it requires measuring the rate of combustion of ethanol or butane (Kalhan and Denne 1990, Shortland et al 1992). This potential error may partially account for the differences in results from the studies described.

Recently, energy expenditure measurement using doubly labelled water (DLW) has been validated for use in term and preterm infants (Roberts et al 1986, Jones et al 1987, Wong et al 1990, Westerterp et al 1991). While called "doubly labelled water", this method actually uses two distinct types of isotopically labelled water,  $^2\text{H}_2\text{O}$  and

$\text{H}_2^{18}\text{O}$ . The labelled waters are introduced into the body water pool, either orally or intravenously. The  $^2\text{H}$  from the  $^2\text{H}_2\text{O}$  equilibrates with only the body water pool, but  $^{18}\text{O}$  from the  $\text{H}_2^{18}\text{O}$  equilibrates with both body water and bicarbonate pools, through the action of carbonic anhydrase. Periodic sampling of the body water pool allows for detection of a change in the ratio of  $^2\text{H}$  to  $^{18}\text{O}$  in the body water pool over time. The difference in the decline in enrichment between the two isotopes provides an estimate of  $\text{CO}_2$  production. Total energy expended can be calculated from the estimate of  $\text{CO}_2$  produced (Lifson et al 1955).

Estimates of  $\text{CO}_2$  production by the DLW method and by indirect calorimetry were compared in five healthy preterm infants as part of a validation study (Roberts et al 1986). Indirect calorimetry was conducted almost continuously throughout the five day DLW period. The difference between methods was less than 1.5%. Westerterp et al (1991) compared indirect calorimetry conducted over eight hours to DLW conducted over a five day period. To estimate the rate of  $\text{CO}_2$  production using the DLW method, they applied the equations established by Jones et al (1987) derived from a study with post-surgical newborn infants. No significant difference was found between methods. DLW may be more representative of true energy expenditure than respiratory gas exchange, since it provides a mean estimation of total energy expenditure derived from a study period which is days long, rather than hours long.

Estimating energy expended by the DLW method without simultaneously

measuring respiratory gas exchange requires the use of an estimate of the respiratory quotient (RQ), to determine the caloric equivalent of the CO<sub>2</sub>. For infants who are on a constant source of nutrition, the RQ can be easily estimated by calculating a food quotient (FQ) from dietary intake. Two validation studies have investigated the impact of this potential error in infants, and found it to be insignificant (Jones et al 1987, Westerterp et al 1991). According to Black et al (1986) this source of error should be less than 2%, even with minimal dietary intake data.

DLW has the potential to be extremely valuable in the determination of energy expenditure of free-living infants and children (Schoeller et al 1986), since only periodic urine sampling is required. The advantages of this method over respiratory gas exchange for use with BPD infants include no restriction of activity, an estimation of energy expenditure that is averaged over days, and no introduction of errors due to supplemental oxygen use. It is the ideal to method to address the question of prolonged elevated energy expenditure in infants with BPD.

## **2.6 Summary**

Infants who survive with BPD beyond hospital discharge experience prolonged growth failure, with greater morbidity and a high frequency of re-hospitalization. While nutritional factors have been implicated in the etiology and pathogenesis of BPD, no controlled studies have been conducted to determine the potential of

nutritional interventions for ameliorating the sequelae of the disease. Furthermore, descriptive studies of sub-optimal growth and bone mineralization may not reflect the status of infants who currently have BPD, since advances in medical care have resulted in the survival of a more immature group of infants. Since no evidence based guidelines for optimal nutritional care of BPD infants exist, it is essential that randomized trials are carried out to delineate the nutrient needs of this vulnerable population. The research presented in this thesis addresses questions regarding etiological factors of growth failure, including the adequacy of nutrient intake, nutrient requirements of BPD compared to non-BPD infants, and the presence of prolonged elevated metabolic demands.

The survival of such extreme prematurity is likely not without cost, especially considering the current use of long term glucocorticoid therapy to treat BPD which may further compromise growth. Possible consequences include disproportionate growth of lean and fat masses, stunting and/or osteopenia. The following chapters discuss the composition of growth of infants with BPD, and the influence of nutritional intervention on the rate and quality of growth. In order to accomplish the body composition measures, the task of validating the new DXA imaging technology for use in infants is the first issue addressed in this thesis.

**VALIDATION OF DUAL ENERGY X-RAY  
ABSORPTIOMETRY TO MEASURE  
BODY COMPOSITION IN SMALL INFANTS**

## **CHAPTER 3**

# **VALIDATION OF DUAL ENERGY X-RAY ABSORPTIOMETRY FOR USE IN CLINICAL STUDIES WITH INFANTS**

**Validation and application of dual energy x-ray absorptiometry (DXA) to measure bone mass and body composition in small infants.**

Janet A. Brunton, B.A.Sc., Henry S. Bayley, Ph.D. and Stephanie A. Atkinson, Ph.D.

The following manuscript is the first paper resulting from the validation study of dual energy x-ray absorptiometry (DXA).

The multiple authorship can be explained as follows:

**Dr. Henry Bayley** was invited to collaborate on this project because of his experience with body composition by chemical analysis. The initial steps of carcass analysis were conducted in Dr. Bayley's research facilities under his supervision.

**The Ph.D. candidate** was the author of this manuscript. Most of the measurements in the study were conducted by her. All analytical work and data analyses were done by the Ph.D. candidate, under the direct supervision of **Dr. Atkinson**.

This chapter was published in *The American Journal of Clinical Nutrition* (1993; 58:839-845). It is reproduced with written permission by the Executive Officer of the journal (Appendix 3).

# Validation and application of dual-energy x-ray absorptiometry to measure bone mass and body composition in small infants<sup>1-3</sup>

Janet A Brunton, Henry S Bayley, and Stephanie A Atkinson

**ABSTRACT** Precision and validity of dual-energy x-ray absorptiometry (DXA) for analysis of whole-body composition in infants were assessed by 1) scanning piglets in triplicate to calculate CVs, and 2) comparing DXA estimates with chemical analysis of whole carcass. The mean CVs for all DXA measures in small piglets and large piglets were < 2.5%, except for fat mass, which were 6.3% and 3.5%, respectively. In large piglets DXA provided reasonable estimates of chemical analysis for bone mineral content (BMC), lean body mass, and fat mass, but only for lean body mass in small piglets. DXA overestimated fat by twofold and underestimated BMC by a third in small piglets. Scans of prematurely born infants ( $n = 17$ ) at term and at 3, 6, and 12 mo corrected age demonstrated that changes in BMC, lean body mass, and fat mass can be quantitated by DXA. However, further refinement of DXA technology is necessary before reliable measures of BMC and fat mass in small infants are attainable. *Am J Clin Nutr* 1993;58:839-45.

**KEY WORDS** Dual-energy x-ray absorptiometry, body composition, premature infants, bone mineral content, total body fat, piglets

## Introduction

The influence of nutrient intake on body composition in growing low-birth-weight infants is essential knowledge for optimizing nutritional management for the support of growth and development. Until recently, indirect methods of measuring body composition were not easily applied to small infants, but the latest generation of whole-body absorptiometers using dual-energy x-ray absorptiometry (DXA) may be a useful tool for this pediatric population.

The application of DXA for quantitative assessment of fat mass, lean body mass, and bone mass should provide measures with precision and accuracy while being safe and noninvasive for use in infants and small children. Previous generations of densitometers using radionuclides were too slow and subjected infants to radiation exposures that were of concern (1). DXA uses a constant potential x-ray energy source, resulting in an increased scan speed with greater resolution and lower radiation exposure compared with dual-photon absorptiometry using <sup>153</sup>Gd (2). Total-body-potassium counting is not sensitive enough to use in small infants (3) and methods of electrical conductivity have not been validated in this population. Skinfold-thickness measure-

ments at one or more sites have been used to estimate total body fat, but these are difficult to measure reliably in very small infants. Single-photon absorptiometry is a precise method for measuring bone mineral content (BMC) at peripheral individual bone sites (4), but may not reflect total skeletal bone mineral mass in infants prone to osteopenia.

To establish DXA as a useful clinical and research tool, the sensitivity of the technology in detecting small changes in body composition must be determined. Validation of DXA for use in infants has been limited to repeated measures of small tissue phantoms or excised bone (5, 6); precision has not been established by repeated in vivo measures. Our objectives were first to establish the precision and accuracy of DXA measurements of whole-body BMC, fat mass, and lean body mass in young piglets, which are similar in body composition to infants (7). Second, serial DXA scans of prematurely born infants were conducted at term and at 3, 6, and 12 mo corrected age to 1) determine the feasibility of the measurement technique in young infants; 2) assess the sensitivity of DXA in quantifying small changes in BMC, lean body mass, and fat mass in rapidly growing infants; 3) compare DXA measures of radial bone mineral density (BMD) to radial BMD determined by single-photon absorptiometry (SPA); and 4) determine the accuracy of DXA in predicting total body weight.

## Materials and methods

### Piglets

Piglets ranging from 2 to 20 d of age were removed from the sow at the Arkell Research Farm (University of Guelph, Guelph, Ontario) and transported to McMaster University Central Animal

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<sup>2</sup> Supported by grants from The Ministry of Health of Ontario (SAA) and the Medical Research Council of Canada (SAA) and by a Career Scientist Award at McMaster University from the Ministry of Health of Ontario (SAA).

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Received December 15, 1992.

Accepted for publication June 21, 1993.

temperature of 30 °C. The piglets were weighed on an electronic scale accurate to 0.1 g (Sartorius, Goettingen, Germany). Two specific weight groups were targeted for study: 1.6 kg (small piglets) and 6.0 kg (large piglets). These weights were chosen to approximate the lowest and midrange weight of infants who would be of clinical interest. Piglets that were within 5% of either targeted weight were given 5% glucose water only, and killed later the same day. Piglets that were underweight were weaned to formula designed to meet their nutrient requirements starting with half-strength feeding and progressing to full-strength feeding by the third day. Piglets weighing < 1.5 kg were intermittently gavage fed formula or glucose water until they were feeding independently. Once the piglets had reached the targeted weight, formula feeding was stopped and glucose water was provided until the scan measurement occurred later that day. The final formula feeding was provided 24 h before the scan measurement and consisted of half of the piglet's daily requirement. If the piglets were studied on the day of arrival to the laboratory, they were killed  $\geq$  8 h after removal from the sow.

To facilitate the scan procedure, piglets were anesthetized with an intraperitoneal injection of 25 mg pentobarbital/kg body wt (65 g/L). This was necessary to ensure that the animal remained completely motionless for the DXA scan.

#### *DXA scan procedure*

Scans were performed by using a Hologic QDR-1000/W (Hologic Inc, Waltham, MA). The principles of dual-photon absorptiometry to estimate tissue composition are described in detail elsewhere (9). The Hologic system uses fast kV<sub>p</sub> switching of an x-ray tube to generate both high- and low-energy photon beams (140 and 70 kV<sub>p</sub>). The beam is transmitted in a rectilinear raster throughout the entire body. Measurements of photon transmission are made for the high- and low-energy beams along the scan path. At all measurement locations the beam passes through a wheel containing tissue and bone simulants for continuous internal calibration during the scan. The relative attenuation of the two photon beams can be related to the mass of either component in a two-component system. Consequently, if the body is considered to consist of bone mineral and soft tissue, then total-body bone mineral mass can be measured. If a second dual-photon analysis is performed at sites that contain only soft tissue, then soft tissue mass can be divided into lean tissue mass and fat mass.

Triplicate whole-body scans were conducted by using the pediatric scan mode. The piglets were placed uncovered on the scan bed on their stomachs in the spread-eagle position, with the legs extended from the body. Generally, scans were performed without repositioning but if the piglets started to arouse during scanning, more anesthetic was given and the scan restarted. On completion of the scans the piglets were killed with an injection of sodium pentobarbital. The whole carcass was immediately frozen for subsequent chemical analysis. To determine whether fluid shifts that occur postmortem would influence the DXA estimate of soft tissue, six piglets (three small, three large) were frozen in the scanning position and rescanned. This variable was important for future studies in animals.

Individual scans were analyzed by using the *Pediatric Whole-Body* software (version 6.01, Hologic Inc.). The soft tissue analysis in the pediatric mode assumes a lean tissue hydration factor

To determine the influence of covers and blankets in the subject scan field, one piglet was scanned three times with 1) a flannel sheet under and over it in a single layer (total blanket weight 92 g), 2) a flannel sheet under and over it in a single layer with the piglet also wrapped in a flannel receiving blanket (total blanket weight 220 g), and 3) a double layer of flannel under and over it with the piglet wrapped in a receiving blanket (total blanket weight 265 g). This experiment was conducted after an upgrade to the software had been installed (version 6.02).

#### *Carcass preparation and analysis*

The whole frozen carcasses were individually ground to a coarse homogenate (small piglets: Hobart model M803, Don Mills, Ontario; large piglets: Autio model 801CH25, Astoria, OR) then reground to ensure a fine homogeneous mixture. Two samples of tissue homogenate from each piglet were weighed to 0.1 g, frozen, and then lyophilized. Total body water of each animal was determined by the difference between the wet tissue weight and the lyophilized weight. The lyophilized tissue was reground in a blender to facilitate sampling.

Total carcass BMC was determined by weighing  $\approx$  0.5 g dried tissue to a reproducibility of 0.1 mg. The weighed tissue was heated to 500 °C in a muffle furnace for 72 h, dried in a desiccator to a constant weight, then reweighed to determine ash weight. The CVs of triplicate samples from each piglet were 7.0% in the small piglets and 7.6% in the large piglets. Total carcass lean body mass was determined by the following equation:

#### *Lean body mass*

$$= \text{total carcass nitrogen} \times 6.25 + \text{total body water}$$

Nitrogen was analyzed by the micro-Kjeldahl method (10) by using 0.5 g dry tissue. The CVs for repeated samples for the nitrogen analysis were 2.6% and 5.7% in the small and large piglets, respectively. Total carcass fat mass was determined by the lipid-extraction method adapted from Folch et al (11). Approximately 1.5 g dried tissue was homogenized (Polytron; Brinkman, Lucerne, Switzerland) with a mixture of chloroform, water and methanol for 5 min. Separation of chloroform and methanol layers was facilitated by centrifugation at 10 000  $\times$  g for 10 min at 4 °C. The chloroform layer was drawn off and evaporated in a nitrogen stream. Samples were heated in an oven at 100 °C for 0.5 h then placed in a desiccator. The lipid was then weighed. The CVs by this method were 2.2% and 2.9% in the small and large piglets, respectively.

#### *Infant scans*

Infants (birth weight < 1500 g) who were diagnosed with bronchopulmonary dysplasia (BPD) were recruited from the neonatal intensive care unit at the Children's Hospital at Chedoke-McMaster, Hamilton, Ontario, as part of an ongoing clinical nutrition trial. The study was approved by the Research Project Advisory Committee and informed parental consent obtained. During routine visits to the Growth and Development Follow-up Clinic at term and at 3, 6, and 12 mo corrected age, serial DXA measures were conducted. Infants were laid on the scan bed on their stomachs. When sleeping quietly, the infants were scanned once by using the pediatric scan mode. No sedation was used.



Precision of whole-body scans by dual-energy x-ray absorptiometry in small and large piglets

	Mean	Mean CV%*	Range
	g	%	%
Small piglets (n = 10)			
Weight	1572	0.2	0.0-0.3
BMC	27	2.3	0.6-4.3
Lean mass	1371	0.8	0.3-1.6
Fat mass	174	6.3	2.8-12.2
Large piglets (n = 10)			
Weight	5984	0.2	0.0-0.5
BMC	116	1.5	0.1-3.4
Lean mass	5217	0.6	0.3-1.0
Fat mass	651	3.3	0.9-5.7

\* From triplicate scans on individual piglets.

During the scan infants were covered by a single layer of flannel blanket and were wearing only a disposable diaper. Each infant was weighed to 1 g on an electronic balance. Mean values of the measurements of fat mass of infants were compared with literature values for full-term infants (12) at similar corrected ages to compare percent body fat values obtained by DXA vs those obtained by skinfold-thickness measurement.

Triplicate measures of BMD of the left radius at the one-third distal site were conducted by using SPA (Norland, Fort Atkinson, WI). The pediatric software allowed the DXA scans to be regionally analyzed so that the radius could be isolated and the

of the radius was of interest to determine whether the radius could be compared with previously published values for infants, obtained by using SPA technology. All regional analyses were conducted by one person with a mean intraobserver CV of 4.0% (determined from repeated analyses of scans of three infants of varying size). Infant scans were analyzed as two groups (< 5 kg and ≥ 5 kg body weight) to determine whether infant size influenced the relationship between the methods.

Statistics

CVs were calculated for the triplicate measures. Differences between mean DXA estimates for the groups of pigs and measured values were determined by paired *t* tests (Minitab, version 7.1; Minitab Inc, Pittsburgh). The relationship between the two methods was determined by regression analyses (Fig P, 6.0; Biosoft, Ferguson, MO). The level of significance for all tests was *P* < 0.05.

Results

The mean weights and CVs for whole body and individual body compartments as measured by DXA for each group are presented in Table 1. Figure 1 shows linear regressions of whole-body composition determined by DXA compared with the scale, or chemically analyzed carcass weights in the small piglets. Only total-body weight (Fig 1, A) and lean body mass (Fig 1, C) measured by the two methods were significantly correlated. DXA-determined BMC (Fig 1, B) and fat mass (Fig 1, D) were not

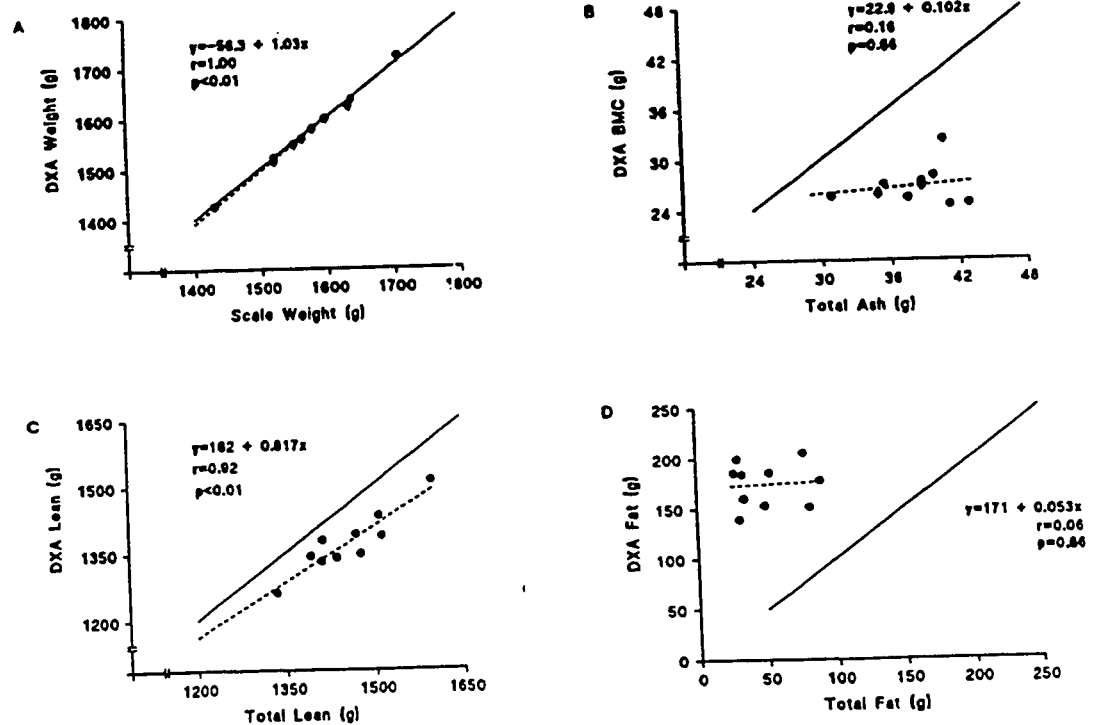


FIG 1. Linear regression comparing dual-energy x-ray absorptiometry (DXA)-determined total body weight (A), bone mineral content (BMC) (B), lean body mass (C), and fat mass (D) with measured values in small piglets. Hatched line represents regression line, solid line is the line of identity.

correlated with the measured values. Figure 2 represents linear regressions comparing the two methods in the large piglets. DXA-estimated total body weight, BMC, total lean body mass, and fat mass were all significantly correlated with the measured values.

Comparisons between DXA-determined weights and tissue analysis for body weight, BMC, lean body mass, and fat mass for both small and large piglets are presented in Table 2. In the small piglets, DXA-determined values were significantly different from measured values for all indexes except total body weight. DXA consistently underestimated total bone and lean body mass and overestimated fat mass. In large piglets, DXA marginally overestimated the total body weight and total lean body mass. Total fat mass was overestimated by approximately one-third.

In the large piglets, when the lean tissue hydration assumption was changed from 86% to 69%, further errors occurred in the soft tissue measurements. Overestimation of total fat mass increased the error from 35% to  $\approx 57\%$ . Lean body mass was underestimated by  $< 1\%$  when compared with chemically analyzed values.

Table 3 represents the results of the three scans with different coverings. Additional layers of flannel consistently increased the DXA-determined weight by 33% of the measured blanket weight. The addition of blankets reduced the precision of the measurements compared with scans in small piglets (Table 1) for total body weight (CV 1.7 vs 0.2%) and lean body mass (CV 1.9 vs 0.8%).

There were no significant differences between live and frozen piglets for DXA estimates of weight (small  $1565 \pm 54$  vs  $1569$

weights vs measured tissue weights in small and large piglets)

	DXA	Measured	P*	Percent difference†
	g	g		%
Small piglets (n = 10)				
Weight	$1572 \pm 76$ (1426-1720)‡	$1575 \pm 73$ (1431-1710)	0.13	0.2
Bone mineral	$27 \pm 2.2$ (25-32)	$38 \pm 3.3$ (31-43)	$< 0.01$	29.7
Lean tissue	$1371 \pm 62.8$ (1261-1509)	$1456 \pm 70.6$ (1335-1599)	$< 0.01$	5.9
Fat tissue	$174 \pm 20.9$ (139-205)	$52 \pm 22.5$ (27-89)	$< 0.01$	234.6
Large piglets (n = 10)				
Weight	$5984 \pm 211$ (5704-6415)	$5894 \pm 208$ (5630-6293)	$< 0.01$	1.5
Bone mineral	$116 \pm 14.0$ (98-141)	$116 \pm 19.9$ (90-147)	0.85	0.6
Lean tissue	$5217 \pm 203.3$ (4866-5612)	$5151 \pm 188.5$ (4779-5505)	$< 0.01$	1.3
Fat tissue	$651 \pm 82.7$ (485-736)	$480 \pm 78.8$ (325-585)	$< 0.01$	35.6

\* Determined by paired *t* test.

† Percent difference between measured value and DXA estimate.

‡  $\bar{x} \pm SD$ ; range in parentheses.

$\pm 57$  g; large  $5984 \pm 207$  vs  $5984 \pm 200$  g), BMC (small  $25.7 \pm 1.4$  vs  $25.6 \pm 2.8$  g; large  $114.8 \pm 5.0$  vs  $118.6 \pm 9.4$  g), fat mass (small  $172 \pm 29$  vs  $136 \pm 24$  g; large  $624 \pm 101$  vs  $614$

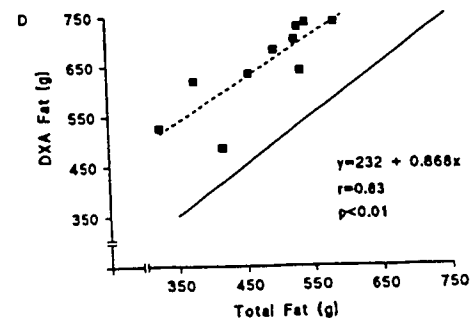
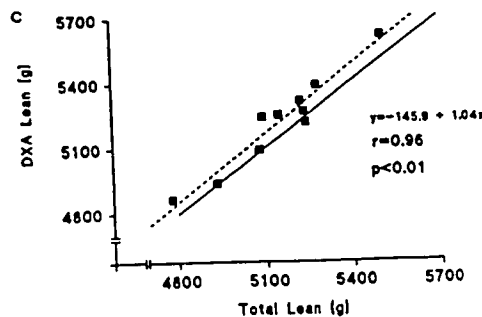
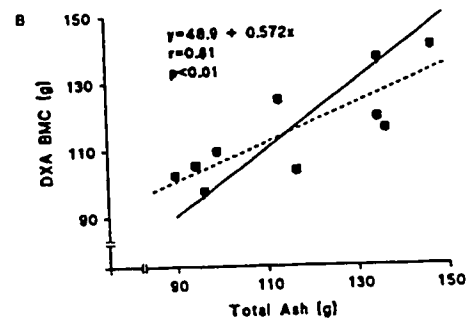
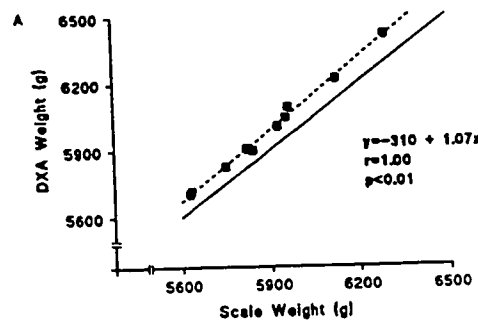


FIG 2. Linear regression comparing dual-energy x-ray absorptiometry (DXA)-determined total body weight (A), bone mineral content (BMC) (B), lean body mass (C), and fat mass (D) to measured values in large piglets. Hatched line represents regression line, solid line is the line of identity.

	Scan 1*	Scan 2†	Scan 3‡	$\bar{x} \pm SD$	CV
	g	g	g	g	%
Weight	1749	1787	1809	1782 ± 30.4	1.7
BMC	42.4	40.5	41.3	41.4 ± 0.96	2.3
Lean	1592	1639	1649	1627 ± 30.4	1.9
Fat	115	108	119	114 ± 5.6	4.9

\* Single flannel sheet under and over piglet; blanket weight 92 g.

† Single flannel sheet under and over piglet with piglet in flannel receiving blanket; blanket weight 220 g.

‡ Doubled flannel sheet under and over piglet with piglet in receiving blanket; blanket weight 265 g.

± 107 g), or lean body mass (small 1366 ± 32 vs 1409 ± 42 g; large 5247 ± 147 vs 5252 ± 144 g). The CVs of triplicate scans on the frozen animals were similar to those calculated for the same animals when scanned alive.

The characteristics of the infants are presented in Table 4. A total of 37 scans were conducted in 17 infants. There was a high correlation between DXA-estimated total body weight and the scale-measured weight of infants weighing < 5 kg ( $y = 0.024 + 1.039x$ ,  $r = 0.997$ ,  $P < 0.01$ ) and ≥ 5 kg ( $y = 0.206 + 1.014x$ ,  $r = 0.995$ ,  $P < 0.01$ ).

The correlation between the DXA and SPA measures of radial BMD in infants weighing < 5 kg and ≥ 5 kg is depicted in Figure 3. There was no correlation between methods in the smaller infants, but there was a significant correlation in infants weighing ≥ 5 kg. In most infants weighing ≥ 5 kg the DXA-determined radial BMD was higher than the SPA measurement.

Figure 4 depicts the proportion of fat and fat-free body mass (FFBM) as determined by DXA in infants scanned during follow-up visits. Compared with reference infants (12), the infants with BPD had lower body weights and a higher proportion of total body weight as fat at all ages except 12 mo.

## Discussion

Measures of whole-body composition in infants, especially those of low birth weight, require an accurate tool. For longitudinal measures the precision of measurement must be small enough to allow detection of small differences in BMC and soft tissue compartments.

Our data established that repeated whole-body measures of BMC by DXA were repeatable with CVs < 2.5% in live animals as small as 1.6 kg, but in these animals the quantitation of BMC by DXA was not accurate compared with total body ash. Other investigators have assessed the accuracy of DXA-determined BMC but used in vitro rather than in vivo models. In one study by Brailon et al (5), femurs excised from premature infants were submersed in water, scanned in triplicate (Hologic QDR-1000), then ashed. In contrast with our findings, they found a high correlation between BMC measured by DXA and ash weight, but DXA consistently overestimated BMC. Chan (6) used a Norland XR-26 (Fort Atkinson, WI) and found a high correlation between

which is similar to our results in the small piglets.

Ashing whole-carcass tissue homogenate will result in a slight overestimation of BMC because of the inclusion of nonbone mineral, but this should not influence the results significantly. The discrepancy between our study results and previously published in vitro findings may be partially explained by different instrumentation. Brailon et al (5) used the same device on bones that were similar in size to those found in our piglets, but the scan type and software used in the study were not clearly defined. Thus, it is difficult to compare their findings with ours, determined by using the pediatric whole-body scan mode.

When the body weight of an animal (and presumably an infant) is ≈ 6 kg, both the accuracy and precision of DXA estimates of BMC are acceptable. At 1.6 kg body wt, DXA consistently underestimated BMC by a range of 17–40%. The failure of this device to accurately measure BMC in small piglets is likely multifactorial. The partial volume effect of determining bone content in pixels containing bone and soft tissue will be exaggerated in very small animals. As well, skeletal density varies; therefore, regions of low density may be below the threshold for detection resulting in artificially low whole-body BMC. The small and large piglets had 38 ± 3.3 and 116 ± 19.9 g whole-body BMC, respectively. Healthy full-term infants have whole-body BMC comparable to that of the small piglets (13). The infants we studied had an average DXA-estimated whole-body BMC of 58 ± 18 g at full-term corrected age. Infants at 3 mo corrected age had almost double this mass (112 ± 26 g), a value similar to that of the large piglets. Our results illustrate the difficulties that will be encountered when DXA is used for in vivo measurement of whole-body composition in small infants in early life.

The small piglet is an ideal model for studying soft tissue composition of preterm infants. Both have lean tissue hydration of ≈ 80% and both have low body-fat stores that are well under 10% (7, 13). DXA-estimated total body fat was the measure with the most variability and least accuracy. In small piglets, DXA overestimated total body fat by 2.5-fold. In absolute terms, this corresponds to ≈ 120 g fat. DXA calculates a fat fraction for the pixels containing no bone. This is extrapolated to total body fat from the soft tissue mass. In small piglets and preterm infants, their distinct body composition (ie, minimal body fat) may be beyond the capabilities of the current pediatric software. A further explanation for the large error in estimation of total body fat may be the absolute thickness of the animal being measured. In

TABLE 4  
Infant characteristics\*

	Weight	Corrected age
	g	wk
Birth (n = 17)	847 ± 152	24.6 ± 1.6†
Follow-up age		
Term (n = 9)	3089 ± 422	4.3 ± 1.9‡
3 mo (n = 14)	4675 ± 824	15.1 ± 1.9‡
6 mo (n = 10)	5924 ± 952	26.8 ± 0.8‡
12 mo (n = 4)	8140 ± 1291	51.8 ± 1.8‡

\*  $\bar{x} \pm SD$ .

† Postconceptional age at birth.

‡ Age counted from term (40 wk postconceptional age).

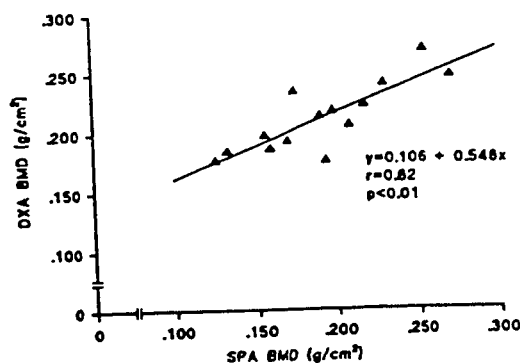
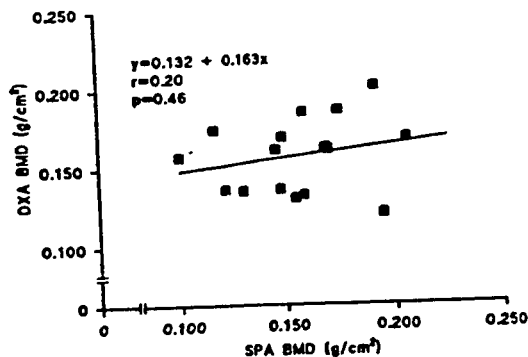


FIG 3. Linear regression comparing dual-energy x-ray absorptiometry (DXA)-determined radial bone mineral density (BMD) to single-photon absorptiometry (SPA)-determined radial BMD in infants weighing  $< 5$  kg ( $n = 16$ ) (A), and  $\geq 5$  kg ( $n = 14$ ) (B).

vivo studies of tissue phantoms mimicking various tissue thicknesses showed the greatest accuracy between 10 and 20 cm (14, 15). At thicknesses outside this range, the attenuation error caused by the fractional crossover of the high- and low-energy beams increases. A large proportion of the total body in a 1.6-kg piglet was  $< 10$  cm thick in the scanning position, particularly in the appendages. Specific calibration to account for this effect is likely required when small animals or infants are measured.

Although the precision and accuracy of DXA was better in the large piglets than in the small piglets, DXA still overestimated total body fat by  $\approx 35\%$ . DXA measures of large piglets would also be subject to the error introduced by minimal tissue thickness but to a lesser degree than in the small piglets.

The 6-kg piglet is not an ideal model for an infant of the same size because of differences in body fat content and regional dis-

tribution. Large piglets were  $8.2 \pm 1.3\%$  fat by carcass analysis; an infant of the same weight has  $\approx 25\%$  fat (12). The distribution of fat in infants would likely influence the accuracy, i.e., a high proportion of fat in the appendages could be overestimated because of the low tissue thickness.

The hydration of the lean tissue decreases in both piglets and infants during early neonatal life (7, 13). Theoretically, the lean tissue hydration assumption should be altered in the software program to account for this change in body hydration. In practice, the use of a lower hydration factor (69% vs 86%) increased the error in the soft tissue analysis by further overestimating total body fat. The relationship between the ratio of attenuation coefficients at the low and high energy levels ( $R_{\mu}$ ) and the percent fat value of soft tissue will be altered as the tissue hydration changes. Further study is required to determine the effect of hydration state on DXA estimates of soft tissue, because a change in the lean tissue hydration factor did not improve accuracy.

The numerous potential sources of error in DXA technology dictate that all DXA instruments must be validated before reliable in vivo measures of whole-body composition can be achieved in small infants. Previous technological refinements of the pediatric software program for the QDR-1000/W resulted in improved precision in small piglets; the CVs for total fat decreased from 8.6% to 6.3% when scans were reanalyzed with upgraded software (version 6.01). Further improvement in accuracy is necessary before DXA estimates of whole-body composition in infants or animals weighing  $< 6$  kg can be interpreted with confidence. Appropriate blanket coverings and positioning will influence DXA estimates, thus standardization of the DXA scanning procedure is essential.

DXA scanning to determine whole-body composition has proven to be a feasible technology for use in infants. Precision was not assessed because repeated measures unnecessarily expose infants to further radiation and would require sedation for the infants to remain still for a prolonged period of time. In our experience, scanning of infants without the use of sedation is certainly feasible; 40 infant scans were attempted, of which 37 were completed successfully. The average scan time for whole-body-composition analysis of an infant is  $\approx 8$  min.

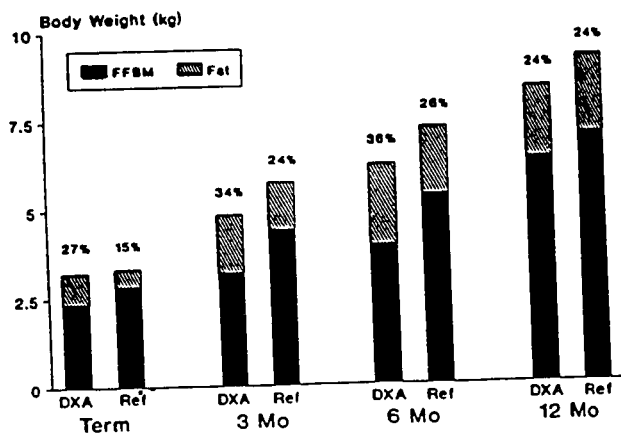



FIG 4. Dual-energy x-ray absorptiometry (DXA) estimates of body composition in prematurely born infants (DXA) ( $n = 17$ ) at specific corrected ages during the first year of life compared with skin-fold-thickness estimates of body composition in a reference population (Ref) (12). The values represent percent body fat. Term ( $n = 9$ ), 3 mo ( $n = 14$ ), 6 mo ( $n = 10$ ), and 12 mo ( $n = 4$ ). FFBM, fat-free body mass.

Analysis of the accuracy of DXA in infants resulted in similar findings to those for the piglets. DXA consistently overestimated total body weight by  $\approx 3.5\%$ , a value slightly greater than in the piglets (0.2–1.5%). This is likely due to the use of blanket coverings and disposable diapers on the infants during scanning, which were not used on the piglets. The use of flannel coverings in the scan field increases the DXA-determined weight of the subject by  $\approx 33\%$  of the weight of the blanket in small piglets. Unpublished data from our laboratory showed that in large piglets the same blanket coverings increased the DXA-determined weight, representing 50% of the blanket weight. Therefore, the use of a correction factor to accommodate blanket use must be done with caution.

We analyzed the infant bone-density-scan data in two groups (those weighing  $< 5$  kg and those  $\geq 5$  kg) based on the differences in accuracy of DXA observed in the large piglets vs that in the small piglets. In infants weighing  $< 5$  kg, the mean DXA- and SPA-determined BMD were 0.211 and 0.192 g/cm<sup>2</sup>, respectively. The greater value for radial BMD by DXA over SPA is as expected because the DXA analysis includes more dense cortical bone in its measurement than would be present at the distal one-third radius site. There was no association between the DXA and SPA measures in infants weighing  $< 5$  kg. In the smaller infants it was more difficult to isolate the radius for the regional analysis by DXA. The potential error in isolation of the radius in the DXA scan field likely contributes to the lack of agreement between measures by DXA and SPA.

If measures of body fat in infants taken by using DXA technology are validated, this technique could become the standard for body-composition analysis because hydrostatic weighing is not ethical in this population and skinfold-thickness measures do not represent total body fat (16). Body-composition data for a reference population of infants used two-site skinfold-thickness measures to estimate total body fat (12). Compared with these reference data, our infants had lower weights (by  $\approx 1$  kg) and had a greater percent of total body fat (Fig 4). The largest discrepancy between our measures of percent body fat and the published values (12) was in the smallest infants. If our values are corrected by the 35% overestimation of fat compared with chemical analysis that we observed in the large piglets, then the percent body fat of our infants is similar to the reference population at term and at 3 and 6 mo corrected age. Further study is required to determine whether body composition of term infants is a reasonable reference standard of comparison for prematurely born infants when they reach similar corrected ages.

DXA technology is noninvasive and relatively safe for use in small infants and children. We believe that further software improvements could result in a technology that has the potential to be the standard for estimation of body composition in small infants. 

We gratefully acknowledge the assistance of Robert Bertolo, Michelle Whelan, and Colin Webber, and especially appreciate the cooperation of the parents of the infants who were scanned.

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**Improvement in the accuracy of dual energy x-ray absorptiometry for whole body and regional analysis of body composition: validation using piglets and methodological considerations in infants.**

Janet A. Brunton B.A. Sc., Hope Weiler Ph.D., Stephanie Atkinson, Ph.D

The following manuscript is the second paper resulting from the validation study of dual energy x-ray absorptiometry (DXA). This represents a re-evaluation of DXA after the software (which was provided with the system by the manufacturer) was upgraded. The paper also addresses methodological issues regarding scanning protocols.

The multiple authorship can be explained as follows:

**Dr. Hope Weiler** was Ph.D. candidate in the lab of Dr. Atkinson at the time of this research. For her own work, she assessed the accuracy and precision of DXA for regional analyses of bone. It was a valuable addition to this manuscript. She also assisted in collecting data regarding the influence of artifacts on DXA measures. Hope wrote the sections of the manuscript that specifically refer to regional analyses of femurs in piglets, and participated in the review and editing of the whole manuscript.

**The Ph.D. candidate** was the primary writer of this manuscript. Most of the measurements in the study were conducted by her. The collection of data referring to artifacts in the scan field was a collaborative effort by many members of our research group (since it was labour intensive, but the information was needed immediately to aid in on-going studies) All analytical work and data analyses were done by the Ph.D. candidate, under the direct supervision of **Dr. Atkinson**.

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# Improvement in the Accuracy of Dual Energy X-ray Absorptiometry for Whole Body and Regional Analysis of Body Composition: Validation Using Piglets and Methodologic Considerations in Infants

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## ABSTRACT

Previously, we conducted dual energy x-ray absorptiometry (DXA) (Hologic QDR-1000/W) scans and carcass analysis of piglets to evaluate the Pediatric Whole Body software (PedWB) (V5.35) for use in infants. A software upgrade designed for infant whole body (InfWB) (V5.56) led to a reassessment of DXA by: 1) reanalysis of the original scans using InfWB software and 2) comparison of InfWB-estimates of bone mineral content (BMC) and lean and fat mass with chemical analysis. Other assessments included 1) methods of regional analysis and 2) artifacts and the Infant Table Pad in the scan field. The mean coefficients of variation for InfWB whole body measures in small piglets ( $n = 10$ , weight  $1575 \pm 73$  g) and large piglets ( $n = 10$ , weight  $5894 \pm 208$  g) were less than 2.6% except for fat mass which was higher (8.0% versus 6.3% and 6.6% versus 3.5%, respectively) compared with PedWB. In large piglets InfWB produced good estimates of BMC, lean and fat masses. In small piglets, fat mass by InfWB was correlated with chemical analysis, but not by PedWB. There was improvement in the estimation of BMC with InfWB, from  $27 \pm 2.2$  g to  $32 \pm 2.3$  g (carcass ash =  $38 \pm 3.3$  g). Femur BMC analysis by InfWB was precise

and was accurate when compared with chemical analysis. Artifacts in the DXA scan field (diapers and blankets) resulted in an increase of the DXA-estimated fat and lean masses. The Infant Table Pad increased the estimate of fat mass in a small piglet by 50%, thus further study is required before it is used routinely. Improvements of the DXA technology have resulted in a more accurate tool, if scanning procedures are carefully implemented. (*Pediatr Res* 41: 590-596, 1997)

## Abbreviations

BMC, bone mineral content  
CV, coefficient of variation  
DXA, dual energy x-ray absorptiometry  
F, performance right hip software (V4.47P)  
FFM, fat-free mass  
InfWB, infant whole body software (V5.56P)  
PedWB, pediatric whole body software (V5.35)  
SEE, standard error of the estimate  
TOBEC, total body electrical conductance

A "gold standard" method is needed to measure total body composition including bone and soft tissue components, which has been validated and universally accepted for clinical assessment in infants. DXA is a relatively new imaging approach with analytical software programs specifically designed to assess the composition of small bodies, including human infants and rats. DXA estimates total BMC and total body soft tissue, the latter of which can be separated into total body fat and lean masses by virtue of using factory-installed calibration materials that simulate the attenuation properties of lean and fat (2). Other imaging

techniques that can measure bone and soft tissue include computed tomography, with a radiation exposure too high to permit safe use in human infants, and magnetic resonance, which generally has limited access for research purposes. TOBEC has recently been investigated for use in infants (3, 4), but is limited because it measures only the lean soft tissue compartment. Other indirect measures of fat free mass such as  $^{40}\text{K}$  counting and estimating dilution space by stable isotope methodology are difficult to conduct accurately (5, 6).

Despite the growing use of DXA in small infants for clinical and research purposes (1, 7-10), validation of the precision and accuracy of DXA in infants has been mostly limited to *in vitro* repeated measures of small tissue phantoms or excised bone (9, 11). In our previous validation study (1), using the original PedWB from the manufacturer (Hologic Incorporated,

Received February 12, 1996; accepted November 7, 1996.

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Supported in part by the Ontario Ministry of Health.

(approximately 200%) compared with chemical carcass analysis. However, in small piglets (1.6-kg body weight) the accuracy of DXA-estimated whole body BMC was low, and total body fat was overestimated by greater than 200%. Subsequent to these findings, the manufacturer provided an upgrade to the PedWB, which is identified as InfWB.

The primary objective of this study was to assess the precision and accuracy of DXA estimates of whole body weight, BMC, and fat and lean mass in the piglets when analyzed with the revised software (InfWB) compared with the original findings (PedWB and chemical carcass analysis). Also the effect of the precision of DXA on the interpretation of changes in fat mass and BMC over time was assessed in infants. DXA also offers the option to conduct regional analysis of identified areas of interest on a scan, but whether these estimates have the same accuracy and precision as obtained with specialized software for specific bone regions has not been evaluated in small infants. Therefore, the second aim was to assess the accuracy and precision of regional analyses of BMC in small bones by comparing *in vivo* measurements in piglet femurs with ash weight.

To optimize the conditions for DXA measures of whole body composition, consideration must be made for artifacts that may interfere with the attenuation of the photons. For ethical and practical purposes, infants must wear diapers and be kept warm with blankets. To minimize the radiation dose delivered to the infant, a device called an Infant Table Pad was provided as an option for use with the InfWB. It was designed to improve the linearity within the thin regions of the infant (product literature). Variations in the estimates of body composition as a result of such artifacts in the scan field have not been thoroughly assessed. Therefore, the third objective was to determine the influence of artifacts in the scan field, including the Infant Table Pad, on the precision and absolute results of DXA estimates of whole body composition.

## METHODS

Piglets used in the present analysis were involved in different studies (1, 12), but all were obtained from the Swine Research Facility, Arkell Farms (Guelph, ON) and transported to the McMaster University Central Animal Facility. Details of the methods of piglet care and chemical carcass analysis have been published (1, 13). In the previous study two specific weight groups of piglets were targeted for measurements, 1.6 kg (small piglets,  $n = 10$ ) and 6.0 kg (large piglets,  $n = 10$ ) (1). These weights were chosen to approximate the lowest and mid-range weight of infants who would be of clinical research interest.

**DXA scan procedure.** Scans were performed using a Hologic QDR-1000/W (Hologic Inc., Waltham, MA). The principles of dual photon absorptiometry to estimate tissue composition are described in detail elsewhere (14). Triplicate whole body scans were conducted with piglets uncovered and with legs extended from the body. The Infant Table Pad was not used. Upon completion of the scans, the piglets were killed with an injection

previously (1).

**Whole body scan analysis in piglets.** Individual scans were originally analyzed using the PedWB Option (V5.35). The software upgrade (InfWB V5.56) had the capability to reanalyze scans from previous software versions, thus the original scans were loaded into the program and re-analyzed by a single operator (J.A.B.). According to the manufacturer, the new InfWB software included a revised algorithm which separated the image into small local regions of tissue in which was evaluated the bone mineral content. The estimates of soft tissue mass were supposed to be the same between the two software versions.

**Accuracy and precision of InfWB regional analysis of BMC.** Thirty-nine male piglets between 2.5 and 15 kg were anesthetized and measured at 22 ( $n = 6$ ) and 37 ( $n = 33$ ) d of age to estimate left femur BMC by two different DXA scan modes: 1) InfWB-estimated BMC was determined by regional isolation of the left femur by a single operator (H.A.W.), 2) subsequently, the F program V4.47P was used to estimate BMC of the left femur. After the DXA measures, the piglets were killed by an injection of sodium pentobarbital, and the left femurs were removed. The femurs were cleaned with a scalpel then ashed at 600°C for 14 d in a muffle furnace and ash weights determined. To evaluate whether the accuracy of DXA is influenced by body weight, the piglets were divided into weight categories: 1) less than 5.99 kg, 2) 6.00–7.99 kg, and 3) greater than 8.00 kg. Precision of InfWB and F was determined by triplicate scans of four piglets without repositioning. Average intraobserver precision (*i.e.* femur isolation) was determined by triplicate analyses of individual InfWB and F scans by the same operator (H.A.W.).

**Infant whole body scans.** Serial DXA whole body measurements of infants were conducted to determine whether DXA was sensitive enough to detect significant or true changes. This was evaluated using the CV% determined from the piglet experiments in the equation (15):

$$\text{Percent change} > 2\sqrt{2CV\%}$$

Prematurely born female infants ( $n = 26$ , gestational age =  $25.7 \pm 1.7$  wk, birth weight =  $800 \pm 151$  g) were recruited from the neonatal intensive care unit at the Children's Hospital at Chedoke McMaster, Hamilton, ON, as part of an ongoing clinical nutrition trial. The study was approved by the Research Project Advisory Committee, and informed parental consent was obtained. During routine visits to the Growth and Development Follow-up Clinic at term and at 3, 6, and 12 mo (adjusted for prematurity), serial DXA measures were conducted. Caregivers were asked to bring their infants to the clinic hungry and were asked not to let their infants sleep on the way to the clinic visit. An experienced pediatric research nurse fed and cuddled the infants until sleep was induced. Infants were laid on the scan bed on their stomachs and were scanned once using the InfWB software without the Infant Table Pad. No sedation was used.



piglets (1.7 and 6.4 kg) were killed by an injection of sodium pentobarbital and weighed on an electronic scale accurate to 1 g (Sartorius, Germany), then immediately frozen in the scanning position. In our previous study, we assessed the effect of scanning a live anesthetized piglet *versus* the frozen carcass, and found no difference (1). In each of the conditions tested, the small piglet was scanned four times, and the large piglet was scanned three times. The conditions included measurements with and without: 1) the Infant Table Pad under the piglets, 2) a flannel receiving blanket wrapped tightly around the piglets, 3) a disposable diaper on the piglets, or 4) a cotton flannel nightgown on the piglets.

**Statistics.** CV% were calculated for the repeated measures. The relationship between DXA estimates and whole carcass or femur analysis was determined by regression analyses (FigP, 6.0, Biosoft, Ferguson, MO). Also, the percent difference of DXA estimates of weight, fat and lean mass, and BMC compared with chemical analysis and with and without artifacts was calculated. The method of Bland and Altman (16) was used to assess the agreement between scan modes (InfWB and F) for interpretation of DXA small bone analysis. For the infant studies, the percent change necessary to detect a true change (calculated from the CV%) was compared with the actual mean percent change (% $\Delta$ ) determined from serial scans of infants. The level of significance for all tests was  $p < 0.05$ .

## RESULTS

**Piglet whole body scan analysis.** With the revised software a loss of precision in the measurement of fat was observed (Table 1). The range of CV values using InfWB for fat mass for the 10 small and large piglets, respectively, was 0.40–16.4% and 0.9–16.9%.

Reanalysis of scans using the InfWB software resulted in an improvement in the accuracy of the estimation of total BMC and fat mass in the small piglets, and a slight improvement in the fat measurement in large piglets relative to chemical carcass analysis (Table 2). As the overestimation of fat mass in the small piglet was reduced, the estimate of lean tissue improved reciprocally.

Table 1. CV% of triplicate scans\* by PedWB and InfWB

DXA measure	Mean CV%	
	PedWB	InfWB
Small piglets (weight: 1575 $\pm$ 73 g)†		
Total weight	0.2 $\pm$ 0.1	0.3 $\pm$ 0.2
Total BMC	2.3 $\pm$ 1.3	2.3 $\pm$ 1.1
Total BMD	1.4 $\pm$ 0.7	2.6 $\pm$ 1.1
Total lean	0.8 $\pm$ 0.3	0.6 $\pm$ 0.3
Total fat	6.3 $\pm$ 2.5	8.0 $\pm$ 5.3
Large piglets (weight: 5894 $\pm$ 208 g)		
Total weight	0.2 $\pm$ 0.2	0.2 $\pm$ 0.1
Total BMC	1.5 $\pm$ 0.8	1.1 $\pm$ 0.5
Total BMD	1.1 $\pm$ 0.4	1.1 $\pm$ 0.4
Total lean	0.6 $\pm$ 0.3	0.6 $\pm$ 0.4
Total fat	3.5 $\pm$ 1.7	6.6 $\pm$ 4.7

\* Scans were conducted without the Infant Table Pad.

† Mean  $\pm$  SD of the CV for the 10 piglets in each group.

InfWB was still overestimated in the small piglets, but was significantly correlated to values from chemical analysis. For the large piglets, the correlations between the InfWB and carcass analysis changed very little compared with PedWB, and all were highly significant (Table 3).

**Accuracy and precision of InfWB regional analysis of BMC.** The InfWB regional analysis of piglet femurs more accurately estimated the total ash content than did the F analysis, regardless of piglet size (Table 4). The mean femur BMC was 5.71  $\pm$  1.99 g by InfWB and 4.87  $\pm$  1.71 g by F, compared with the mean ash weight of 6.39  $\pm$  2.49 g. The regression equation for InfWB BMC *versus* ash weight was  $y = 0.73x + 1.06$ ,  $r = 0.91$ ,  $p < 0.01$ , and  $y = 0.62x + 0.93$ ,  $r = 0.89$ ,  $p < 0.01$  for F BMC *versus* ash weight. The intercepts were significantly different from zero. When body weight was not considered, regression analysis of InfWB femur BMC *versus* ash weight demonstrated that single InfWB scans are preferred for estimating BMC of small bones rather than performing two separate scans. InfWB and F estimates of femur BMC were highly correlated (Fig. 2A). However, using the method of Bland and Altman (16), it was evident that the greater the femur BMC the greater the difference was between DXA estimates (Fig. 2B).

The average CV for femur BMC measures were similar between InfWB and F (3.3  $\pm$  1.8% and 2.0  $\pm$  1.2%, respectively). The intraobserver variation for the isolation of the femur was 1.5  $\pm$  1.2% for the InfWB and 1.2  $\pm$  0.9% for the F.

**Whole body scans in infants.** In the group of premature infants, fat accretion as estimated by DXA between term (40 wk) and 3 mo, and 3 and 6 mo adjusted age exceeded the variability of the measurement (Fig. 3A). Between 6 and 12 mo the rate of fat deposition appeared to decline; thus DXA-estimated changes in fat mass may be confounded by the degree of imprecision of the measurement. Changes in whole body BMC of the premature infants (Fig. 3B) between all time points measured exceeded the variability of InfWB measurement of BMC.

**Influence of artifacts in the scan field.** Scanning the small piglet with the Infant Table Pad appeared to improve the CV of the measurement of total body fat (2.8% *versus* 8.9% without the pad), but the absolute weight of fat increased by almost 50%. The measurement of BMC was more variable (higher CV%) with all artifacts tested (Table 5). Part of the weight of each artifact was reflected in higher DXA-estimated body weights when the piglets were scanned with an artifact compared with without an artifact. Heavier artifacts produced a greater error in the measurements (Table 5).

## DISCUSSION

Both technical and biologic factors have a bearing on our ability to accurately interpret changes in body composition in rapidly growing infants. Currently, there is no method for measuring total body fat in small infants that has been demonstrated as safe, accurate, and precise. Methods used include

	PedWB	InfWB	Carcass analysis	Difference%*	
				PedWB	InfWB
Small piglets, n = 10					
Weight (g)	1572 ± 76†	1581 ± 77	1575 ± 73	-0.2	+0.4
Bone mineral (g)	27 ± 2.2	32 ± 2.3	38 ± 3.3	-29.7	-15.8
Lean tissue (g)	1371 ± 63	1433 ± 75	1456 ± 71	-5.9	-1.6
Fat tissue (g)	174 ± 21	114 ± 25	52 ± 23	+235	+119
%Fat	11.0 ± 1.1	7.2 ± 1.6	3.2 ± 1.4	+244	+125
Large piglets, n = 10					
Weight (g)	5984 ± 211	5999 ± 211	5894 ± 208	+1.5	+1.8
Bone mineral (g)	116 ± 14	116 ± 12	116 ± 20	+0.6	+0.1
Lean tissue (g)	5217 ± 203	5264 ± 204	5151 ± 189	+1.3	+2.2
Fat tissue (g)	651 ± 83	619 ± 94	480 ± 79	+35.6	+29.0
%Fat	10.9 ± 1.4	10.3 ± 6.6	8.1 ± 1.3	+35	+27

\* Percent difference = (InfWB or PedWB value - chemical analysis value) ÷ chemical analysis value × 100.

† Values are mean ± SD.

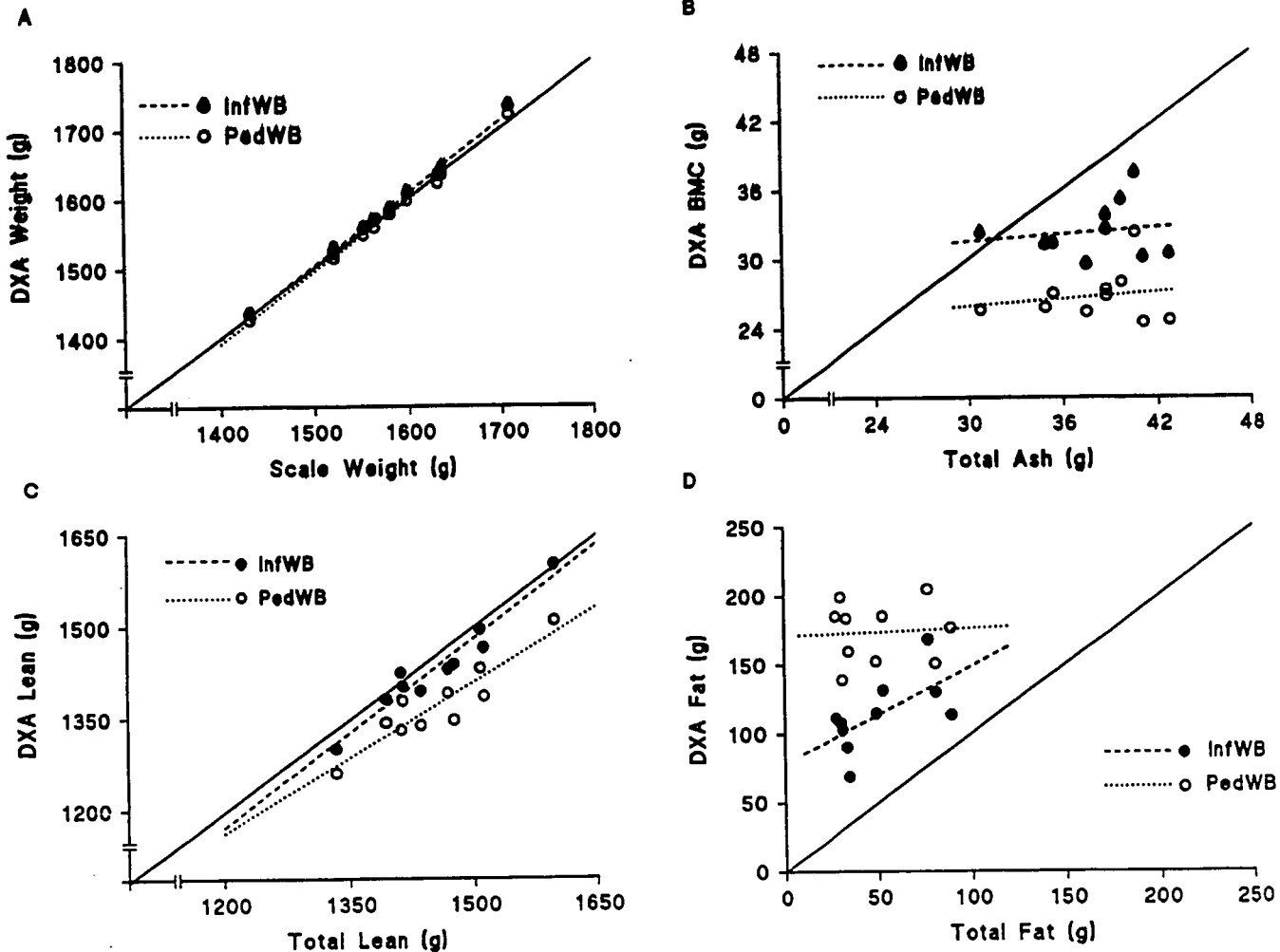


Figure 1. Linear regression comparing PedWB and InfWB scan results with (A) body weight measured by scale, (B) total carcass ash, (C) total carcass lean mass, and (D) total carcass fat mass in small piglets. Solid line represents the line of identity, indicating exact agreement.

skin fold measurements, dilution space, TOBEC, and DXA. However, these methods often do not agree (17) and may misrepresent patterns of change in body fat in individual infants over time. TOBEC measures FFM, but unlike DXA,

estimates total fat by subtracting FFM from body weight. Fiorotto *et al.* (3) reported that TOBEC estimates of FFM in infants less than 2.8 kg were unreliable and often greater than the body weight. In addition, the difference in small piglet

Table 3. Regression equations and SEE by chemical carcass analysis (or scale weight) in small and large piglets

	Software	Equation*	r	p	SEE
<b>Small piglets</b>					
Weight (g)	PedWB	$y = -55.9 + 1.03x$	1.00	<0.01	4.8
	InfWB	$y = -64.4 + 1.04x$	1.00	<0.01	5.6
BMC (g)	PedWB	$y = 22.9 + 0.102x$	0.16	0.66	2.3
	InfWB	$y = 28.5 + 0.101x$	0.15	0.66	2.5
Lean (g)	PedWB	$y = 182.2 + 0.82x$	0.92	<0.01	26.5
	InfWB	$y = -57.0 + 1.02x$	0.97	<0.01	20.7
Fat (g)	PedWB	$y = 171.0 + 0.053x$	0.06	0.86	20.1
	InfWB	$y = 78.5 + 0.702x$	0.63	0.05	20.8
<b>Large piglets</b>					
Weight (g)	PedWB	$y = -310 + 1.07x$	1.00	<0.01	16.3
	InfWB	$y = -310 + 1.07x$	1.00	<0.01	11.9
BMC (g)	PedWB	$y = 48.9 + 0.572x$	0.81	<0.01	8.7
	InfWB	$y = 62.1 + 0.466x$	0.77	<0.01	7.9
Lean (g)	PedWB	$y = -145.9 + 1.04x$	0.96	<0.01	56.2
	InfWB	$y = -181.1 + 1.06x$	0.98	<0.01	43.8
Fat (g)	PedWB	$y = 232.0 + 0.868x$	0.83	<0.01	49.1
	InfWB	$y = 143.3 + 0.922x$	0.83	<0.01	54.9

\* y denotes the DXA-estimated values, and x denotes the values determined from chemical carcass analysis.

Table 4. Percent difference between InfWB or F scan and femur ash weight

Weight range	Weight (kg)	Percent difference*	
		InfWB	F
<5.99 kg, n = 6	4.6 ± 1.1†	0.9 ± 21.6	-14.0 ± 18.9
6.00-7.99 kg, n = 13	7.1 ± 0.5	-3.6 ± 14.1	-18.2 ± 14.4
>8.00 kg, n = 20	10.4 ± 1.9	-11.7 ± 11.7	-24.3 ± 10.7
All pigs, n = 39	8.4 ± 2.6	-12.5 ± 11.6	-24.7 ± 11.1

\* Percent difference = (InfWB or F BMC - femur ash) ÷ femur ash × 100.  
† Values are mean ± SD.

(1-10 kg) between chemical carcass analysis and TOBEC ranged from -8.2 to 10.2% (3), which would translate into significant errors in total body fat mass. In our study, DXA was accurate in estimating lean mass and suggests that DXA rather than TOBEC may be the more appropriate method of measuring FFM in small infants. The new software for DXA measurements provided with the Hologic QDR1000W machine improved the estimates of both lean and fat mass; however, absolute fat mass was still overestimated compared with fat extraction from the carcass in all piglets. Unlike the previous software, the values for fat determined by InfWB and chemical analysis were significantly correlated. The overestimation of fat mass by DXA produced a systematic error, in contrast to TOBEC in which the error in measurement was variable (3). A significant correlation does not imply accuracy (18), but provides a basis from which a mathematical model may be developed to correct InfWB fat values. However, such a model would be limited in its application to animals of similar size and body composition. Further validation is required to determine whether the error in accuracy of fat mass by DXA in a piglet with less than 6% body fat can be extrapolated to human infants who normally have a much higher proportion of body weight as fat (*i.e.* >20%).

DXA estimates of fat are subject to low precision (CV = 6.6%) even in large piglets. Li *et al.* (10) reported a CV of

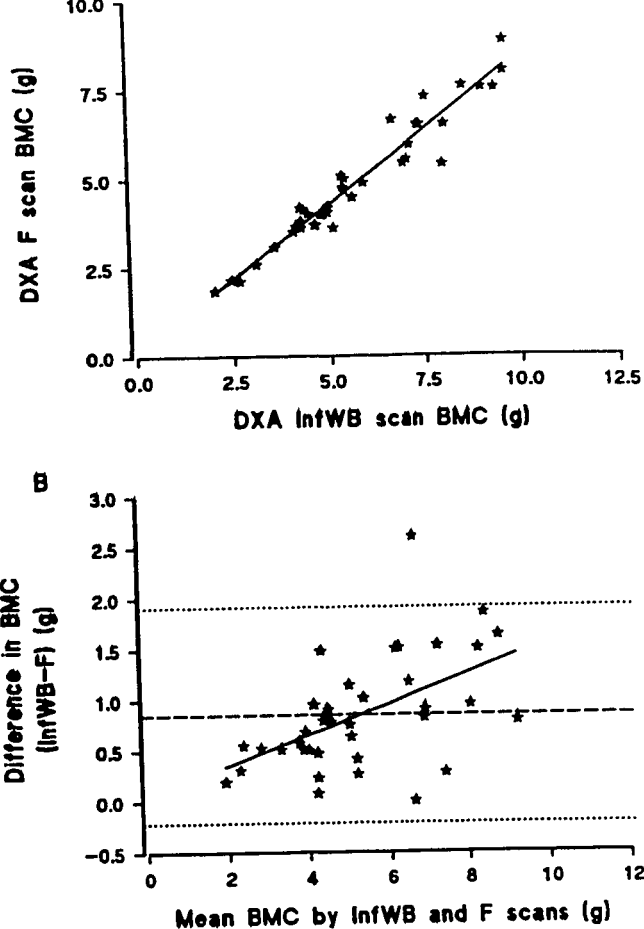


Figure 2. Linear regression comparing (A) InfWB BMC to F BMC ( $y = 0.83x + 0.10$ ,  $r = 0.97$ ,  $p < 0.01$ ) and (B) the difference between InfWB and F BMC plotted against the mean BMC by the method of Bland and Altman (16). Hatched lines in B represent the mean ± 2 SD,  $y = 0.15x + 0.04$ ,  $r = 0.52$ ,  $p < 0.01$ .

7.0% for total body fat calculated from duplicate scans in infants of 3-17 mo of age using a Hologic QDR 1000W machine. If one accepts this precision value in infants, then it appears that measurement error is not influenced by differences in the proportion of body fat between piglets and infants. Unless fat accretion between DXA scans exceeds the variability of the measurement, interpretation of serial DXA measures may be difficult. Our longitudinal measures of body composition in small infants demonstrated the importance of timing of serial DXA measurements for total body fat (Fig. 3A). Between 6 and 12 mo the mean delta fat was less than the variability of the measurement. However, when the motive is to collect data to develop reference standards, or to conduct a clinical intervention trial, it is possible to calculate a sample size large enough to overcome the lack of precision (15). For our infants measured at ages 6 and 12 mo, it would take only four infants to confirm that the 14% change in body fat is not an error of precision.

In contrast to other validation studies (8, 9, 11, 19), we did not find a significant correlation between InfWB and carcass

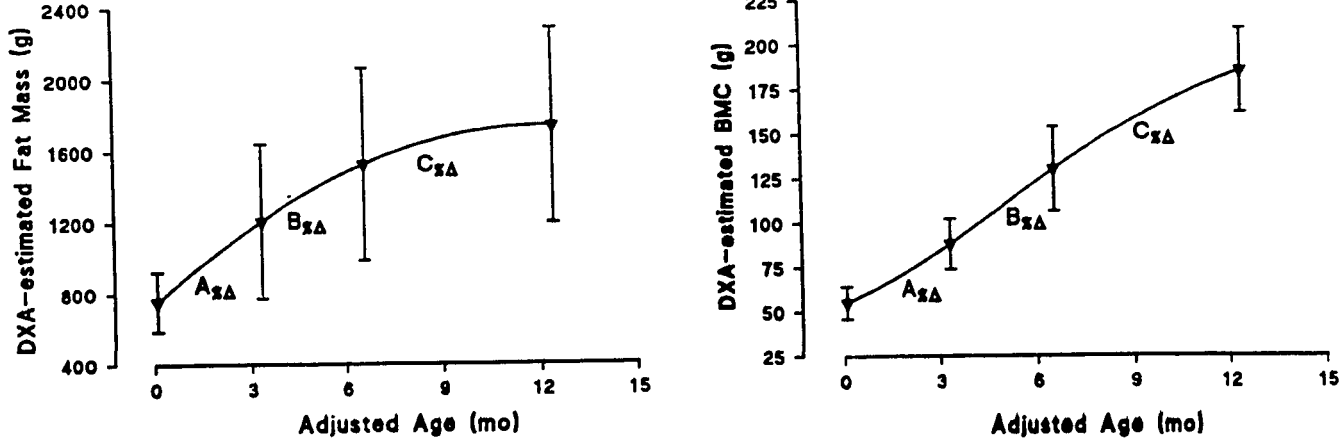


Figure 3. The mean DXA-estimated (A) fat mass and (B) whole body BMC of a group of prematurely born infants measured serially from term age to 12 mo adjusted age. (A) To detect a true or significant change in fat mass, the percent difference (% $\Delta$ ) between measurements must be  $>18.7\%$  (based on a CV of 6.6%) (15). Premature infants:  $A_{\% \Delta} = 60.2\%$ ,  $B_{\% \Delta} = 26.5\%$ ,  $C_{\% \Delta} = 14.0\%$ . (B) To detect a true or significant change in BMC, the percent difference (% $\Delta$ ) between measurements must be  $>3.1\%$  (based on a CV of 1.1%) (15). Premature infants:  $A_{\% \Delta} = 60.0\%$ ,  $B_{\% \Delta} = 47.7\%$ ,  $C_{\% \Delta} = 42.3\%$ .

Table 5. Repeated whole body scans (using InfBW) of a small piglet (1731 g) and a large piglet (6416 g) with and without artifacts in the scan field

Condition	Mean weight (g)	CV%	Mean BMC (g)	CV%	Mean lean (g)	CV%	Mean fat (g)	CV%	Mean %fat	CV%
Small piglet										
No artifact	1727	0.1	40	0.7	1582	0.5	105	8.9	6.1	8.9
Infant Table Pad	1728	0.1	39	1.1	1535	0.3	155	2.8	8.9	2.6
Flannel blanket*	1772	0.1	42	1.1	1612	0.5	119	6.9	6.7	6.8
Disposable diaper†	1740	0.1	40	1.3	1589	0.4	111	4.6	6.4	4.2
Flannel nightgown‡	1762	0.1	42	2.4	1605	0.4	114	6.7	6.5	5.6
Large piglet										
No artifact	6532	0.2	109	0.9	6042	0.04	381	0.8	5.8	0.8
Infant Table Pad	6553	0.1	110	1.7	6037	0.4	408	7.1	6.2	7.0
Flannel blanket*	6618	0.02	110	2.7	6124	0.3	384	4.4	5.8	4.2
Disposable diaper†	6565	0.1	110	2.5	6038	0.1	417	1.0	6.4	0.7
Flannel nightgown§	6624	0.1	112	3.2	6106	0.3	406	4.5	6.1	4.7

\* Flannel blanket (100% cotton) weight = 130 g.

† Disposable diaper weight = 37 g.

‡ Small flannel nightgown weight = 88 g.

§ Large flannel nightgown weight = 137 g.

ash in small piglets. The small piglets were within a narrow body weight range (1431–1710 g), thus the range of total mineral ash was also quite narrow (31–43 g). This minimizes the possibility of a significant correlation between DXA-estimated BMC and carcass analysis (16). Even though the new InfWB is improved over the PedWB software (Table 2), our results and others (8, 9) suggest that DXA underestimates BMC in small subjects. The lack of accuracy in estimating BMC has been attributed to nonosseous mineral in total carcass ash (8, 19). However, DXA underestimated BMC for small piglet whole body (16%, Table 2) and femur (12.5%, Table 4) to a similar degree implying that nonosseous mineral does not account for differences between DXA-estimated BMC and carcass ash. It does, however, imply that the InfWB program for this DXA instrument has a detection limit for estimating BMC and as mineral content decreases, the error of estimation increases.

The high correlation but lack of absolute agreement between InfWB and F estimates of femur BMC demonstrates the inappropriateness of comparing two nonreference methods by regression analysis (16). To compare InfWB and F regional scan results, the difference in DXA-estimated BMC between the two methods was compared with the mean of the two estimates (16) (Fig. 2). This revealed a trend for the difference between scan results to increase as the size of the femur increased. However, by expressing the difference between scan estimates of femur BMC as a percent, the relationship between the error of estimate and bone size disappears, implying a systematic error.

Reliable measures of body composition in infants using DXA are further confounded by movement artifacts and clothing such as diapers and covers. Although it might be easier to restrict movement while scanning a single appendage such as the femur rather than the whole body, our data show that the accuracy of the femur bone mineral content was better using

size is likely a reflection of the differences in bone thickness in small and large piglets in comparison with the whole body and to that found in adults for whom the software was intended. Furthermore, both our data and that of Koo *et al.* (8) showed that artifacts such as the Infant Table Pad provided by Hologic or diapers and blankets do not interfere with the accuracy or precision of bone mineral measurements. Based on these results, we conclude that any regional analysis of bone in infants is likely best estimated from whole body scans.

Measures of fat and lean by DXA are more influenced than bone by artifacts. The Infant Table Pad demonstrated no convincing evidence of an advantage with its use. The radiation exposure for an infant whole body scan is very low at  $7\mu\text{Sv}$  (0.7 mrem) without the Infant Table Pad, and  $3\mu\text{Sv}$  (0.3 mrem) with the Pad (8). This minimal change in safety value must be weighed against measurement differences, because InfWB-estimated total fat mass increased from 105 to 155 g with the use of the Pad. Further study of the influence of the Infant Table Pad on the measurement of soft tissue is required before the Pad should be used for routine use with infants. Until accurate measures of soft tissue are demonstrated with the Pad, single infant scans will minimize radiation exposure while avoiding the use of sedatives to accommodate prolonged scan times.

It is apparent that larger artifacts in the scan field have a more profound effect than smaller artifacts, likely affecting both the precision and accuracy of soft tissue estimates. When blankets or nightgowns are tightly bundled around the piglet, InfWB cannot completely distinguish between animal and artifact. It is likely that only the portion of the artifact that is very close to the subject's body (*i.e.* under and over the subject) is included in the scan as soft tissue. The flannel material was divided as lean and fat tissue, although it was not a consistent division, with lean mass accounting for 63–95% of the weight added by the various flannel artifacts tested. Diapers also altered fat and lean mass to variable degrees depending on the size of the diaper (data not shown). This may be the result of different manufacturing practices, or it may also be a reflection of the lack of precision and accuracy of InfWB measurement of fat mass.

DXA technology has improved such that it is a useful tool for estimating the total body composition of rapidly growing infants if used with caution. The experiments with various artifacts exemplify the need to conduct measurements in a consistent manner with respect to blanketing practices and

tissue measures are now possible. Clinically useful data can be obtained in small growing subjects using DXA with a specially designed software application program such as InfWB, if serial measures of body composition are at intervals which take into account the change expected, and the precision of the instrument.

**Acknowledgments.** The authors thank Ine Wauben and Michelle Whelan for assisting with the scans in piglets and infants.

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**NUTRITIONAL INTERVENTION  
FOR INFANTS  
WITH BRONCHOPULMONARY DYSPLASIA**

**CHAPTER 5                    GROWTH DURING NUTRITIONAL INTERVENTION: A  
RANDOMIZED, CONTROLLED CLINICAL TRIAL**

**Long term growth, nutrient accretion and body composition in infants with bronchopulmonary dysplasia: A randomized trial of a high energy nutrient enriched formula versus a high energy standard formula fed after hospital discharge.**

Janet A. Brunton, BAsC., Saroj Saigal, MD and Stephanie A. Atkinson, PhD

The following manuscript is the first paper resulting from a large randomized nutrition intervention trial. The multiple authorship can be explained as follows:

**Dr. Stephanie Atkinson** is the principal investigator of this study, and is the thesis advisor to the Ph.D. candidate.

**Dr. Saigal** is also a principal investigator. She was responsible for the long term developmental follow-up of the infants who participated in this study, which was assessed as an outcome, but will be published in a separate manuscript, and is not part of this thesis. Dr. Saigal also acted as an informal advisor to the Ph.D. candidate, and participated in the evolution of these data into the manuscript.

**The Ph.D. candidate** was the writer of this manuscript, and was involved in the design and the process of attaining funding for the study. Most of the measurements in the study were conducted by her, with the assistance of a skilled research nurse. All analytical work and data analyses were done by the Ph.D. candidate, under the direct supervision of Dr. Atkinson.

This chapter has been submitted to the Journal of Pediatrics (February 1997).

Long term growth, nutrient accretion, and body composition in infants with bronchopulmonary dysplasia: A randomized trial of a high energy nutrient enriched formula versus a high energy standard formula fed after hospital discharge.

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Funded by a grant from the Ontario Ministry Health, with formula manufactured to investigators' specifications and donated by Wyeth-Ayerst Int., Radnor, PA..



**ABSTRACT**

*Objectives:* 1) To determine if feeding infants with BPD with a nutrient enriched formula (EF) to 3 months corrected (CA) age would improve rate of growth with greater lean and bone mass accretion when compared to infants fed an isoenergetic standard infant formula (SF). 2) To determine whether nutrient malabsorption and/or inadequate nutrient intake were involved in the etiology of growth delay observed in BPD infants.

*Study Design:* A blinded, nutrition intervention trial of 60 preterm infants with BPD (birth weight =  $866 \pm 169$  g, gestational age =  $26 \pm 1.5$  weeks) who were randomized prior to hospital discharge to either EF (n=26) or SF (n=30). Growth, body composition and nutrient retention were compared between groups by Student's t-tests, and ANCOVA.

*Results:* Infants fed the EF had significantly greater nitrogen and mineral retention at 38 wk post-menstrual age compared to the SF fed infants, and only the EF infants had zinc retention similar to the intrauterine accretion. At 3 mo CA EF infants attained greater length ( $p < 0.05$ ), greater radial bone mineral content ( $p < 0.01$ ), and greater lean mass ( $p < 0.01$ ) compared to the SF infants. The male EF infants had greater whole body bone mineral content than SF males ( $p = 0.02$ ).

*Conclusions:* Enhanced linear growth, lean and bone mass in the EF group suggests that BPD infants utilize high concentrations of protein and minerals beyond hospital discharge.

**ABBREVIATIONS**

BPD - bronchopulmonary dysplasia

CA - corrected age (age adjusted for prematurity based on last menstrual period)

DXA - dual energy x-ray absorptiometry

EF - enriched formula

PMA - post-menstrual age

SF - standard formula

## INTRODUCTION

Both early (1) and late (2,3,4,5) growth failure, as well as delayed skeletal mineralization (6) have been documented in preterm infants who survived with BPD. Such infants are born nutritionally compromised with limited body stores due to a shortened gestation. Inadequate provision of nutrients during the acute phase of lung disease will quickly deplete meagre stores (7). Current medical management of BPD infants, which employs fluid restriction and prolonged courses of catabolic steroids (8) and diuretics (9), further jeopardizes the nutritional status and growth.

Factors previously identified which may contribute to growth failure include elevated metabolic rate secondary to the "work of breathing" (10), and inadequate intake and/or malabsorption of nutrients (11), but none of these factors has been thoroughly investigated in a controlled study. Furthermore, while catch-up growth has not been achieved within 3 years of age (adjusted for prematurity) (2,4), no long term, controlled nutritional intervention study has been conducted in an attempt to induce catch-up growth during recovery from BPD.

Since elevated metabolic rate has been identified at post-term age in infants with BPD who were growth delayed (12), supplemental energy (as carbohydrate or lipid) added to standard term infant formulas has been recommended (13). But the efficacy of such diets in promoting normal growth and body composition has not been established. Data on nutrient accretion in healthy premature infants during early neonatal life fed varying protein/energy ratios support the need for a high protein to

energy ratio (relative to term infant formulas) in order to deposit lean tissue as opposed to fat mass (14,15,16,17). However, whether the same protein/energy ratio is beneficial beyond term age in infants with growth failure is uncertain. If metabolic rates are indeed elevated for a prolonged period of time, then energy alone may compensate and support growth after hospital discharge, without adding further metabolic stress from added protein and minerals.

We have conducted the first randomized, blinded, intervention trial in infants with BPD to evaluate two different infant formulas: 1) with a composition based on the current feeding practice of increasing the energy concentration of standard term infant formula or 2) a formula with a high protein to energy ratio and greater concentration of minerals. Our primary goal was to assess the effect of the formulas on the rate of growth and composition of growth (lean, fat and bone mineral accretion) when groups were compared at 3 months corrected age. Our secondary goal was to determine whether nutrient malabsorption, and/or inadequate nutrient intake contributed to long term growth failure previously observed in infants with BPD.

## **PATIENTS AND METHODS**

Infants were recruited from the Neonatal Intensive Care Units at The Children's Hospital of Chedoke-McMaster, and St. Joseph's Hospital, Hamilton, Ontario between January 1991 to November 1994. The study was approved by the Research Project Advisory Committees at both hospitals. Infants were eligible for the study if they

weighed < 1500 g at birth, had BPD and were formula fed by parental choice. Infants were diagnosed with BPD by the attending physician using the following criteria (18): 1) oxygen or ventilatory support at day 28 post-natal age, 2) radiographic changes indicative of BPD (19), and 3)  $p\text{CO}_2 > 50$  mm Hg. Infants were excluded if they were small for gestational age, had major congenital anomalies or gastrointestinal surgery or resection. Informed consent for enrollment was obtained only after the diagnosis of BPD was confirmed. An overview of the complete study protocol, including in-hospital and post-discharge procedures, is presented in Figure 1.

*In hospital study protocol* The study protocol was initiated only when the infants were stable, growing and receiving full oral feeds. A baseline 72 hour mass balance for nutrient retention was conducted while infants were fed standard premature formulas (SMA Premie [3200 kJ/L or SMA [3800 kJ/L], Wyeth-Ayerst Ltd, Toronto, Ontario). Distal 1/3 radial bone mineral content using single photon absorptiometry (SPA) (Norland Instruments, Fort Atkinson, WI) was also measured at baseline. SPA measures were conducted in triplicate without repositioning. Our precision (CV = 3.4%) was similar to that reported in the literature (20).

Bi-weekly measures of weight, length using a polyacrylic recumbent board, and head circumference using a non-stretchable plastic tape were conducted by the trained study team until the infants reached approximately 38 weeks PMA . After 37 weeks PMA infants were randomized to an enriched high energy (3760 kJ/L), high protein,

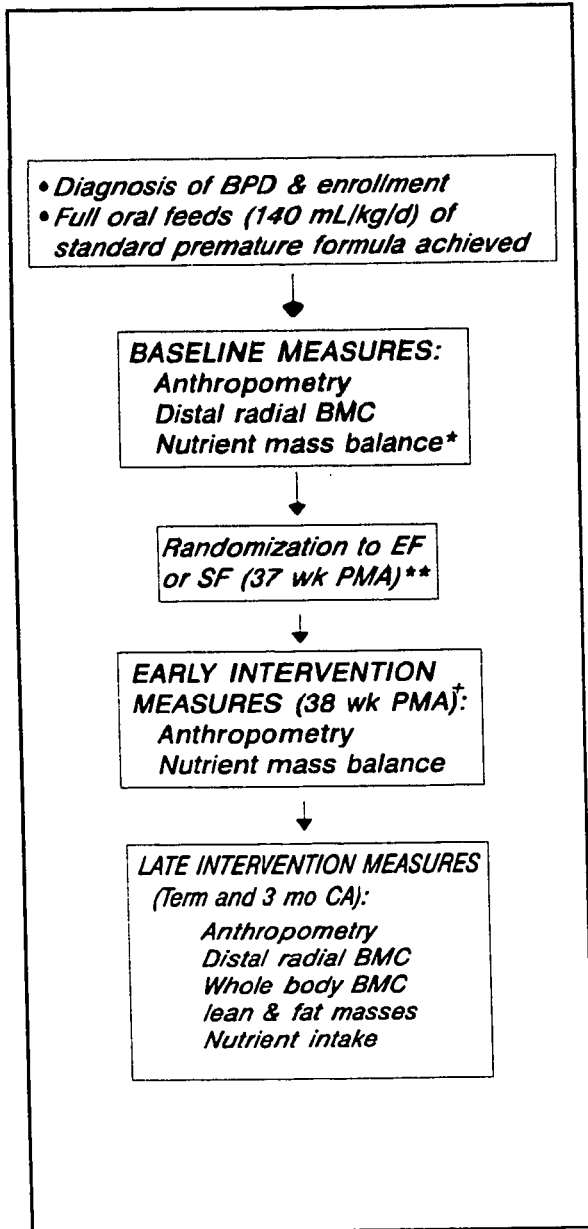


Figure 1. Overview of the study protocol.

BMC = bone mineral content

EF = enriched formula

SF = standard formula

PMA = post-menstrual age

CA = corrected age

\*Nutrient mass balance was conducted on a subset of infants at baseline.

\*\*Randomization was stratified for birth weight (< or ≥ 1000 g) and severity of disease, determined by respiratory support that was provided (ventilation or oxygen) on day 28 of life.

+Early intervention measures were conducted on a subset of infants.

high mineral formula (EF), or a standard isoenergetic (3760 kJ/L) formula (SF). The composition of the formulas (Table 1), which were provided by Wyeth-Ayerst International (Radnor, PA) was verified by analysis. Hospital personnel were requested to prescribe a volume of formula of 140 mL/kg·d<sup>-1</sup>; if fluid restriction was necessary for the treatment of BPD, this superseded the study protocol.

Randomization was ensured by the use of sealed envelopes. Treatment groups were stratified by birth weight, and severity of disease as determined by the use of supplemental oxygen alone versus mechanical ventilation on day 28 post-natal age. Parents, NICU staff and all research team members were blinded to the nutritional intervention. For the duration of the study, all outcome assessments including measurements of growth and body composition, mass balance collection procedures and biochemical analyses were conducted under blinded conditions.

A second mass balance period was conducted a minimum of seven days after randomization. Infants who were discharged to a community hospital prior to adaptation to the study formula did not participate in the second balance period. Also, some infants recruited into the study were too medically unstable, or discharged to a level II nursery too early to participate in the full study. These infants were randomized to study formula and participated only in the long term growth follow-up, with no in-hospital nutrient balance studies.

Table 1 Label claim nutrient composition\* of the enriched and the standard study formulas.

<u>Nutrient (per L)</u>	<u>Standard</u>	<u>Enriched</u>
Energy (kJ)	3760	3760
Protein (g)	15	23
Fat (g)	50	47
Carbohydrate (g)	90	90
Calcium (mmol)	10	25
Phosphorus (mmol)	9	20
Zinc (umol)	77	165
Vitamin D (IU)	2430	2430
Vitamin A (IU)	8100	8100
Osmolality (mOsm/L)	380	450

\*Formula concentrations of osmolality, energy, protein and minerals were confirmed by chemical analysis.



*Nutrient balance* The 72 hour mass balances were accomplished by using a condom-like urine collector (21), with stools collected on ashless filter papers fitted into the diaper. All feeds were pre-measured by the study team, and all feeding apparatus (syringes, bottles, feeding tubes) were collected and rinsed to correct intake for residual losses. Protein deposition during the balance periods was calculated as nitrogen retained ( $\text{g/kg}\cdot\text{d}^{-1}$ )  $\times$  6.25.

*Post-hospital discharge protocol* Infants were discharged from hospital with a supply of study formula in 120 mL ready-to-feed bottles and a vitamin D<sub>3</sub> supplement, D-Vi-Sol (Mead Johnson Ltd, Ottawa, ON). Parents were instructed to give 400 IU of vitamin D per day. No other vitamin supplements were prescribed; however, attending physicians were informed that the formulas were not iron fortified, so virtually all infants received supplemental iron. Parents were instructed to feed their infants ad libitum.

Infants returned to the Growth and Development Clinic at The Children's Hospital at Chedoke-McMaster at approximately term and three months corrected age as part of the routine follow-up care. During these visits, measurements of weight, length, head circumference and distal 1/3 radial bone mineral content by SPA were repeated. Measures of weight and length were converted to National Center for Health Statistics (NCHS) standard deviation scores (Z-scores) (22), to normalize the data for sex and variability in timing of follow-up measures. Total body composition including lean and fat masses, and whole body bone mineral content was measured using dual

energy x-ray absorptiometry (DXA) (Hologic QDR 1000/W, Waltham, MA). Single scans were conducted with infants dressed only in a disposable diaper, wrapped in a cotton flannel receiving blanket, while sleeping prone on the scan bed. No sedation was used. Previous validation studies of DXA using piglets demonstrated that the CV% for the measurement of lean, fat and bone mineral masses were 0.6, 6.6 and 1.1%, respectively (23).

At the clinic visit, parents were provided with a five day supply of pre-weighed bottles of ready-to-feed study formula. Unused portions of formula were subsequently returned to the laboratory and re-weighed to assess nutrient intake. Parents were instructed not to introduce foods other than the formula provided. However, if infant cereal was used, then parents were instructed to use measuring spoons to accurately record the amount of cereal consumed.

*Biochemical analyses* Aliquots of 24 hour urine collections were frozen at -20°C for analysis. Urine calcium and zinc concentrations were measured directly by flame atomic absorption spectrophotometry (Perkin-Elmer, Model 703, Norwalk, Connecticut). Stools were scraped off the papers and pooled (72 hours). Stool and residual calcium and zinc were also measured by flame atomic absorption spectrophotometry after digestion as follows. Samples were lyophilized and ground to a fine powder, then 0.25 g was wet ashed in 5 mL concentrated nitric acid under pressure using microwaves at full power for 10 minutes, after a gradual stepwise increase in power over 40 minutes (CEM Microwave Digestion System, model MDS

2000, CEM Corporation, NC). The stool remaining on the filter papers after scraping was leached off by soaking in 500 mL of 10% HCl for 48 hours. The effluent was analyzed for calcium and zinc by atomic absorption spectrophotometry. A second soaking resulted in negligible mineral remaining (<5% of the amount leached in the first soaking for both calcium and zinc) and ashing the papers after soaking resulted in less than 0.001 mmol Ca and less than 0.8  $\mu$ mol zinc remaining. Triplicate testing of known quantities of stools added to filter papers demonstrated that this method had a precision (as measured by CV%) of 3.3% and recovery of 94% for calcium.

Phosphorus concentration was measured in the same digested samples and in urine using a colorimetric assay (24). Nitrogen content of the formulas, lyophilized stools and feeding apparatus residuals, urine, and filter paper effluent was measured by the micro-Kjeldahl method (25). All mineral and nitrogen analyses were done in duplicate or triplicate, and the mean %CV was less than 5%. Accuracy was ensured by the use of reference materials including desiccated bovine liver (1577b, National Bureau of Standards, Washington DC, 1991) and certified non-fat milk powder (1549, National Institute of Standards, Washington DC, 1984) for nitrogen and mineral analyses of lyophilized material, and urea standards for urine nitrogen analysis. Recovery of nitrogen and mineral for all assays was >90%. Energy content of liquid formulas was measured by combustion bomb calorimetry (Parr Instruments, Moline, IL) (26).

*Statistics* The sample size for this study was calculated based on the outcome of bone mineral content by SPA, and a difference of 25 mg/cm was used as the  $\delta$  value, which

represents the difference in radial bone mineral content between healthy preterm infants (27) and infants with BPD at 6 mo CA (6). Using a pooled standard deviation of 16.2 mg/cm (6, 27) with  $\alpha = 0.01$  and  $\beta = 0.2$ , then nine infants per group were required. Sixty infants were required to assess developmental outcomes at 12 mo CA (to be reported separately). This was calculated based on detecting a group difference in Bayley Scores of 10 points (standard deviation = 15 points), with an  $\alpha = 0.02$  and  $\beta = 0.2$ . Since no data were available to estimate sample size for body composition measures after hospital discharge, growth, DXA and SPA measures were conducted on all recruited infants. Differences in nutrient intake, absorption and retention were determined by Student's t-test. Weight, length and head circumference at 3 months corrected age, as well as rates of change between randomization and term, and term and 3 mo were compared between groups by ANCOVA (BMDP Student Version, BMDP Statistical Software, Inc., Los Angeles CA), taking into consideration birth weight, gestational age at birth, cumulative dexamethasone therapy, days to regain birth weight, days to full oral feeds, days on a ventilator, length of intervention and sex. Radial bone mineral accretion was determined by calculating individual slopes from measures at randomization, term and 3 mo CA, and comparing mean slopes between groups by ANOVA. Absolute differences in body composition as measured by DXA at 3 mo CA were compared by ANOVA, grouped by treatment and sex.

## RESULTS

A total of 60 infants were randomized to one of the two nutritional interventions. Four infants in the EF group did not complete the study for the following reasons: 1) suspected lactose intolerance, which ultimately was not demonstrated, 2) parental request to discontinue because of "colic", 3) recurrent excoriated buttocks and 4) infant death resulting from respiratory complications. These infants participated in the in-hospital balance periods, and their data are included. The two groups of infants were similar in neonatal characteristics (Table 2).

### *Baseline Measures of Growth and Nutrient Balance Studies*

Outcomes of growth and nutrient retention for infants prior to randomization (while fed standard premature formula) are presented in Table 3. Post-hoc analysis demonstrated that there were no differences between treatment groups in formula intake, energy intake, daily weight gain, net daily protein accretion, calcium, phosphorus, zinc or nitrogen absorption or retention (Table 3).

### *Nutritional Intervention Period*

At the time of randomization, the EF and SF groups of infants were similar (age =  $36.8 \pm 1.7$  wk PMA, body weight =  $2.01 \pm 0.33$  kg, length =  $41.4 \pm 2.2$  cm head circumference =  $31.3 \pm 1.7$  cm, mean  $\pm$  SD). After randomization, EF and SF infants received similar daily volumes of formula and energy intake (by study design) (Table 4). The early metabolic responses by the EF fed group included significantly greater absolute absorption and retention of all nutrients analyzed, but the percent absorption was different only for phosphorus (Table 4).

Table 2 Characteristics of infants studied as randomized to the standard and enriched formulas.

	<u>Standard</u>	<u>Enriched</u>
n (m/f)	30 (13/17)	30 (18/12)
Birth weight (g)	858 ± 167 <sup>†</sup>	874 ± 173
Gestational age (wk)	26.0 ± 1.6	26.0 ± 1.4
Mechanical ventilation (d)	38 ± 18	38 ± 18
Birth weight re-gained (d)	20 ± 7	20 ± 6
Full oral feeds attained (d)	22 ± 11	23 ± 10
Cumulative dexamethasone dose (mg/kg)	6.0 ± 3.7	6.4 ± 4.1
Age at term visit (mo) <sup>‡</sup>	0.7 ± 0.4	0.8 ± 0.5
Age at 3 mo visit (mo) <sup>‡</sup>	3.2 ± 0.4	3.4 ± 0.6
Duration of intervention (mo)	4.0 ± 0.5	4.2 ± 0.7

<sup>†</sup>Values are mean ± SD

<sup>‡</sup>Age adjusted for prematurity

Mean daily weight gain was not different between groups, however the EF fed group had a net daily protein accretion that was significantly greater than the SF fed group (Table 4).

**Nutrient Intake Post-Hospital Discharge** There were also no significant differences in volume of formula intake or energy intake between groups at term or 3 mo CA, when intake was ad libitum (Table 5). During this time, EF fed infants continued to have significantly greater intakes of protein, calcium, phosphorus and zinc (Table 5). Reflux was identified in 5 EF and 10 SF fed infants, therefore the higher osmolality of the EF formula did not appear to induce formula intolerance.

**Growth and Body Composition During Nutritional Intervention** Z-scores-for-age were similar between groups at the first follow-up visit (1 mo CA) (Table 6), but at 3 mo CA the infants fed the EF were significantly longer (Table 6). There were no group differences in mean head circumference at 1 or 3 mo CA.

Secondary analyses demonstrated that the EF group had fewer infants below the 3rd percentile for length at 3 mo CA (38% versus 70 %,  $p < 0.05$ ). Also, the EF and SF groups had similar weight-for-age Z-scores at 1 and 3 mo CA, but only the SF group had a mean positive weight-for-length Z-score which was different than zero ( $0.51 \pm 0.8$ ,  $p < 0.01$ ).

Rates of length and weight gain were different between formula groups prior to but not after the first follow-up visit at 1 mo CA. From the point of randomization to

Table 3 Baseline measures of energy and formula intake, growth, and nutrient balance for all infants while receiving standard premature formula.

<b>At initiation of nutrient mass balance period:</b>	
n (m/f) <sup>†</sup>	27 (14/13)
Post-menstrual age at balance (wk)	34.5 ± 1.4 <sup>‡</sup>
Weight (kg)	1.51 ± 0.2
Length (cm)	38.9 ± 1.9
Head circumference (cm)	29.0 ± 1.3
<b>Intake:</b>	
Formula volume (ml/kg·d <sup>-1</sup> )	144 ± 9
Energy (kJ/kg·d <sup>-1</sup> )	470 ± 31
<b>Growth:</b>	
Weight gain (g/kg·d <sup>-1</sup> )	15.5 ± 12.4
Net protein accretion (g/kg·d <sup>-1</sup> )	1.97 ± 0.49
<b>Nutrient balance:</b>	
<b>Calcium (mmol/kg·d<sup>-1</sup>):</b>	
Intake	2.76 ± 0.26
% Absorption	78 ± 13
Retention	2.07 ± 0.37
<b>Zinc (μmol/kg·d<sup>-1</sup>):</b>	
Intake	17.2 ± 1.8
% Absorption	14 ± 39
Retention	1.7 ± 6.8
<b>Phosphorus (mmol/kg·d<sup>-1</sup>):</b>	
Intake	1.74 ± 0.16
% Absorption	96 ± 3
Retention	1.54 ± 0.20
<b>Nitrogen (mg/kg·d<sup>-1</sup>):</b>	
Intake	447 ± 46
% Absorption	96 ± 3
Retention	316 ± 79

<sup>†</sup>There were no differences in birth weight or gestational age at birth between the infants who participated in the balance studies, and the total group of infants in the growth study (see text for details).

<sup>‡</sup>Values are mean ± SD



Table 4 Early metabolic responses by infants to the enriched or the standard formulas.

	<u>Standard</u>	<u>Enriched</u>
<b>At initiation of nutrient mass balance period</b>		
n (m/f) <sup>‡</sup>	13 (6/7)	10 (8/2)
Post-menstrual age (wk)	37.9 ± 1.6	38.2 ± 1.4
Weight (kg)	2.15 ± 0.2	2.25 ± 0.2
Length (cm)	42.3 ± 2.0	42.3 ± 2.0
Head circumference (cm)	31.5 ± 1.2	32.1 ± 1.2
<b>Intake:</b>		
Formula volume (mL/kg·d <sup>-1</sup> )	145 ± 12	142 ± 22
Energy (kJ/kg·d <sup>-1</sup> )	560 ± 53	555 ± 54
<b>Growth:</b>		
Weight gain (g/kg·d <sup>-1</sup> )	11.9 ± 2.9	10.8 ± 3.0
Net protein accretion (g/kg·d <sup>-1</sup> )	1.51 ± 0.41	2.21 ± 0.17**
<b>Nutrient balance:</b>		
<b>Calcium (mmol/kg·d<sup>-1</sup>):</b>		
Intake	1.53 ± 0.14	3.87 ± 0.70**
Absorption	1.00 ± 0.25	2.60 ± 0.78**
% Absorption	65 ± 16	67 ± 16
Retention	0.95 ± 0.25	2.52 ± 0.78**
% Retention	62 ± 16	65 ± 16
<b>Zinc (μmol/kg·d<sup>-1</sup>):</b>		
Intake	13.7 ± 1.13	23.7 ± 3.82**
Absorption	-0.12 ± 7.5	6.60 ± 6.9*
% Absorption	-1 ± 54	29 ± 30
Retention	-0.51 ± 7.5	6.30 ± 7.0*
% Retention	-4 ± 54	28 ± 31

Table 4 continued...

Table 4 Continued

	<u>Standard</u>	<u>Enriched</u>
<b>Nutrient balance (continued) :</b>		
<b><i>Phosphorus</i> (mmol/kg·d<sup>-1</sup>):</b>		
Intake	1.52 ± 0.12	3.11 ± 0.56**
Absorption	1.44 ± 0.13	2.76 ± 0.48**
% Absorption	94 ± 4	89 ± 5*
Retention	1.20 ± 0.18	2.39 ± 0.54**
% Retention	77 ± 7	76 ± 7
<b><i>Nitrogen</i> (mg/kg·d<sup>-1</sup>):</b>		
Intake	366 ± 28	490 ± 51**
Absorption	327 ± 37	446 ± 38**
% Absorption	89 ± 6	91 ± 5
Retention	262 ± 48	365 ± 34**
% Retention	72 ± 10	75 ± 8

†Values are mean ± SD

\*There were no differences in birth weight or gestational age at birth between the infants who participated in the balance studies, and the total group of infants in the growth study (see text for details)

\*p < 0.05 and \*\* p < 0.01, EF versus SF .

Table 5 Nutrient intakes calculated from five days of weighed formula intake for infants fed the enriched or standard formula at 1 and 3 mo corrected age.

	Standard	Enriched
<u>1 mo CA</u>		
Formula volume (mL/kg·d <sup>-1</sup> )	140 ± 35 <sup>†</sup>	130 ± 25
Energy (kJ/kg·d <sup>-1</sup> )	536 ± 133	491 ± 95
Protein (g/kg·d <sup>-1</sup> )	2.1 ± 0.6	2.8 ± 0.6*
Calcium (mmol/kg·d <sup>-1</sup> )	1.7 ± 0.5	3.8 ± 0.9*
Phosphorus (mmol/kg·d <sup>-1</sup> )	1.6 ± 0.5	3.0 ± 0.7*
Zinc (μmol/kg·d <sup>-1</sup> )	13.2 ± 3.2	22.9 ± 4.8*
<u>3 mo CA</u>		
Formula volume (mL/kg·d <sup>-1</sup> )	130 ± 30	116 ± 24
Energy (kJ/kg·d <sup>-1</sup> )	496 ± 116	438 ± 92
Protein (g/kg·d <sup>-1</sup> )	2.0 ± 0.5	2.5 ± 0.5*
Calcium (mmol/kg·d <sup>-1</sup> )	1.7 ± 0.5	3.2 ± 1.1*
Phosphorus (mmol/kg·d <sup>-1</sup> )	1.6 ± 0.6	2.6 ± 0.9*
Zinc (umol/kg·d <sup>-1</sup> )	11.6 ± 3.0	17.9 ± 4.7*

† Values are mean ± SD

\* Enriched versus standard,  $p < 0.001$

Table 6 Z-scores-for-age and mean head circumference at term (1 mo CA) and 3 mo CA for infants fed SF or EF study formulas.

	Standard	Enriched
<u>1 mo CA</u>		
Weight-for-age	-1.49 ± 0.8 <sup>†</sup>	-1.14 ± 0.8
Length-for-age	-2.60 ± 1.1	-2.36 ± 1.1
Head circumference	35.4 ± 0.7	35.8 ± 1.9
<u>3 mo CA</u>		
Weight-for-age	-1.42 ± 0.9	-1.05 ± 1.1
Length-for-age	-2.36 ± 0.9	-1.80 ± 1.2*
Head circumference (cm)	39.8 ± 1.6	40.3 ± 1.6

<sup>†</sup> Values are mean ± SD

\* EF versus SF, p < 0.05

the measurement at 1 mo CA, length growth for infants fed EF was  $1.0 \pm 0.2$  cm/wk compared to  $0.8 \pm 0.2$  cm/wk for SF infants ( $p < 0.01$ ). Length of time on the formulas, sex or age at randomization did not influence the outcome. Weight gain during the period from randomization to 1 mo CA was also significantly enhanced by the EF compared to SF ( $28 \pm 9$  versus  $21 \pm 6$  g/d,  $p < 0.01$ ). Weight at randomization was a significant co-variable ( $p < 0.01$ ), having an inverse correlation with rate of weight gain. There was no effect of the treatment on head circumference growth in the period prior to 1 mo CA, or on growth rates (weight, length or head circumference) during the period from 1 mo CA to the end of the intervention at 3 mo CA. The rate of length growth in the EF group between term and 3 mo CA was significantly less than the rate between randomization and 1 mo CA ( $0.8 \pm 0.2$  versus  $1.0 \pm 0.2$  cm/wk,  $p < 0.01$ ). In contrast, the SF group did not demonstrate a change in the rate of length growth after the 1 mo CA visit ( $0.8 \pm 0.2$  versus  $0.8 \pm 0.1$  cm/wk,  $p = 0.21$ ). The daily rate of weight gained declined significantly between 1 and 3 mo CA for the EF infants to  $22 \pm 9$  g/d ( $p < 0.001$ ), while the SF group remained constant ( $22 \pm 8$  g/d).

The absolute radial bone mineral content was similar between treatment groups at entry into study, but both the rate of bone mineral accretion and the absolute bone mineral content were significantly greater in the EF group compared to the SF group at 3 mo CA (Figure 2). When distal radial BMC at 3 mo CA was expressed as a function of body weight, the difference between EF and SF was no longer significant ( $0.0173$  versus  $0.0160$  mg/cm $\cdot$ kg $^{-1}$ ,  $p = 0.15$ )

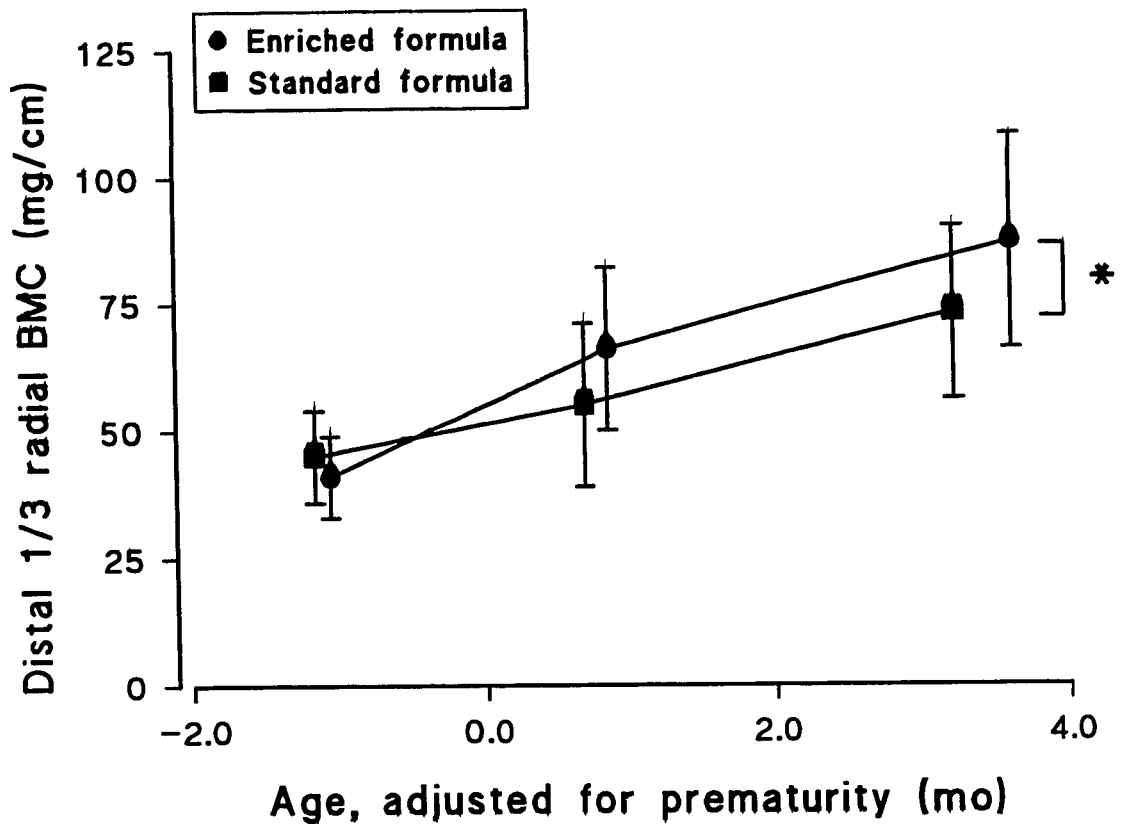


Figure 2. Changes in radial bone mineral content from 37 wk post-menstrual age to 3 mo corrected age for infants fed the enriched (EF) or the standard formula (SF). The EF and SF group means for slope (from slopes calculated for individual infants) are  $10.0 \pm 5.0$  and  $7.0 \pm 5.0$  mg/cm, respectively ( $p = 0.01$ ). The asterisk (\*) represents a significant absolute difference between groups at the end of the intervention ( $87 \pm 21$  versus  $74 \pm 17$  mg/cm,  $p = 0.01$ ).

At the end of the study, DXA measures of whole body composition were successfully completed in 23 infants fed the SF (10 males, 13 females), and 22 infants fed the EF (13 males, 9 females). Since no sedation was used, occasionally infants would not sleep, and the DXA scan was not acquired. Re-admission to a hospital other than the research centre also resulted in missed scans. The inability to acquire DXA scans in all infants resulted in an uneven distribution of males and females between treatment groups. For this reason, analyses of body composition data have included sex as a grouping factor, but have been presented separated in the Figures.

The infants in the EF group appeared to benefit from the high protein:energy ratio formula, having significantly greater lean mass ( $p < 0.001$ )(Figure 3), but male infants benefitted to a greater extent, as there was a significant interaction between treatment and sex ( $p = 0.01$ ). The EF formula feeding resulted in significantly greater whole body bone mineral content ( $p = 0.028$ ) (Figure 4), but the analysis did not detect gender differences or an interaction between treatment and gender. As a total group, the EF compared to the SF group had significantly lower mean % body fat ( $p = 0.019$ ) (Figure 3).

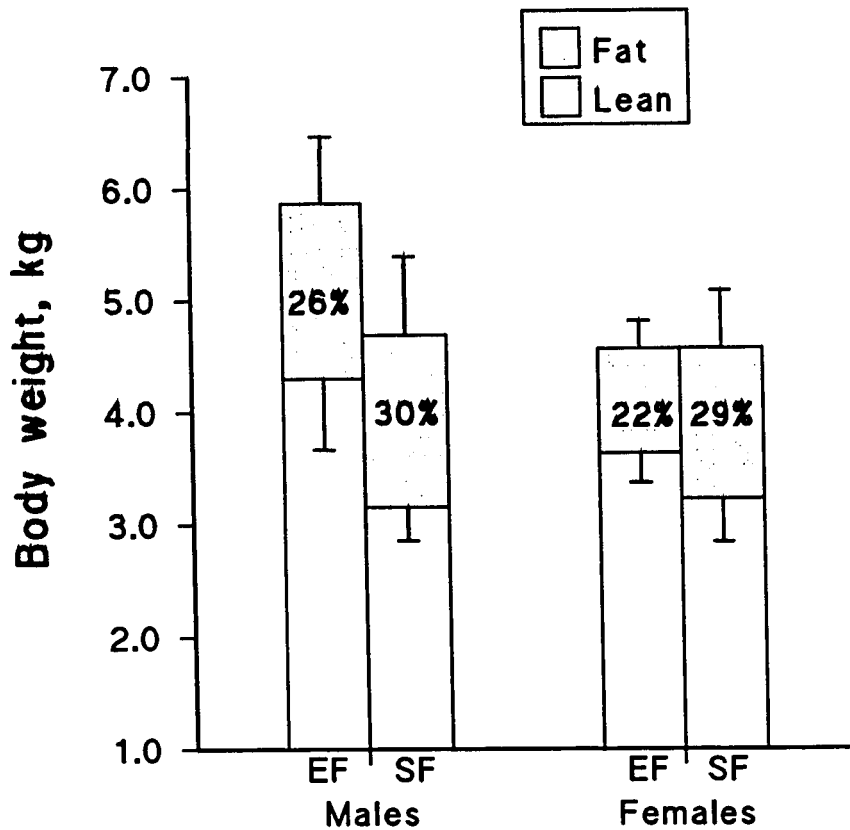


Figure 3. DXA measures of whole body lean and fat masses in infants fed enriched (EF) or standard formula (SF), partitioned by sex (males:  $n = 13$  EF, 10 SF and females:  $n = 9$  EF, 13 SF) at 3 mo CA. Bars are means  $\pm$  SD; values in the bars are % body fat. Absolute lean mass of male and female EF infants was greater than SF infants ( $p < 0.001$ ), and the % body fat was significantly less ( $p = 0.019$ ).



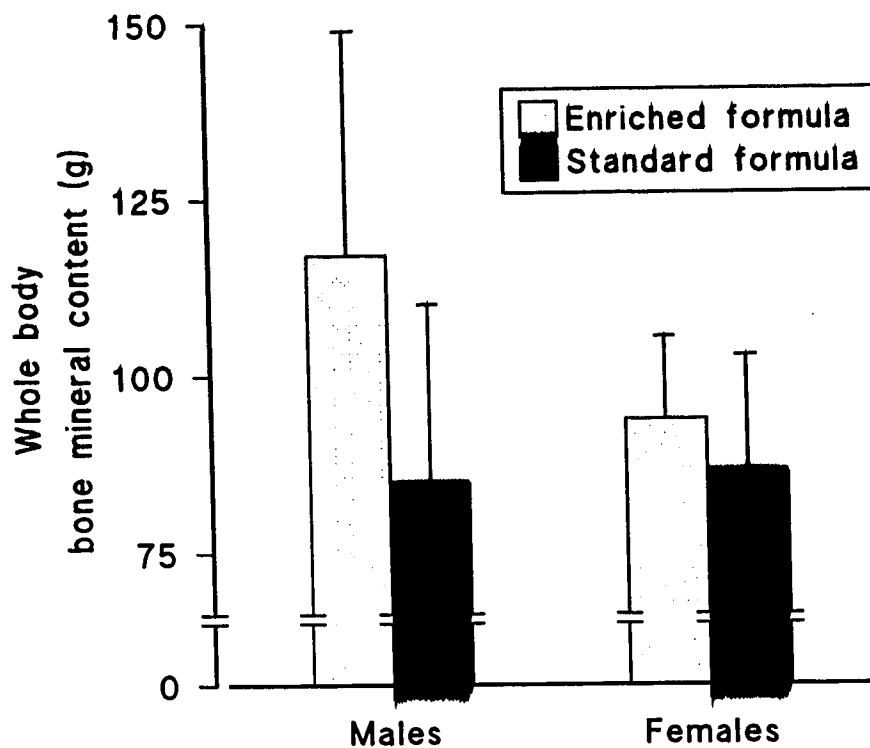


Figure 4. DXA measures of whole body bone mineral content of enriched (EF) and standard formula (SF) fed infants, partitioned by sex (males:  $n = 13$  EF, 10 SF and females:  $n = 9$  EF, 13 SF) at 3 mo CA. Bars are means + SD. EF infants had greater whole bone mineral content than infants SF infants ( $p = 0.028$ ).

## DISCUSSION

The improved velocity of both linear growth and weight gain associated with the feeding of EF is perhaps the most important result of the study. The period of most improved growth was from approximately 37 wk PMA to 1 mo CA (the term visit). During this time, linear growth rate was 0.7 cm/mo faster, with 25% greater weight gain in the EF infants compared with SF fed infants receiving standard concentrations of protein and minerals. This critical time for growth appears to be close to term age when infants are normally discharged home on a standard term infant formula. After this time, the velocity of length gain declined in the EF group, and plateaued in the SF group, while the velocity of weight gain declined in both groups, when calculated on a per kilogram basis.

The fact that we employed a randomized, double blind study design provides convincing evidence that feeding an enriched formula does indeed facilitate enhanced growth in infants with BPD. An adequate sample size was achieved and careful control of variables within the study protocol was maintained. Examples of such control include the use of a ready-to-feed formulas (to ensure exact formula composition) and the use of pre- and post-weighed formula to estimate nutrient intake. Furthermore, routine contact with families minimized drop-outs from the study and ensured timely follow-up of subjects. Complete anthropometric measures and distal radial BMC were obtained in virtually all infants enrolled; however, this was not the case for whole body composition data. Missed whole body scans resulted in smaller

sample sizes that were not truly randomized. It is possible that a common characteristic exists amongst the infants in whom DXA scans were unsuccessful, which could potentially bias the results. Weight or length did not differ between infants who were and were not scanned. It is also important to note that the four infants who did not complete the study (for four different reasons) were all in the EF group. The infant who died was the smallest infant enrolled in the study (birth weight 460 g), and the mean birth weight of all four drop-out infants was  $616 \pm 65$  g which was significantly less than the total EF group. EF and SF groups were not significantly different in birth weight, gestational age at birth, or characteristics at randomization when these infants were excluded from the total group demographics. As more is learned about the influence of birth weight on growth outcomes, it may become apparent that stratification for birth weight using the criterion of  $< 1000$  g was not ideal to control bias introduced by size at birth, and that the "micro-preemie" (ie  $< 750$  g) must be assessed separately.

While this is the first reported nutritional intervention study in BPD infants conducted after the neonatal period, accelerated growth in response to a protein, mineral and energy enriched formula was also recently demonstrated in healthy preterm infants by Lucas et al (28). In a randomized, blinded trial of an enriched versus a standard formula that continued to 9 mo CA, the difference in length growth velocity was significant throughout the study. Similar to our findings, the greatest deviation in length growth between groups appeared to occur immediately after

randomization at 37 wk PMA. In contrast to our findings, the infants studied by Lucas et al (28) did not appear to have the large differences in rate of weight gain shortly after randomization. This may be a birth weight effect (the infants in the study by Lucas et al (28) being almost twice the birth weight of the BPD infants, about 1500 versus 850 g) since we found an inverse correlation between birth weight and rate of weight gain during this early period. Another recent report of post-discharge nutritional intervention demonstrated accelerated length growth with no difference in weight gain between groups of preterm infants randomized to a standard term formula, or an isoenergetic, protein and mineral enriched preterm formula (29). A significantly lower formula volume consumed by the group of infants receiving the enriched formula negated any difference in protein intake between treatment groups (29). This suggests that supplemental minerals alone may enhance length growth. In our study, the EF infants consumed lower volumes of formula than SF infants after hospital discharge, but the difference in intake between groups was not significant. Therefore, it is not possible to discern from our study whether supplemental protein, minerals, or both are responsible for the improved length growth performance of the EF group.

The appropriateness of comparing longitudinal growth of extremely low birth weight infants to reference standards derived from term born infants is controversial. Compared to the NCHS growth standards, most of the BPD infants were severely growth delayed by term corrected age, with greater delay in length compared to weight, resulting in stunting. This disproportionate growth continued after term, with

the majority of infants (>70%) having weight-for-length z-scores greater than zero at 3 mo CA. The interpretation of adequate or proportionate growth depends upon the reference standards used for comparison. If the mean weight and length growth of infants with BPD are plotted on the Infant Health and Development Program (IHDP) Growth Percentiles for premature infants with birth weights < 1500 g (30), then the apparent stunting disappears, and the infants are appropriate or slightly long for their weight. Thus, differences in growth patterns between term and preterm infants exist beyond post-term age. Whether preterm infants can grow to fulfil their genetic potential remains uncertain, but is likely a desirable goal, justifying the use of growth standards for term infants when assessing preterm infant growth.

Almost all infants in this study received the glucocorticoid dexamethasone, which has been demonstrated to inhibit linear growth and weight gain in preterm infants during treatment (31,32,33). Recently, Weiler et al (33) demonstrated disproportionate growth and continued delays in linear growth at term and 3 mo corrected age in preterm infants previously treated with dexamethasone. The impact of the drug on growth was isolated from the influence of the lung disease in studies of dexamethasone treated piglets free of respiratory disease. Growth of the piglets was also impaired with dexamethasone treatment that mimicked therapeutic protocols for infants (34). In our study, the glucocorticoid therapy likely contributed to the growth abnormalities in the BPD infants. Since the provision of EF resulted in improved growth outcomes, it may be an important therapy in the nutritional rehabilitation of

infants treated with dexamethasone.

### *Composition of growth*

The gold standard for the optimal composition of growth for premature infants prior to and after term age is unknown (35). During the first mass balance at 34 wk PMA the BPD infants were consuming 2.8 g/kg·d<sup>-1</sup> protein and exhibited a net daily protein gain (2.0 g/kg·d<sup>-1</sup>) which was similar to healthy preterm infants (14,16,17) and the growing fetus (36). At 38 wk PMA, the SF fed infants had a protein intake of 2.3 g/kg·d<sup>-1</sup>, and showed a decline in protein deposition to a rate similar to the fetus approaching term (1.5 compared to 1.4 g/kg·d<sup>-1</sup>) (36). The infants fed the EF continued to deposit protein at a rate similar to the fetus at 32 to 36 wk PMA (2.2 versus 2.1 g/kg·d<sup>-1</sup>, respectively). It is valid to question when the composition of growth in preterm infants should mimic that of the term infant. Growth delays of these infants with BPD in early life resulted in a mean body weight at 38 wk PMA that was similar to the reference fetus at 33 to 34 wk gestational age (36). de Regnier et al (1) demonstrated that infants with BPD incurred deficits in lean tissue mass early in life, which persisted until hospital discharge. Differences in absolute lean mass between EF and SF groups at 3 mo CA suggest that the infants continued to utilize the higher protein intake beyond hospital discharge. The finding that male infants appeared to respond to the high protein formula with greater lean mass deposition than female infants was an unexpected result; however, both genders did benefit from the additional nutrients in the enriched formula. It seems reasonable then, to try to

facilitate lean tissue deposition at a rate similar to the fetus, at least until body weight and ideally, body composition comparable to term born infants are achieved.

The differences in protein deposition at 38 wk PMA between formula groups may be related to protein intake, but it could also be a reflection of differences in zinc status. Despite intakes deemed adequate compared to current recommendations (37), 16 of 26 infants were in negative zinc balance at 34 wk PMA. At 38 wk PMA, six of 13 infants on the SF and only two of 10 infants on the EF were in negative zinc balance. Others have also found preterm infants in negative zinc balance (38,39). Interestingly, a study which fed preterm infants an amount of zinc that was 2 to 3 times greater than current recommendations demonstrated a net positive zinc retention of only 12% of intake (40). Therefore, high intakes for a prolonged period of time may be necessary to support positive zinc balance and the repletion of zinc stores. Improved growth in response to zinc supplementation has been identified in infant who were not exhibiting classical signs of zinc deficiency (41,42). Friel et al (41) demonstrated improved linear growth in healthy premature infants who were supplemented with zinc post-hospital discharge. In this study, only the EF fed infants at 38 wk PMA had a mean zinc retention similar to intrauterine accretion rates (43).

Greater retention of calcium, phosphorus, and perhaps zinc in the EF compared to the SF infants may be the reason for differences in distal radial bone mineral deposition. The accretion rate during the whole study period was significantly greater in the EF group, however, there appeared to be a period of rapid bone mass accretion

between randomization at 37 wk PMA and the first follow-up measurement at term (1 mo CA) which paralleled the rapid weight and length growth at these times. The greatest period of in utero bone mineral accretion occurs between 36 to 38 weeks gestational age (36,44), so the time close to term may represent a window of opportunity for catch-up growth. During this period, the EF group had a bone mineral accretion rate in the radius that was two fold greater than the SF group (13.3 vs 6.5 mg/cm·mo<sup>-1</sup>). A higher rate of radial bone mineral accretion after 40 weeks post-menstrual age has been identified in descriptive studies of preterm infants compared to term-born infants (45,46,47), but these were infants of greater birth weight and gestational age, with no lung disease.

No controlled nutritional intervention studies have been conducted in infants with BPD; however, in two descriptive studies no difference in radial bone mineral content was found at 40 weeks PMA (6,48), or at one year of age (6) in infants with and without BPD. The current population of infant survivors of BPD are more immature than previously studied, and commonly undergo prolonged courses of diuretics and catabolic steroids, which have been demonstrated to have a negative impact on bone mineral accretion (34, 49). Thus, there is a renewed concern for bone health amongst this population.

Our results are similar to other randomized intervention trials with non-BPD preterm infants. These have demonstrated the effectiveness of high mineral formulas enhancing bone mineral deposition post-term age (50,51,52). In a blinded



intervention trial of healthy preterm infants fed a mineral enriched formula versus a standard formula to 9 mo CA, the rate of mineral accretion was different only during the period between hospital discharge and 3 mo CA (50). Again, this supports the hypothesis that there may be a discreet period when "catch-up" mineralization is possible. Whether the additional mineral provided after 3 mo CA is necessary to maintain bone mineral content is an important question which has not yet been addressed.

#### *Summary and Speculation*

In this group of infants recovering from BPD, we established that a formula concentrated in energy alone did not meet nutrient needs, particularly of protein and zinc; this was reflected in the superior growth achievement of the EF fed infants. Fractional absorption of nutrients by both groups of BPD infants was similar to that reported in the literature for healthy preterm infants. This conclusively eliminates generalized malabsorption as a cause of long term growth failure in BPD infants.

Nutrient intakes deemed adequate for preterm infants post-hospital discharge did not support catch-up growth in the EF fed infants. While their growth was superior to the infants fed the SF, it may be that complete catch-up growth by 3 mo CA is simply not possible for infants with such extremely low birth weights. It is feasible that

shortened gestation alters the development, function or effectiveness of factors which ultimately control body size and stature (53). Additional longitudinal studies of these extremely low birth weight infants are necessary to determine if and when catch-up growth occurs, and whether early aggressive nutritional intervention is beneficial.

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## **CHAPTER 6 THE MEASUREMENT OF ENERGY EXPENDITURE IN INFANTS USING DOUBLY LABELLED WATER**

**Energy expenditure and body composition measured by doubly labelled water in infants with bronchopulmonary dysplasia: Variables related to diet, methods, diet and disease state.**

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The following manuscript is the second paper resulting from a large randomized nutrition intervention trial. The multiple authorship can be explained as follows.

The involvement of **Dr. Saroj Saigal** was the same as described in the preface to Chapter 5.

**Dr. Andrea Winthrop** informally supervised the Ph.D. candidate on one of the technical aspects of this study. She was also one of the investigators involved in the submission of the grant to the Ministry of Health of Ontario, which funded the study.

**Shannon Brady** was a fourth year student in the biochemistry program, who conducted a method development study as her undergraduate thesis project under the supervision of Dr. Atkinson. Informal guidance was provided by the Ph.D. candidate. A portion of Shannon's results have been included in the discussion section of this manuscript, since they are key to some of the methodological issues regarding the use of doubly labelled water in preterm infants. The findings deserve the attention of others working in the field, but do not warrant a separate manuscript.

The **Ph.D. candidate** was the writer of this manuscript, and was involved in the design and the process of attaining funding for the study. Most of the measurements in the study were conducted by her, with the assistance of a skilled research nurse. The only work in this manuscript that the Ph.D. candidate is not responsible for, is Shannon Brady's data on isotope equilibration in one pilot infant, which is addressed in the discussion. All other analytical work and data analyses were done by the Ph.D. candidate, under the supervision of **Dr. Atkinson**.

This chapter is written in the format acceptable for submission to the American Journal of Clinical Nutrition. Information within this manuscript was published in abstract format in *Pediatr Res* 1996; 39:305A and in *J FASEB* 1996; 10:A230.



**Energy expenditure and body composition measured by doubly labelled water in infants with bronchopulmonary dysplasia: Variables related to diet, methods, and disease state.**

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Funded by a grant from the Ontario Ministry of Health, with formula manufactured to investigators' specifications and donated by Wyeth-Ayerst Int., Radnor, PA..

Running Title: Energy expenditure in BPD infants

**ABSTRACT**

**Clinical study:** The doubly labelled water (DLW) method was used to conduct longitudinal measures of energy expenditure (EE) and body composition in infants with bronchopulmonary dysplasia (BPD) up to 3 months corrected age (CA). This was part of a nutritional intervention study aimed at enhancing growth in a population that commonly experiences growth failure (Chapter 5). Using a double blind design, 36 preterm infants with BPD (birth weight =  $836 \pm 216$  g, gestational age =  $26 \pm 1.4$  weeks) were randomized prior to hospital discharge to either a nutrient enriched (protein and energy) formula (EF) (3760 kJ/L) or an isoenergetic standard formula (SF) (3760 kJ/L). Between 34 wk PMA and 3 mo CA, no differences in energy intake (by design) or EE were detected at any of the four times measured. For SF and EF groups the EE at 1 mo CA was  $303 \pm 84$  and  $302 \pm 88$  kJ/kg·d<sup>-1</sup>, respectively and at 3 mo CA was  $331 \pm 113$  and  $303 \pm 129$  kJ/kg·d<sup>-1</sup>, respectively. The high variability within each group precluded detection of any differences due to the treatment (EF or SF). The large SD observed may have been due to variable intensities of ongoing lung disease within each group, or imprecise DLW measurements caused by undetermined factors related to the disease.

**DLW Measures of Body Composition:** Comparison of DLW and dual energy x-ray absorptiometry (DXA) showed poor agreement in the estimation of lean mass in individual infants, despite similar group means ( $69 \pm 10\%$  versus  $73 \pm 8\%$ , respectively). Subsequent investigations of the DLW method included the assessment

of the impact of 1) excluding of dietary water intake in the calculation of total body water, and 2) inaccurate detection of peak isotope enrichment. Both of these conditions were found to alter the estimate %FFM by greater than 5%. This demonstrates that estimates of body composition by DLW are method dependent, which is important to consider when interpreting results from studies using this methodology.

**ABBREVIATIONS**

BPD - bronchopulmonary dysplasia

CA - corrected age (age adjusted for prematurity)

DLW - doubly labelled water

DXA - dual energy x-ray absorptiometry

EE - energy expenditure

EF - enriched formula

EI - energy intake

FFM - fat free mass

$^2\text{H}$  -  $^2\text{hydrogen}$

$^{18}\text{O}$  -  $^{18}\text{oxygen}$

PMA - post-menstrual age

SF - standard formula

TBW - total body water

## INTRODUCTION

Prolonged elevated energy expenditure may have a role in the growth failure consistently observed in infants with bronchopulmonary dysplasia (BPD) (1). However, most studies which demonstrated elevated energy expenditure in infants with BPD were conducted during the perinatal period while infants were receiving supplemental oxygen (2,3). In these studies, oxygen consumption was measured via respiratory gas exchange, a method highly susceptible to errors when oxygen concentrations greater than room air are necessary (4,5). At older post-natal ages, only one study has identified elevated energy expenditure in infants with BPD who were not receiving supplemental oxygen (1); but nutritional management was not controlled. Since that study was conducted, the routine use of surfactant and improved ventilation techniques has changed the clinical course of the disease (6).

The successful application of doubly labelled water (DLW) to measure energy expenditure in human infants (7,8,9,10,11) has provided a methodology to investigate whether prolonged elevated energy expenditure does indeed contribute to growth failure in BPD infants. Simultaneously, DLW provides an estimate of total body water (TBW), thus it is an indirect method of assessing composition of growth.

A lack of evidence regarding optimal feeding practices of BPD infants post-hospital discharge, and the identification of prolonged elevated EE in one study (1), has fostered the addition of supplemental energy (as carbohydrate or lipid) to standard term infant formulas for infants with delayed growth (12). Neither the effectiveness

of this intervention, nor the resulting composition of growth has been investigated. Recent evidence of improved growth without increased adiposity in healthy preterm infants has supported the use of protein, energy and mineral enriched formulas post-hospital discharge (13,14). To our knowledge, no controlled study of a formula enriched with energy alone has been conducted. If metabolic rates are indeed elevated for a prolonged period of time in BPD infants, then energy alone may compensate and support growth, without further metabolic stress from added protein and minerals. Alternatively, excess energy and/or inadequate protein could result in lower energy expended with greater energy stored as fat.

In infants with BPD, we conducted a randomized, blinded, intervention trial to evaluate two different formulas, with compositions based on the feeding practices of high energy in a standard formula, or a high protein to energy ratio (Chapter 5). The primary goals of this report relating to the clinical trial were: 1) to assess the effect of feeding a high energy versus a high protein:energy ratio formula on energy expenditure prior to and after hospital discharge, up to 3 months corrected age, 2) to determine whether prolonged elevated energy expenditure contributed to the long term growth failure observed in infants with BPD and 3) to compare soft tissue composition between treatment groups up to 3 mo CA. Secondly, we evaluated the use of isotope dilution to measure body composition in small infants by means of 1) quantifying the impact of dietary water intake on the estimate of soft tissue composition, and 2) comparing lean mass estimates by isotope dilution to values determined by the imaging technique dual energy x-ray absorptiometry (DXA).

## PATIENTS AND METHODS

Infants were recruited from the Neonatal Intensive Care Units at The Children's Hospital of Chedoke-McMaster, and St. Joseph's Hospital, Hamilton, Ontario from January 1991 to November 1994. The study was approved by the Research Project Advisory Committees at McMaster University and St. Joseph's Hospital. Infants were eligible for the study if they weighed < 1500g at birth, had BPD and were formula fed by parental choice. Infants were diagnosed with BPD by the attending physician using the following criteria (15): 1) oxygen or ventilatory support at day 28 post-natal age, 2) radiographic changes indicative of BPD (16), and 3)  $p\text{CO}_2 > 50$  mm Hg. Infants were excluded if they were small for gestational age, had major congenital anomalies or gastrointestinal surgery or resection. Informed consent for enrollment was obtained only after the diagnosis of BPD was confirmed.

*In hospital study protocol* The study protocol was initiated when infants were stable, growing and receiving full oral feeds as prescribed by the house staff. A baseline 72 hour mass balance for nitrogen retention and energy balance was conducted while infants were fed standard premature formulas (SMA Premie [3200 kJ/L] or SMA [3800 kJ/L], Wyeth-Ayerst Ltd, Toronto, Ontario). Baseline energy expenditure using the DLW method was measured simultaneously (details of DLW method are described below).

At approximately 37-38 weeks post-menstrual age (PMA) infants were randomized via sealed envelope to an enriched high energy (3760 kJ/L) high protein

formula (EF), or a standard isoenergetic (3760 kJ/L) formula (SF) (Wyeth-Ayerst International, Radnor, PA) . The percentage of non-protein energy contributed by fat was approximately 53% in the EF and 56% in the SF. Parents, NICU staff and all research team members were blinded to the intervention.

A second mass balance period/energy expenditure measure was conducted a minimum of seven days after randomization. Infants who were discharged to community hospitals prior to randomization did not participate in the second balance period.

*Nitrogen and energy balance* Details of the mass balance procedures and biochemical analyses are published elsewhere (Chapter 5). In brief, stool, urine and formula were analyzed for nitrogen by the micro-Kjeldahl method (17). Gross energy content of lyophilized stool and liquid formula was determined by combustion bomb calorimetry (Parr Instruments, Moline, IL)(18). Urine was assumed to have negligible energy content (19).

*Post-hospital discharge protocol* Infants were discharged from hospital with a supply of study formula in 120 mL ready-to-feed bottles. Subjects returned to the Growth and Development Clinic at The Children's Hospital at Chedoke-McMaster at approximately term and 3 mo CA as part of the routine follow-up care. Measures of weight and length were obtained by trained members of the research team, and converted to NCHS standard deviation scores (Z-scores) (20).

While in clinic, parents were provided with a five day supply of pre-weighed



bottles of ready-to-feed study formula. Unused portions of formula were subsequently returned to the laboratory for re-weighing, to estimate a mean daily energy and protein intake. Also during the clinic visit infants were dosed with DLW to obtain an ambulatory measurement of energy expenditure.

*Energy expenditure by doubly labelled water* The DLW method has been previously validated and described for use in preterm infants (9,10,11) and term born infants recovering from abdominal surgery (7,8). On day one of the EE period, after at least five days on the same nutritional prescription to ensure a constant background isotope enrichment, the infants were weighed, and a baseline urine sample was obtained. Infants were then given an oral dose of 0.3 g/kg body weight  $^{18}\text{O}$  ( $\text{H}_2^{18}\text{O}$  as one of 99, 97.8, 97, 96.5, 81, 10.2 or 9% atom percent enrichment, Isotec Inc., Ohio) and 0.1 g/kg body weight  $^2\text{H}$  ( $^2\text{H}_2\text{O}$  as 90.5% atom percent enrichment, MDS Isotopes, St. Louis, MO). If the infant was gavage-fed, the isotopes were administered through the feeding tube, followed by formula to flush the tube. Bottle fed infants received the isotopes mixed 50:50 with formula and the bottle was refilled with a similar volume of formula and fed twice to wash out any remaining isotope. Syringes used to measure the isotope doses were weighed pre- and post-dosing on a scale accurate to 1 mg (Sartorius, Germany). In hospital, urine samples were collected at five and 24 hours post-isotope dosing, with a final sample collected on day seven. For infants at home, the caretakers were instructed to collect urine samples at 24 hours and seven days post-dosing. The samples were frozen immediately for pick-up by a

research team member. All urine samples were stored in 15 mL plastic vials with screw cap lids, and were frozen at or below -20 C° until analyzed. <sup>18</sup>O and <sup>2</sup>H enrichment of isotopically labelled water used for dosing and urine samples were measured using gas isotope ratio mass spectrometry (VG-SIRA II and VG 602D, VG Isogas, Cheshire, England) as previously described (7). The precision as measured by CV% was determined for the range of expected values by triplicate analyses of selected samples. For baseline and highly enriched samples, the intra-assay CV% were 2.3% and <1% for <sup>18</sup>O and 2.8% and <0.5% for <sup>2</sup>H, respectively. Details of the sample preparation, analysis and calculations are presented in Appendix 2.

For infants in hospital, the five hour post dose urine was presumed to be at isotopic equilibrium and was used in the calculation of TBW (plateau method) (21). For outpatients (term and 3 mo), the isotopic plateau values for <sup>2</sup>H and <sup>18</sup>O were calculated from the water turnover constants, using the equation (22):

$$E_t = E_p e^{-kt}$$

Where  $E_p$  is enrichment at theoretical plateau (five hours post-dose),  $k$  (the water turnover constant) was derived from the two point method (24 hours and day seven samples), and  $t$  was the difference in time from theoretical plateau (five hours) to the first post-dose urine sample. There is no difference in the estimation of TBW when using the actual measured plateau or the "back extrapolated" theoretical plateau in the calculations (21).

Total dilution space was calculated for both isotopes using the following equation

(23):

$$\text{TBW (kg)} = \frac{d \times \text{APE}_d \times 18.02}{\text{MW}_d \times \text{APE}_{\text{BW}}} \times 10^{-3}$$

Where  $d$  was the isotope dose in grams,  $\text{APE}_d$  was the atom percent enrichment of the dose water determined by mass spectrometry analysis,  $\text{MW}_d$  was the molecular weight of the dose, and  $\text{APE}_{\text{BW}}$  was the enrichment of the urine sample at plateau (ie plateau enrichment minus baseline enrichment). The distribution space of both  $^2\text{H}$  and  $^{18}\text{O}$  are greater than the water dilution space, due to exchange and sequestration of isotope into tissues. Factors of 1.04 and 1.01 were used to correct the dilution space calculations for  $^2\text{H}$  and  $^{18}\text{O}$ , respectively (23). Initially, the calculations for TBW were completed without considering formula (dietary water) intake between isotope dosing (time zero) and the first post-dose urine sample (five hours or 24 hours). However, small infants are fed frequently, and new water intake could potentially result in a significant over-estimation of TBW. To determine the influence of this potential source of error, the formula intake between isotope dosing and urine sampling was weighed, and corrected for solids content, then subtracted from total body water to correct for new water (22,24).

Estimations of fat free mass (FFM) from TBW were calculated using published lean tissue hydration constants. These were determined from term infants at ages two, three, four and six months to correspond with the post-natal ages of the BPD infants at

34 and 38 weeks PMA, and 1 and 3 mo CA, respectively. The values used (0.803 , 0.798, 0.798 and 0.796 for 34 and 38 wk PMA and 1 and 3 mo CA, respectively) were determined from measures of total body potassium and total body water (25). The calculated %FFM for the BPD infants was compared to the reference %FFM for fetuses (26) and term born infants (25), since no longitudinal body composition data for preterm infants are available for comparison.

Subsequent estimates of daily carbon dioxide production ( $r\text{CO}_2$ ) during the measurement period were calculated for infants using two different equations, to accommodate environment and amount of clothing worn. For infants in hospital, the following equation was used (7):

$$r\text{CO}_2 \text{ (L/d)} = 0.445N(1.01k_o) - (1.04k_H)$$

where:

$$N = \text{TBW (moles)}$$

$$k = \text{water turnover constants for oxygen and hydrogen}$$

For infants post-term age, the equation by Schoeller et al (23) was used:

$$r\text{CO}_2 \text{ (L/d)} = \frac{N(k_o - k_H)}{2.08 - 0.015Nk_H}$$

The TBW values used in the above equations were corrected for dietary water intake.

Subsequently, energy expenditure estimates were derived from the Weir equation (28):

$$\text{EE (kJ/d)} = [22.4(1.106 \times r\text{CO}_2 + 3.941 \times r\text{O}_2)] \times 4.18$$

An estimate of oxygen consumption ( $rO_2$ ) was obtained by calculating the food quotient of the infant formulas, which was 0.83, as described by Black et al (27). The formulas were the sole source of nutrition for the duration of the study.

*Composition of weight gain during balance periods* Protein deposition during the balance periods was calculated as nitrogen retained ( $g/kg \cdot d^{-1}$ )  $\times$  6.25. Fat deposition was then calculated from the equation:

$$\text{Fat} = \frac{(\text{EI} - \text{EE}) - (\text{Protein stored} \times 23.6)}{38.9}$$

Where EI and EE are energy intake and energy expended expressed as  $kJ/kg \cdot d^{-1}$ , protein and fat stored are expressed as  $g/kg \cdot d^{-1}$ , and 23.6 and 38.9 are the energy equivalents (kJ/g) of protein and fat respectively (29)

*Measurement of body composition by DXA* In a subgroup of infants, measures of lean and fat masses were obtained via a single DXA scan (Hologic QDR 1000/W, Waltham, MA) during the clinic visit at 3 mo CA. Infants were chosen for this analysis based on the successful acquisition of both DXA and DLW measures. The DXA scan was conducted on same day that the DLW measurement was initiated. The DXA imaging technique and scanning protocol employed in the clinical trial are described in detail elsewhere (Chapter 5).

*Statistics* The sample size for this study was calculated based on the outcome of energy expenditure. A difference of 15% in energy expenditure (determined by respiratory gas exchange) was previously observed in BPD versus non-BPD infants

(3), therefore, it was chosen as the delta value. Since no studies using DLW were available at the initiation of this study to estimate a standard deviation, the variability from the study using respiratory gas exchange was used in the calculation (SD = 49 kJ). An  $\alpha = 0.02$  was used to detect significance because within the whole study, there were three major outcomes of interest (the other two are reported elsewhere). Thus, the level of significance was reduced to accommodate multiple outcomes. With  $\alpha = 0.02$  and  $\beta = 0.2$ , a sample size of 17 infants per group was required.

Differences between groups in nutrient intake, retention and growth at 3 mo CA were determined by Student's t-test (BMDP Student Version, BMDP Statistical Software, Inc., Los Angeles CA). Change in TBW and FFM over time for the combined groups was assessed by one way ANOVA. Change in EE over time was determined by linear regression versus age for individual infants. Repeated measures ANOVA could not be employed due to missing values. The mean slopes for EE derived for EF and SF groups were compared by Student's t-test.

DXA and TBW estimates of FFM were compared by paired t-test. Since neither method is considered to be the 'gold standard' for comparison, the agreement between DXA and TBW was assessed using the method of Bland and Altman (30).

## **RESULTS**

Characteristics of infants are presented in Table 1. Since not all measures were successful in all infants, sample sizes for individual outcomes are presented in the tables of results. The desired goal of 17 infants per group for the energy expenditure

measures was not attained as three infants dropped out prior to the term and 3 month measures. Some planned energy expenditure measures were missed for the following reasons: 1) a world-wide shortage of  $^{18}\text{O}$  oxygen, 2) inadequate urine sample volume collected, 3) a sudden deterioration in the medical condition in some infants during the energy expenditure period, or 4) the parents were unable to obtain the urine samples as requested.

*Nitrogen and Energy Balance Studies* Gross energy intake, metabolizable energy intake (34 and 38 wk PMA) and energy expenditure were not different between formula groups at any of the time points measured (Table 2). There was also no group difference in the rate of change of EE during the period prior to hospital discharge to 3 mo CA (EF vs SF,  $-0.5 \pm 5.4$  versus  $3.2 \pm 2.8$  kJ/kg·d<sup>-1</sup>,  $p = 0.16$ ).

*Growth and Body Composition* Protein intake and net accretion of fat and protein were similar on standard premature formula at 34 wk PMA; after randomization and adaption to the study formulas, infants receiving the EF had significantly greater protein intake and accretion (Table 3). Fat accretion did not differ between groups.

There were no significant differences in weight, length or head circumference between groups of infants at randomization to study formulas, or at 3 mo CA (Table 1). Both groups of infants were greater than one standard deviation below the 50th percentile on NCHS reference growth curves for weight. The SF group mean for length was below the 3rd percentile, while the group mean for the EF group was slightly above the 3rd percentile. Body composition calculated from isotope dilution

space was similar between groups at all four time points (Table 4).

*Body composition estimates from TBW* Since there were no differences between formula groups in body weight, total body water or energy expenditure, groups were combined to investigate methodological issues. Significant declines in %TBW and %FFM from 34 weeks PMA to 3 mo CA were identified whether or not dietary water was included in the calculations (Table 5).

*TBW versus DXA* The two methods were successfully conducted in 19 infants at 3 mo CA. The mean %FFM estimated by the DLW and DXA methods were not significantly different ( $69 \pm 10\%$  versus  $73 \pm 8\%$ , respectively). Agreement within individuals was highly variable, such that 14 of 19 measurements of %FFM differed by greater than 5% (Figure 1).



Table 1 Characteristics of infants in the enriched and stand formula groups.

	<u>Standard (n=17)</u>	<u>Enriched (n=19)</u>
Birth weight (g)	836 ± 260 <sup>†</sup>	841 ± 168
Gestational age (wk)	26.2 ± 1.6	25.7 ± 1.2
Mechanical ventilation (d)	34 ± 18	41 ± 20
Supplemental O <sub>2</sub> at term (n)	3	2
Supplemental O <sub>2</sub> at 3 mo CA (n)	2	1
Birth weight re-gained (d)	21 ± 5	22 ± 6
Full oral feeds attained (d)	24 ± 11	21 ± 11
Cumulative dexamethasone dose (mg/kg)	5.7 ± 3.3	7.2 ± 4.5
Length of intervention (mo)	4.1 ± 0.6	4.1 ± 0.5
PMA at balance 1 (wk)	34.4 ± 1.0	34.2 ± 1.4
PMA at balance 2 (wk)	37.9 ± 1.6	38.2 ± 1.4
Age at term visit (mo)	0.7 ± 0.3	0.8 ± 0.4
Final visit:		
Age (mo)	3.2 ± 0.4	3.4 ± 0.5
Weight Z-score	-1.3 ± 0.7	-1.19 ± 0.9
Length Z-score	-2.3 ± 0.7	-1.8 ± 1.1
Head circumference (cm)	40.0 ± 1.7	40.1 ± 1.7

<sup>†</sup>Values are mean ± SD

**Table 2** Energy intake and expenditure for infants on standard premature formula (34 wk PMA), and after randomization to Standard or Enriched study formula (38 wk PMA, 1 and 3 mo corrected age).

		<u>Standard (n)<sup>†</sup></u>	<u>Enriched (n)</u>
<b>Energy (kJ/kg·d<sup>-1</sup>)</b>			
Intake:			
Metabolizable <sup>‡</sup>	34 wk PMA	464 ± 31 <sup>‡</sup> (12)	479 ± 25 (11)
	38 wk PMA	530 ± 53 (7)	537 ± 53 (7)
Gross <sup>‡</sup>	1 mo CA	500 ± 84 (10)	484 ± 78 (13)
	3 mo CA	512 ± 114 (9)	427 ± 50 (10)
Expenditure:			
	34 wk PMA	237 ± 109	321 ± 112
	38 wk PMA	270 ± 129	239 ± 126
	1 mo CA	303 ± 84	302 ± 88
	3 mo CA	331 ± 113	303 ± 129
% Expended <sup>§</sup>			
	34 wk PMA	51 ± 23	67 ± 23
	38 wk PMA	51 ± 24	45 ± 23
	1 mo CA	61 ± 17	62 ± 18
	3 mo CA	65 ± 22	71 ± 30

<sup>†</sup>n per group the for expenditure are the same as for intake

<sup>‡</sup>gross energy intake was determined by combustion bomb calorimetry of formulas, and metabolizable intake represents gross intake minus energy excreted in the feces

<sup>‡</sup>values are mean ± SD

<sup>§</sup>%Expended represents the percentage of intake that was expended.

**Table 3** Protein intake, net fat and protein accretion and weight gain during mass balance periods for infants on standard premature formula (34 wk PMA) and after adaptation to Standard or Enriched study formula (38 wk PMA).

	<u>Standard</u>	<u>Enriched</u>
<u>A) 34 wk PMA</u>		
Protein intake (g/kg·d <sup>-1</sup> )	2.8 ± 0.2 <sup>†</sup>	2.8 ± 0.3
Net protein gain (g/kg·d <sup>-1</sup> )	1.9 ± 0.6	2.0 ± 0.4
Net fat gain (g/kg·d <sup>-1</sup> )	4.2 ± 3.5	3.2 ± 2.6
Net weight gain (g/kg·d <sup>-1</sup> )	17.3 ± 11.7	14.0 ± 13.0
<u>B) 38 wk PMA</u>		
Protein intake (g/kg·d <sup>-1</sup> )	2.3 ± 0.2	3.1 ± 0.3*
Net protein gain (g/kg·d <sup>-1</sup> )	1.5 ± 0.4	2.2 ± 0.2*
Net fat gain (g/kg·d <sup>-1</sup> )	6.2 ± 3.8	3.9 ± 3.6
Net weight gain (g/kg·d <sup>-1</sup> )	11.9 ± 2.9	10.8 ± 3.0

<sup>†</sup>Values are mean ± SD

\* Enriched versus Standard, p < 0.01.

**Table 4** Percent fat free mass (%FFM), and %fat (determined from total body water) of EF and SF fed infants at 34 and 38 wk PMA, and term and 3 mo CA and compared to %FFM of the reference fetus (26) and infant (25) at similar post-menstrual (PMA) and post-natal ages (PNA).

<u>Age</u>	<u>Formula group (n)</u>		<u>Weight</u>	<u>BPD Infants</u>		<u>Reference Fetus<sup>†</sup>/Infant<sup>‡</sup> %FFM</u>	
				<u>%Fat</u>	<u>%FFM</u>	<u>PMA<sup>‡</sup></u>	<u>PNA<sup>§</sup></u>
34 wk PMA	SF	(12)	1.55 ± 0.2*	15 ± 8	85 ± 8	92	80
	EF	(13)	1.48 ± 0.2	13 ± 8	87 ± 8		
38 wk PMA	SF	(7)	2.08 ± 0.3	21 ± 13	79 ± 13	91	77
	EF	(10)	2.26 ± 0.3	13 ± 6	87 ± 6		
1 mo CA	SF	(13)	3.12 ± 0.3	28 ± 8	72 ± 8	84	75
	EF	(16)	3.40 ± 0.4	23 ± 10	77 ± 10		
3 mo CA	SF	(16)	4.76 ± 0.7	30 ± 12	70 ± 12	76	75
	EF	(11)	5.49 ± 0.7	28 ± 11	72 ± 11		

<sup>†</sup> Determined from cadaver analysis (26).

<sup>‡</sup> Determined from measures of total body potassium and total body water (25).

<sup>‡</sup> %FFM values derived from reference fetus (26) or infant (25) at similar post-menstrual ages (PMA).

<sup>§</sup> %FFM values derived from reference infant (25) at similar post-natal ages (PNA).

\*Values are mean ± SD.

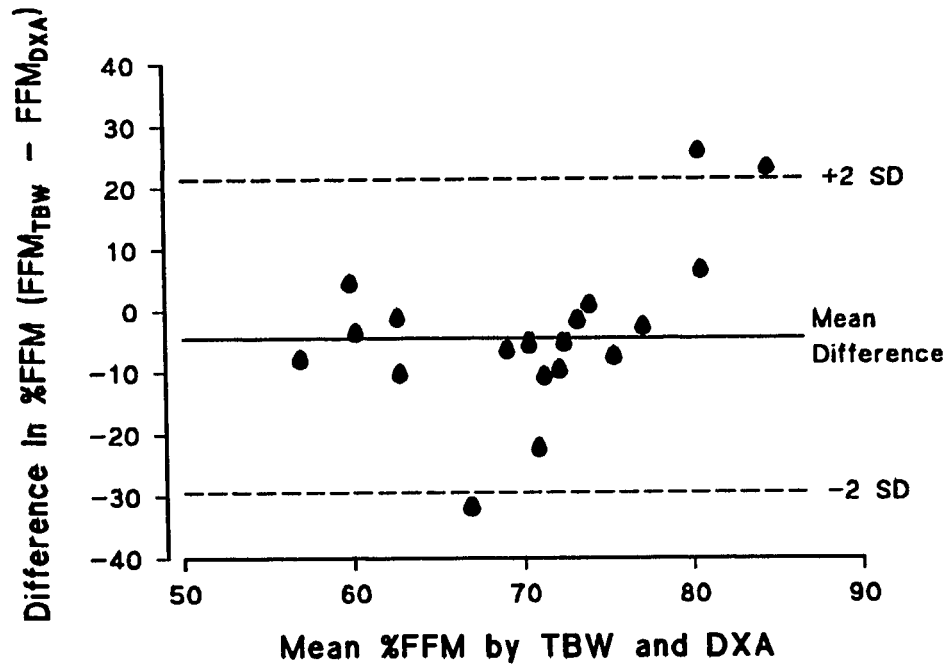
**Table 5** Body composition determined from doubly labelled water calculated with and without correction for dietary water intake at 34 and 38 weeks PMA 1 and 3 mo CA.

Age (n)	Weight	TBW <sup>†</sup> [ <sup>2</sup> H: <sup>18</sup> O]	%TBW		%FFM <sup>‡</sup>		%FAT	
			Not Corrected	Corrected	Not Corrected	Corrected	Not Corrected	Corrected
34 wk (27)	1.51 ± 0.2	1.03	78 ± 8 <sup>§</sup>	72 ± 8 <sup>ab</sup>	97 ± 7	90 ± 10	3 ± 7	10 ± 10
38 wk (18)	2.20 ± 0.3	1.06	73 ± 8	68 ± 9 <sup>cd</sup>	91 ± 9	85 ± 11	9 ± 9	15 ± 11
1 mo (21)	3.24 ± 0.4	1.02	67 ± 7	59 ± 9 <sup>ac</sup>	84 ± 9	73 ± 11	16 ± 9	27 ± 11
3 mo (21)	4.95 ± 0.7	1.03	64 ± 7	55 ± 8 <sup>bd</sup>	80 ± 9	69 ± 10	20 ± 9	31 ± 9

<sup>†</sup>The ratio of total body water as determined by the dilution of <sup>2</sup>hydrogen and <sup>18</sup>oxygen (<sup>2</sup>H:<sup>18</sup>O).

<sup>‡</sup>Calculation of %FFM from %TBW was done by dividing %TBW by published values for lean tissue hydration, which are 0.803 and 0.798 at 34 and 38 weeks PMA and 0.798 and 0.796 and 1 and 3 mo, respectively (25).

<sup>§</sup>Values are mean ± SD, like symbols within the column (for both corrected and not corrected) are significantly different (p < 0.05).



**Figure 1** Comparison of measures of percent fat free mass (% FFM) by isotope dilution (corrected for dietary water intake) (FFM<sub>DLW</sub>) and dual energy x-ray absorptiometry (FFM<sub>DXA</sub>) in a group of BPD infants (body weight = 4.95 kg) at 3 mo CA. Using the method of Bland and Altman (30), the difference between %FFM estimated by DLW and DXA was plotted against the mean of the two methods.

## DISCUSSION

To our knowledge, the use of DLW to measure EE or body composition has not been previously employed in a long term nutritional intervention study with BPD infants. Therefore, we were challenged by methodological issues not yet addressed in the literature. Some of the problems encountered were unique to infants with BPD, such as the relatively large individual variability in EE. Other issues that we have addressed in this study have implications for investigations with all infants, regardless of disease state. These include the impact of dietary water intake on the estimate of soft tissue composition, the high variability between individual DXA and DLW estimates of lean mass, and the difficulty in identifying isotopic equilibrium to measure TBW when using urine to sample the body water pool.

### *BPD and energy expenditure by DLW*

We hypothesized that we would observe greater energy expenditure in the EF group, due to rapid growth and the greater cost of lean tissue synthesis compared to fat tissue synthesis. Greater lean tissue storage in the EF group was observed compared to the SF group (by mass balance technique), but this was not accompanied by significantly higher energy expenditure or energy stored.

Whether energy expenditure is elevated in infants with BPD has not been conclusively established, perhaps due to the limitations of the indirect calorimetry method used in previous studies. Studies which measured respiratory gas exchange suggest that infants with BPD have energy expenditures up to 30% greater than non-

BPD infants, due to the "work of breathing" (1,2,,31). However, when infants are measured while receiving supplemental oxygen (1,2,31), small errors in the measurement of ambient oxygen will translate into large errors in the estimation of oxygen consumed (4,5). Furthermore, indirect calorimetry measures are generally conducted over a period of six to eight hours (10), therefore may not accurately represent average daily EE.

Metabolic costs may be elevated in infants with BPD; one possibility is the influence of drugs on energy metabolism. Using DLW, Fjeld et al (33) investigated the impact of theophylline, a xanthine derivative, on energy expenditure in preterm infants, since xanthines can stimulate thermogenesis. No difference was found between infants treated with the drug versus control infants. In preterm infants with BPD, Leitch et al (34) determined that dexamethasone therapy had no effect on energy expenditure.

The impact of the "work of breathing" on energy expenditure has also been investigated. Kao, Durand and Nickerson (32) studied infants with lung disease treated with diuretics, bronchodilators or placebo to assess the influence of the mechanical power of breathing on oxygen consumption. They found no decline in oxygen consumption with improved pulmonary mechanics. The authors speculated that the elevated requirements were due to the inflammatory and repair processes occurring in the lungs. Elevated oxygen consumption by injured lung tissue has been demonstrated in dogs (35) and adults (36). Oxygen consumption measured by



respiratory gas exchange was 19% greater than the estimation by the arteriovenous oxygen concentration differential ( the "reverse Fick" method) (36). The latter method does not include oxygen extracted by the lungs. Schulze et al (37) recently demonstrated this relationship in newborn very low birth weight infants. The infants were ventilated for respiratory distress syndrome and had pulmonary oxygen consumption which represented 27% of whole body consumption. Therefore, in infants with BPD, the ongoing disease may be responsible for the elevated energy expenditure previously identified.

The combined EE from both groups of study infants at baseline (34 wk PMA) was  $280 \pm 117$  kJ/kg·d<sup>-1</sup>. This was similar to the EE of  $288 \pm 50$  kJ/kg·d<sup>-1</sup> in healthy infants of comparable post-menstrual age (receiving no supplemental oxygen) measured in a DLW validation study by Jensen et al (9). Westerterp et al (10) also validated DLW against indirect calorimetry in healthy, non-oxygen supplemented premature infants 32-35 wk PMA, with a resultant mean EE by DLW that was lower than ours at  $241 \pm 24$  kJ/kg·d<sup>-1</sup>. Our findings and Jensen's (9) also tended to be 10-15% higher than other EE measures in healthy premature infants using indirect calorimetry (29,38,39,40,41). However, comparisons of the two methods within controlled studies resulted in DLW differing from indirect calorimetry by only +0.4% (9) and -4.5% (10). Therefore the difference in EE between our study and others with healthy infants may be partially due to the disease state; but the higher values determined in healthy infants by Jensen et al (9) could imply that the procedures and

calculations chosen to estimate EE from DLW may alter the interpretation of the results.

The variability of EE among our infants with BPD was two to three times greater compared to other studies of healthy preterm infants and term infants which used DLW (9,10,42). There are two other reports published in abstract form of wide variability in EE measured by DLW in BPD infants in the early neonatal period (34, 43). This suggests that the pathophysiology of the disease state may influence the precision or the accuracy of the methodology. The use of the DLW method in healthy preterm infants is susceptible to errors, due to high body water content, and rapid body water turnover (9, 11). These factors may be exaggerated in infants with BPD who appear to experience a hypermetabolic state. One assumption of the method is that subjects are in a steady state of body composition throughout the study period; infants violate this assumption to a small degree due to growth, but the resultant error in the estimation of  $r\text{CO}_2$  is negligible (44). However, in infants with BPD, the accumulation of fluid or sudden diuresis would alter body composition and may amplify the error in the estimation of  $r\text{CO}_2$ .

Another source of variability in our study may have been an inappropriate correction for fractionation of water vapour, and insensible water losses. Correction factors included in the calculation of  $r\text{CO}_2$  for infants in hospital assumed that 75% of the skin surface was exposed (7). As infants approached term, some were moved from enclosed, thermoneutral isolettes into open air bassinets in preparation for hospital

discharge. If this occurred during the EE measurement period it could result in a large error in the estimated CO<sub>2</sub> production rate (11). Similarly, BPD infants who experience chronic rapid breathing may have water vapour losses in excess of healthy preterm infants; thus correction factors for the fractionation of water vapour which were derived from healthy infants may not be applicable to the infant with BPD.

The DLW method uses isotopes to measure body water, but isotope dilution space does not equal body water (24). Therefore, we adjusted the dilution space for isotope sequestration into non-aqueous tissue by using correction factors of 1.04 for <sup>2</sup>H and 1.01 for <sup>18</sup>O, resulting in a fixed ratio (<sup>2</sup>H:<sup>18</sup>O) of 1.03 (7,23). The ratio of <sup>2</sup>H:<sup>18</sup>O that we actually observed was 1.03 at 34 wk PMA and 3 mo CA, but was higher at 38 wk PMA and lower at 1 mo CA (Table 5). The use of a fixed ratio to correct isotope dilution space in calculations of rCO<sub>2</sub> is controversial, and when used for preterm infants can theoretically lead to errors as great as 40% (24). Ritz and Coward (44) recently demonstrated that using the observed ratio as opposed to a fixed ratio may improve the accuracy of the EE estimation. Furthermore, the final observed <sup>2</sup>H:<sup>18</sup>O ratio is physiologically variable, and dependent upon analytical method (44). Therefore the use of a literature derived fixed ratio for all of our infants across all ages and body weights may have contributed to the high variability in EE that we observed.

The large standard deviations in EE may also have been due to stratification of our treatment groups according to the severity of disease. In a descriptive study, de Gamarra (45) reported elevated metabolic demands in five BPD infants (ages 38 to 51

wk PMA) compared to non-BPD infants who had recovered from respiratory distress syndrome. The highest EE was exhibited by infants with the most severe growth deficits. Kurzner et al (46) also identified elevated oxygen consumption in BPD infants with growth failure, compared to BPD infants without growth failure and size matched controls, indirectly supporting a relationship between severity of disease and energy expenditure. At 3 mo CA, we found no correlation between energy expenditure and body weight. However, without a measure of pulmonary function, it was not possible to discriminate between infants who had elevated metabolic demands due to the disease state from those with elevated demands to accommodate rapid growth.

Since our variability in EE measures was much greater than expected, it follows that the sample size that was calculated for the study was found to be inadequate. An estimate of the variance was derived from BPD infants in-hospital, measured by indirect calorimetry (3). Ultimately we had less than a 30% chance of detecting group differences (47), if indeed there was one. The sample size was also calculated based on a difference in EE of 15% identified in BPD versus non-BPD infants during the preterm period. Perhaps it would have been more representative to calculate a sample size based on the 30% difference reported by Kurzner et al (1) in BPD infants with growth failure versus BPD infants without growth failure at 6 mo CA.

*The use of DLW to measure body composition in BPD infants*

It was surprising that the DLW method did not identify differences in body

composition between treatment groups at 3 mo CA, since a significant difference in the rate of accretion of protein was apparent at term. Fat accretion between groups was not different, but the calculation incorporated energy expended, therefore the variability of EE measures was reflected in the fat accretion measurement as well. Comparing the BPD infants to the reference fetus (26) and infant (25), based on post-menstrual age, the BPD infants have a much greater proportion of body weight as fat. It is possible that fat deposition is triggered post-natally for adaptation to extra-uterine life. For this reason, it may be more appropriate to compare the infants by post-natal age. Using this criterion, at 3 mo post-natal age (38 wk PMA) the BPD infants actually appear to have a greater proportion of weight as lean mass than the reference infant (85 versus 76% FFM) (Table 5).

DLW may not have been sensitive enough to detect differences between treatment groups in soft tissue composition. The body composition findings from this study are in contrast to the results from a larger group of BPD infants (including most of the infants in this study) measured by DXA (Chapter 5). DXA identified significantly greater lean mass in EF compared to the SF infants, and the female EF infants had a lower percentage body weight as fat compared to the SF females. Our comparison of DXA and DLW measures in this study clearly identifies poor agreement (Figure 1). The use of  $^{18}\text{O}$  to measure dilution space was validated in newborn piglets, and when administered orally had a random error of 13% (48). Thus, the sensitivity to detect small changes appears limited. We have previously demonstrated that DXA

does have the sensitivity to detect small changes in soft tissue composition of infants between term and 3 mo CA (49).

There is no discussion in the literature regarding the need to correct for dietary water intake to estimate TBW. The correction resulted in a greater decline in TBW with advancing age than when it was not included in the calculations (17% versus 14%). Without correcting for water intake, %FFM was greater than total body weight (>100%) in 33% of the measurements at 34 weeks PMA and 16% of the measurements at 38 weeks PMA, signalling a methodological error. After correcting for water intake, the estimates of %FFM exceeded weight in 19% and 6% of infants at 34 and 38 weeks PMA, respectively. In tiny premature infants it is likely important to consider dietary water intake since feeding patterns of every two or three hours contribute to a high water volume intake. Also, the practical difficulties of collecting urine makes the timing of samples unpredictable. It has been stated that dietary water consumed between isotope dosing and isotopic plateau in infants is small compared to TBW (<5%) (24), therefore, correction for new water intake is not necessary when calculating EE. We demonstrated that correcting for dietary water intake made considerable difference in the estimation of TBW at all times measured. In turn, this dramatically altered the interpretation of soft tissue composition (Table 5).

Establishing the isotopic equilibration point is crucial in order to accurately assess soft tissue composition of premature infants. The length of time required to reach "plateau" is variable ranging from 2 to 20 hours (22,50), but it is commonly

assumed to be less than three hours due to the rapid rate of water turnover in infants. Salazar et al (22) demonstrated that  $^2\text{H}$  equilibration took up to 6.5 hours (mean  $4.9 \pm 1.3$  hours) in infants less than one year of age, as determined by saliva samples. This method should identify isotopic plateau earlier than urine sampling. To determine when peak isotopic enrichment is achieved, a complete collection of all voided urine was obtained from a 33 week gestation, 13 day old premature infant weighing 1.9 kg (unpublished observations). The 24 hour collection consisted of 21 voids, reflecting incomplete bladder emptying and likely urine mixing. Alternate sequential urine samples were analyzed for isotopic enrichment, and demonstrated that the calculation of TBW varied up to 5%, depending upon which sample within a four hour time span was used as the point of isotopic equilibrium (Table 6). Sequential sample analysis is neither practical nor economically feasible for most studies using isotope dilution. However, faulty timing of the sample collected to represent peak enrichment will result in erroneous estimates of lean and fat masses.

In a few of the BPD infants studied, the initial TBW calculation was greater than total body weight (after correcting for dietary water intake), suggesting that the urine sample obtained five hours post-dosing did not represent isotopic plateau. Extrapolation of the peak isotopic enrichment from the water turnover constant confirmed that this was not the result of a dosing error (ie undetected drooling or vomiting), but that in some infants, the five hour urine sample was not representative of isotopic plateau.

### *Conclusions*

While nutritional management has emphasized the provision of extra energy to support elevated metabolic demands of BPD, it is apparent that energy alone will not ameliorate the growth delay in infants with BPD, or facilitate catch-up growth. Compared to healthy reference infants, the BPD infants appeared to have slightly elevated EE after hospital discharge. On average, the BPD infants were expending 11% greater energy at 1 mo, and 8-18% greater energy at 3 mo than reference EE data by DLW in term born infants of similar ages (42). These differences are much smaller than the 30% difference in EE between infants with BPD who were failing to grow compared to healthy size matched controls observed by Kurzner et al (1). The discrepancy between studies may be partially due to the changing nature of BPD, since the Kurzner study (1) was conducted prior to the routine use of surfactant and glucocorticoid therapy. It may also be due to imprecise measurements with DLW in infants with BPD for reasons that have not yet been identified. Recent evidence of high oxygen demands by lung tissue undergoing inflammation and/or repair (36,37) dictates that further studies of EE in infants with BPD are warranted. Within these studies, the severity of disease must be considered, in order to determine which infants require the most aggressive nutritional intervention.



**Table 6** Comparison of estimates of %TBW calculated using varying equilibration points, from three to 11 hours post-dosing, in one healthy preterm infant at 34 wk PMA.

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<b>Sampling</b>	<b>%TBW</b>	<b>%TBW</b>
<b><u>Time (hr)<sup>†</sup></u></b>	<b><u>(<sup>18</sup>O)</u></b>	<b><u>(<sup>2</sup>H)</u></b>
3	87.6	85.4
4.6	84.8	82.9
5.9	86.5	84.2
7.2	86.8	85.3
8.7	88.4	86.3
9.5	89.8	88.4
11.2	90.9	89.2

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<sup>†</sup>Isotope dosing is time zero.

Our study has identified that measurements of body composition by DLW must be interpreted with caution. Details about methodological issues such as determination of isotopic plateau or consideration of dietary water intake must be provided. Otherwise, comparisons of body composition of infants between studies may be confounded by different methodology.

### **ACKNOWLEDGMENTS**

We are grateful to the families and the infants who participated in this study, and to Michelle Whelan, RN, for her patience and skill in acquiring anthropometric and body composition measures. We are also appreciative of the staff of the Growth and Development Clinic at the Children's Hospital of Chedoke-McMaster for assisting in the timely follow-up of the infants in this study.

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**CHAPTER 7                    LONG TERM OUTCOMES AFTER EARLY  
NUTRITIONAL INTERVENTION**

**Enhanced nutrition for infants with bronchopulmonary dysplasia to 3 months corrected age does not improve growth and body composition at one year: Follow-up of a randomized intervention trial.**

**Janet A. Brunton, B.A. Sc., Saroj Saigal, MD and Stephanie A. Atkinson, Ph.D.**

The following manuscript is the third paper resulting from a large randomized nutrition intervention trial. The multiple authorship can be explained as follows: **Dr. Stephanie Atkinson** and **Dr. Saroj Saigal** were the principal investigators of the study. **Dr. Saigal** was responsible for the long term developmental follow-up of the infants who participated in this study, which was assessed as an outcome, but is not included in this manuscript. **Dr. Saigal** also acted as an informal advisor to the Ph.D. candidate, and actively participated in the evolution of these data into the manuscript.

The Ph.D. candidate was the writer of this manuscript, and was involved in the design and the process of attaining funding for the study. Most of the measurements in the study were conducted by her, with the assistance of a skilled research nurse. All analytical work and data analyses were done by the Ph.D. candidate, under the direct supervision of **Dr. Atkinson**.

This manuscript is in the format required for submission to the Journal of Pediatrics. These data have been published in abstract format in **Am J Clin Nutr 1995;61:909 (abst)**.

This manuscript is in preparation for submission to the Journal of Early Human Development.



Enhanced nutrition for infants with bronchopulmonary dysplasia to 3 months corrected age does not improve growth and body composition at one year: Follow-up of a randomized intervention trial.

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Funded by a grant from the Ontario Ministry of Health, with formula manufactured to study specifications and donated by Wyeth-Ayerst Int., Radnor, PA.

**ABBREVIATIONS**

**BPD - bronchopulmonary dysplasia**

**CA - corrected age**

**DXA - dual energy x-ray absorptiometry**

**EF - enriched formula**

**PMA - post-menstrual age**

**P-RNI - Canadian recommended nutrient intakes for premature infants**

**SF - standard formula**

**SPA - single photon absorptiometry**

**ABSTRACT**

*Objective:* To determine whether a formula enriched in energy, protein and minerals (EF), compared to an isoenergetic standard formula (SF), would influence growth attainment and body composition of infants with BPD after cessation of the intervention at 3 mo CA.

*Study Design:* A prospective, longitudinal follow-up study was conducted in 56 infants with BPD (birth weight =  $866 \pm 169$  g, gestational age =  $26 \pm 1.5$  wk) at 6, 9 and 12 mo CA after completion at 3 mo CA of a randomized, blinded nutrition intervention trial. Outcome measures included nutrient intake, anthropometry, body composition (lean, fat and bone masses by dual energy x-ray absorptiometry) and distal radial bone mineral content.

*Results:* There were no differences between groups in any growth or body composition measure at 12 mo CA. Within treatment groups, the SF infants had significantly higher length z-scores ( $p = 0.004$ ) at 12 mo CA compared to 3 mo CA. Infants fed the EF had significantly more negative weight z-scores ( $p = 0.04$ ) at 12 mo versus 3 mo CA. Infants fed the SF also had a significant decline in % body fat from 3 to 12 mo CA ( $p = 0.01$ ). Growth patterns differed between SF and EF groups, despite similar self-selected nutrient intakes.

*Conclusions:* Growth benefits provided by the EF were not sustained after the intervention ended. A longer period of supplemental nutrition is likely necessary to facilitate catch-up growth in infants recovering from BPD.

## INTRODUCTION

Very little is known about the long term growth, or growth potential of extremely low birth weight infants with bronchopulmonary dysplasia (BPD). High morbidity and sub-optimal growth performance have been reported in survivors of BPD at two, three and eight years (1,2,3,4,5,6). However, reports have been limited to descriptive studies of infants who were larger at birth than the infants who currently develop BPD. Improvements in respiratory support and pharmacotherapy have not resulted in a decline in BPD, but rather a more immature surviving population (7), likely at even greater risk for growth failure.

Prolonged parenteral nutrition, fluid restriction, and lengthy courses of diuretics (8) and catabolic steroids (9,10) may invoke nutrient deficiencies that exacerbate long term growth delays associated with BPD (11,12). The shortened gestation results in limited stores of nutrients essential for normal growth and tissue repair, and these nutrients, including non-protein energy stores, are likely depleted early in neonatal life (13). Whether the provision of adequate nutrients in the early or late stage of BPD can ameliorate growth failure has not been investigated, largely because "adequate" levels of nutrient have not been determined for this population.

Randomized, blinded studies of healthy premature infants have demonstrated that supplementation with minerals alone (14, 15), or protein, energy and minerals (16,17,18) after term corrected age supported accelerated rates of mineral deposition and/or growth. Until recently, no such intervention studies had been conducted in

infants with BPD. In clinical practice, aggressive nutritional management generally ends with hospital discharge, unless overt failure to thrive elicits further medical attention. Since elevated resting energy expenditure has been described in BPD infants with growth failure (19), nutritional intervention is often limited to the provision of extra energy (20). We recently identified the ineffectiveness of sole energy supplementation in ameliorating growth failure in preterm infants post-hospital discharge in a randomized, blinded trial of two different nutritional therapies (Chapter 5).

In infants with BPD, we conducted a nutritional intervention trial of a high energy formula enriched in protein and minerals compared to an isoenergetic formula with standard concentrations of protein and minerals, fed from 37 weeks post-menstrual age (PMA) to three months corrected age (3 mo CA). The enriched formula supported greater lean and bone mass accretion, with a greater attained length at the end of the intervention, compared to the standard formula supplemented with energy alone (Chapter 5).

This study presents the long term effects of the intervention on rate and composition of growth. We hypothesized that after the intervention ended, the infants fed the enriched formula would maintain the growth advantage, and would have a greater lean:fat mass ratio and greater bone mass when assessed up to 12 mo CA, compared to the infants fed the standard formula.

## PATIENTS AND METHODS

Details of the methods during the "intervention period" of the study have been published elsewhere (Chapter 5). In brief, infants were recruited from the neonatal intensive care units at The Children's Hospital, Hamilton Health Sciences Corporation, and St. Joseph's Hospital in Hamilton, Ontario. The study was approved by the Research Project Advisory Committees at both hospitals. All infants were formula fed by parental choice. Infants less than 1500 g birth weight and appropriate for gestational who were diagnosed with BPD as previously described (Chapter 5) were identified for the study, and informed consent for enrollment was obtained.

At 37-38 weeks PMA, infants were randomized via sealed envelope to an enriched formula (EF) (3760 kJ, 23 g protein, 25 mmol calcium, 20 mmol phosphorus and 165  $\mu$ mol zinc per litre) or standard formula (SF) (3760 kJ, 15 g protein, 10 mmol calcium, 9 mmol phosphorus and 77  $\mu$ mol zinc per litre). The composition of the formulas, which were provided by Wyeth-Ayerst International (Radnor, PA) was verified by analysis, as previously described (Chapter 5). Randomization was stratified by birth weight and severity of BPD. Parents and all research team members were blinded to the intervention, which continued until the infants reached 3 mo CA. After this time, choices regarding the nutritional management were made by the parents and the primary care physician. Infants returned to the Growth and Development Clinic at The Children's Hospital of

Chedoke-McMaster at 3, 6 and 12 mo CA as part of the routine follow-up care. During these visits, measurements of weight, length, head circumference, distal 1/3 radial bone mineral content and whole body composition were conducted by the trained study team as previously described (Chapter 5). Measures of weight and length were converted to NCHS standard deviation scores (21). Whole body composition including lean mass, % body fat, and whole body bone mineral content were measured using dual energy x-ray absorptiometry (DXA) (Hologic QDR 1000/W, Waltham, MA).

Prior to the clinic visits, and at 9 mo CA, parents were contacted to determine each infant's diet history. Subsequently, nutrient intake was determined from five day weighed food intake records, which was accomplished as follows. The families of the subjects were provided with pre-weighed jars of infant foods (H.J. Heinz Company of Canada Ltd., North York, ON) and proprietary formula, based on the dietary information obtained from the parents. Unconsumed portions of food and formula were returned to the laboratory for post-weighing. Pre-weighed dry foods such as cereals and biscuits were also provided. If infants were receiving table foods or finger foods, the parents were instructed to use measured volumes. Dietary composition was analyzed using a software program based on the Canadian Nutrient File (Nutrient Analysis Program, E. Warwick, PEI), with the nutrient composition of the infant foods used in this study added manually to the data base.

*Statistics* Differences in nutrient intake were determined by Student's t-test (BMDP

Student Version, BMDP Statistical Software, Inc., Los Angeles CA). Weight, length, head circumference and radial bone mineral content at 3, 6 and 12 mo CA were compared between groups by repeated measures ANOVA (BMDP Student Version, BMDP Statistical Software, Inc., Los Angeles CA). Differences in body composition as measured by DXA at 3, 6 and 12 mo CA were compared by ANOVA, since measures at all three times were not successful in all infants (BMDP Student Version, BMDP Statistical Software, Inc., Los Angeles CA). Within group changes over time were assessed by one way ANOVA (Minitab Statistical Software, Minitab Inc., State College, PA).

## RESULTS

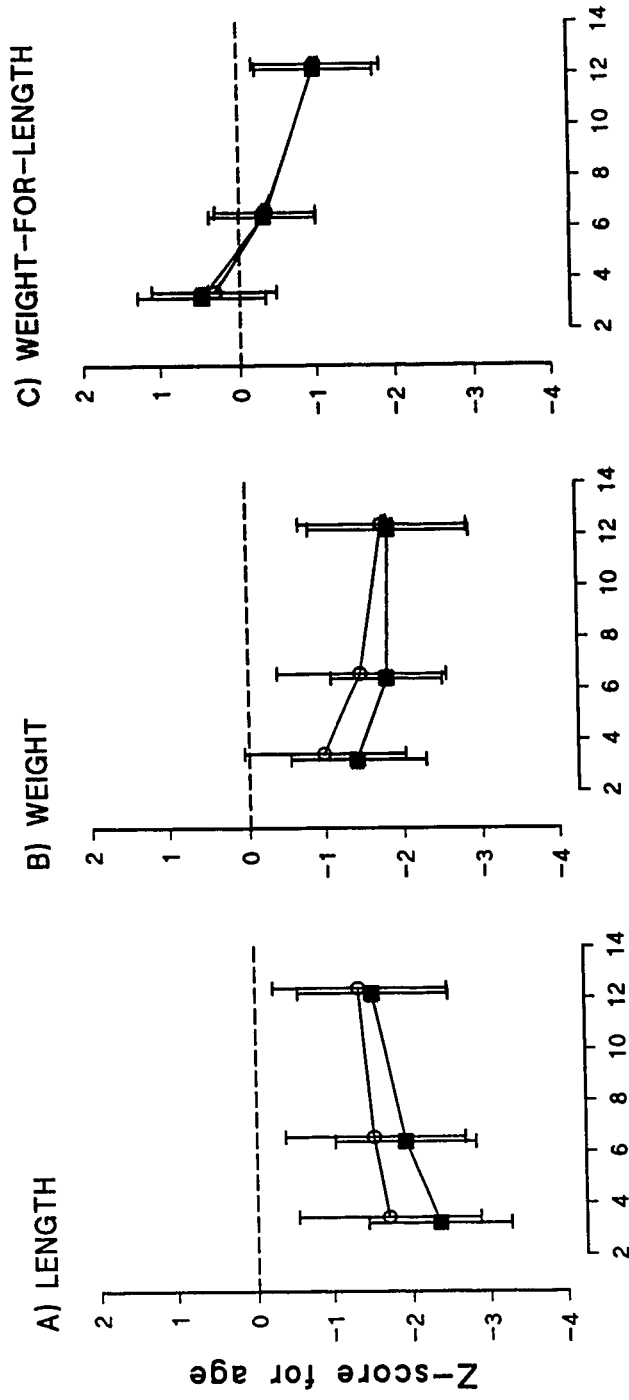
Fifty-six infants were participating in the study at the end of the intervention period at 3 mo CA, with 30 infants in the SF group, and 26 infants in the EF group. The infants were  $866 \pm 169$  g (mean  $\pm$  SD) birth weight, and  $26.0 \pm 1.5$  weeks gestational age at birth. Details of infant characteristics are published elsewhere (Chapter 5). There were no differences between treatment groups in birth weight, gestational age, days on a ventilator, days to regain birth weight, days to full oral feeds, age, weight or length at randomization, or total length of time on intervention. Seven infants in the EF group had neurological impairments at 12 mo CA. Specifically, cerebral palsy was diagnosed in five infants, blindness and deafness in one infant, and unilateral blindness in one infant. Three infants in the SF group had



neurological impairments, including one infant with cerebral palsy and two infants with deafness.

*Growth from 3 to 12 months*

At 3 mo CA, the infants in the EF group were significantly longer than the infants in the SF group. There was no sustained improvement of length growth in the EF group, since Z-scores did not change significantly from 3 mo to 12 mo CA (Figure 1a). The infants in the SF group were clinically stunted at 3 mo CA (22) but exhibited a significant increase in the mean length Z-scores ( $p = 0.004$ ), catching up to the EF group by 12 mo CA (Figure 1a). There were no differences in weight Z-scores between treatment groups at any time during the study (Figure 1b). Inclusion of the presence of neurological impairment as a covariate did not alter these results. The mean weight Z-score of the EF group became significantly more negative from 3 to 12 mo CA ( $-1.0 \pm 1.0$  versus  $-1.8 \pm 1.1$ ,  $p = 0.04$ ). There was no significant change in the SF group, which ultimately was  $-1.9 \pm 1.0$  at 12 mo CA. Both SF and EF infants were short for their weight at 3 mo CA with weight-for-length Z-scores of  $0.47 \pm 0.8$  and  $0.31 \pm 0.8$ , respectively. This pattern of growth changed significantly from 3 to 12 mo CA, as the weight-for-length Z-scores went from a positive value to a negative value ( $-1.0 \pm 0.8$  in both groups,  $p < 0.001$ ) (Figure 1c). There were no differences between formula groups in mean head circumference at any time during the study (Figure 2).



## Age adjusted to term (mo)

Figure 1 Z-scores of length-for-age (A), weight-for-age (B), and weight-for-length (C) for infants fed the EF (○) or SF (■) to 3 mo CA, measured from 3 to 12 mo CA. The mean length-for-age z-score of the SF group increased significantly from 3 to 12 mo CA ( $p = 0.004$ ). The mean weight-for-age z-score of the EF group declined significantly ( $p = 0.04$ ). Both the EF and the SF groups had significant declines in weight-for-length z-scores from 3 to 12 mo CA ( $p < 0.001$ ).

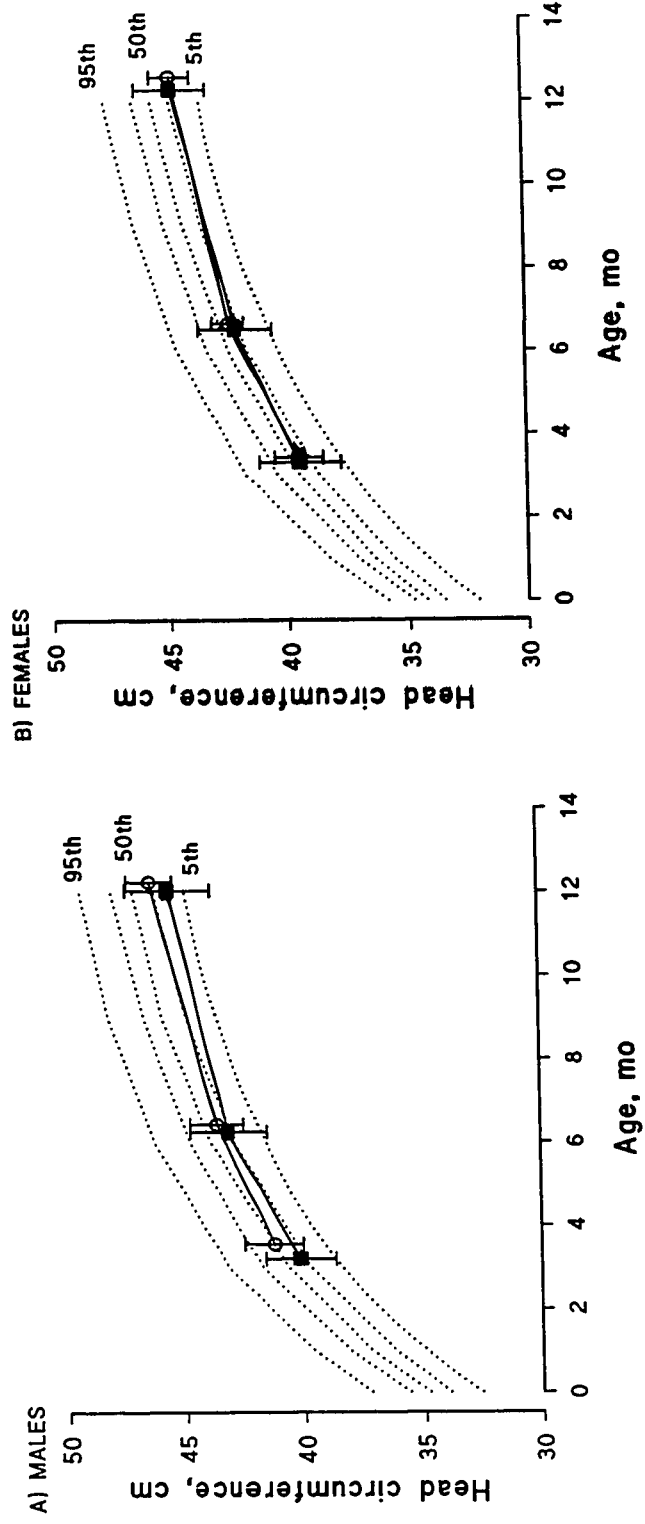
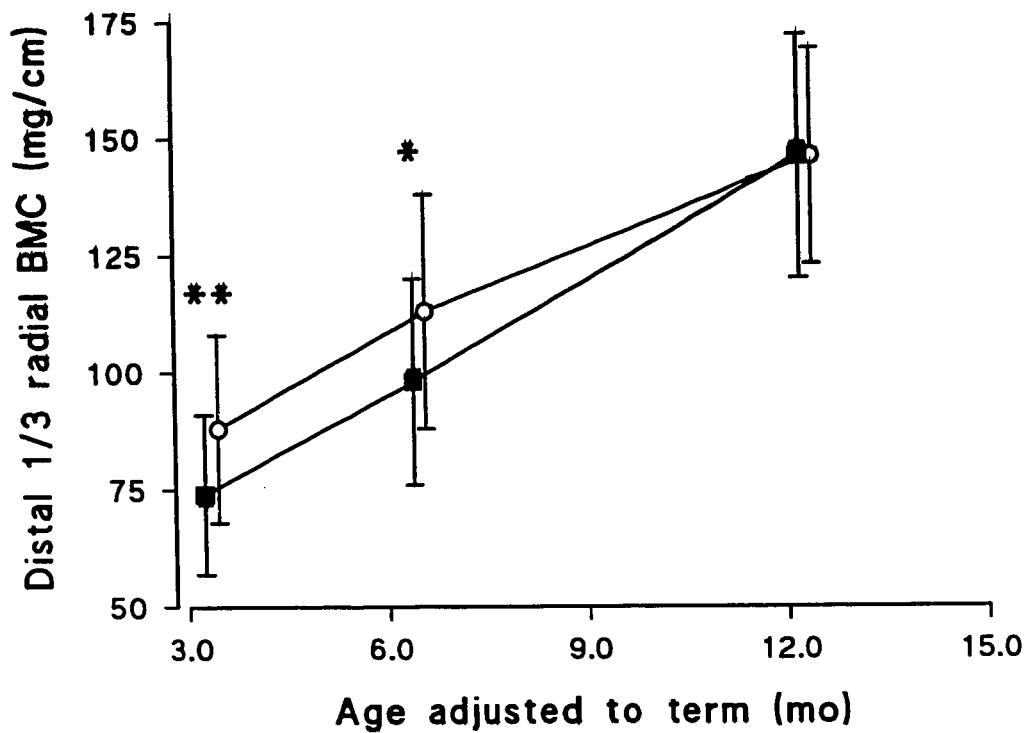


Figure 2 Head circumferences of EF (○) and SF (■) infants compared to the NCHS centile standards (-----) for male (A) and female (B) infants.

### *Whole body composition*

The enriched formula, fed until 3 mo CA, had a sustained effect on distal radial bone mineral content ( $p = 0.04$ ), with significant differences between the treatment group means at 3 ( $p = 0.003$ ) and 6 mo CA ( $p = 0.02$ ), but not at 12 mo CA (Figure 3).

Whole body composition measures were successful in 22 EF and 23 SF infants at 3 mo CA, 16 EF and 20 SF infants at 6 mo CA, and 18 EF and 18 SF infants at 12 mo CA. Since no sedation was used, occasionally infants would not sleep, and the DXA scan was not acquired. Whole body bone mineral content was significantly greater only in the males in the EF group at 3 mo CA ( $p = 0.02$ ), but not at 6 or 12 mo CA ( $p = 0.04$ ) (Figure 4a). Total body lean mass was significantly greater in the EF males and females at 3 mo ( $p < 0.01$ ), but not at 6 or 12 mo CA (Figure 4b). The SF females had significantly greater % body fat at 3 mo CA than the EF females (Figure 4c), and only the SF infants demonstrated a significant decline in % body fat from 3 to 12 mo CA ( $p = 0.01$ ).



**Figure 3** Distal one third radial bone mineral content (BMC) in infants fed EF (○) or a SF (■) to 3 mo CA, measured serially from 3 to 12 mo CA. Infants fed EF had significantly greater bone mineral content at 3 mo CA (\*\*p = 0.003), and at 6 mo CA (\*p = 0.02).

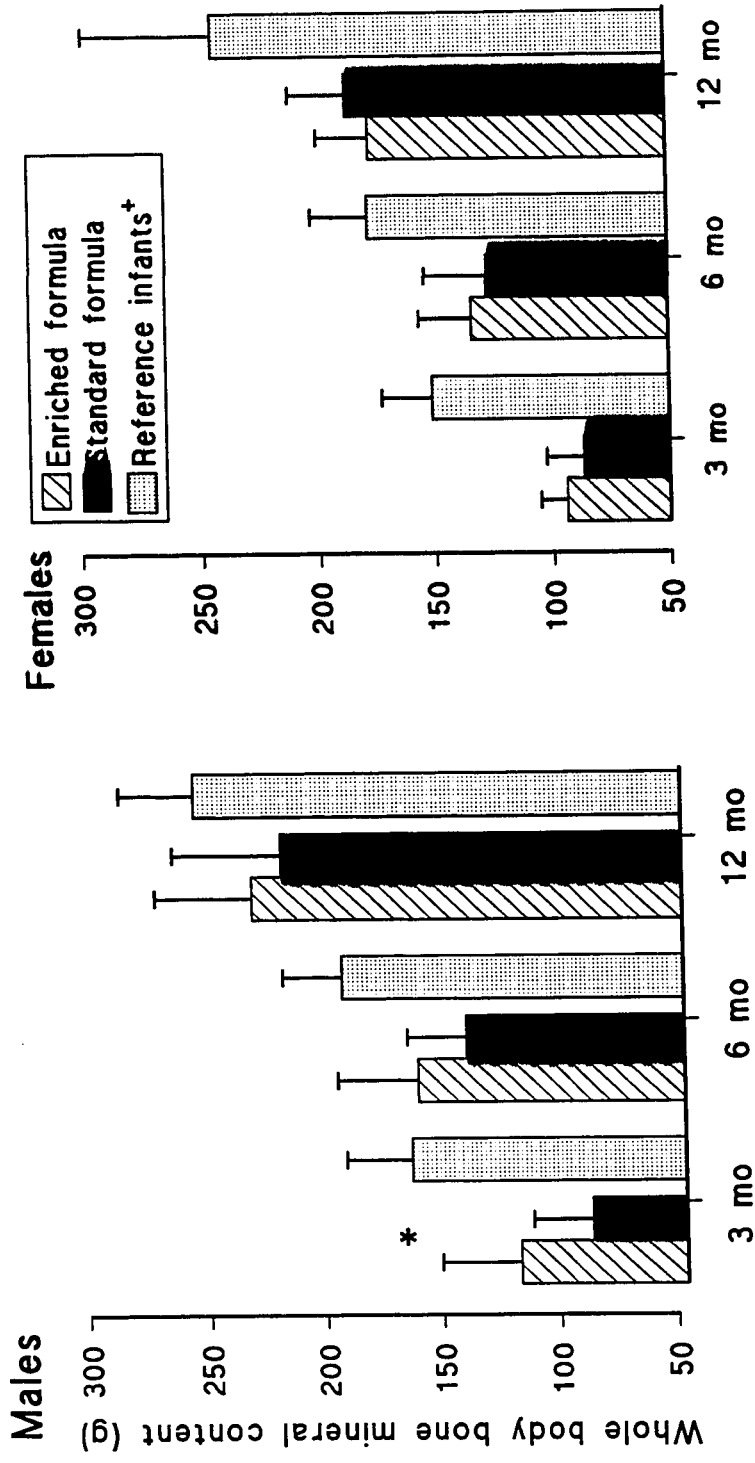
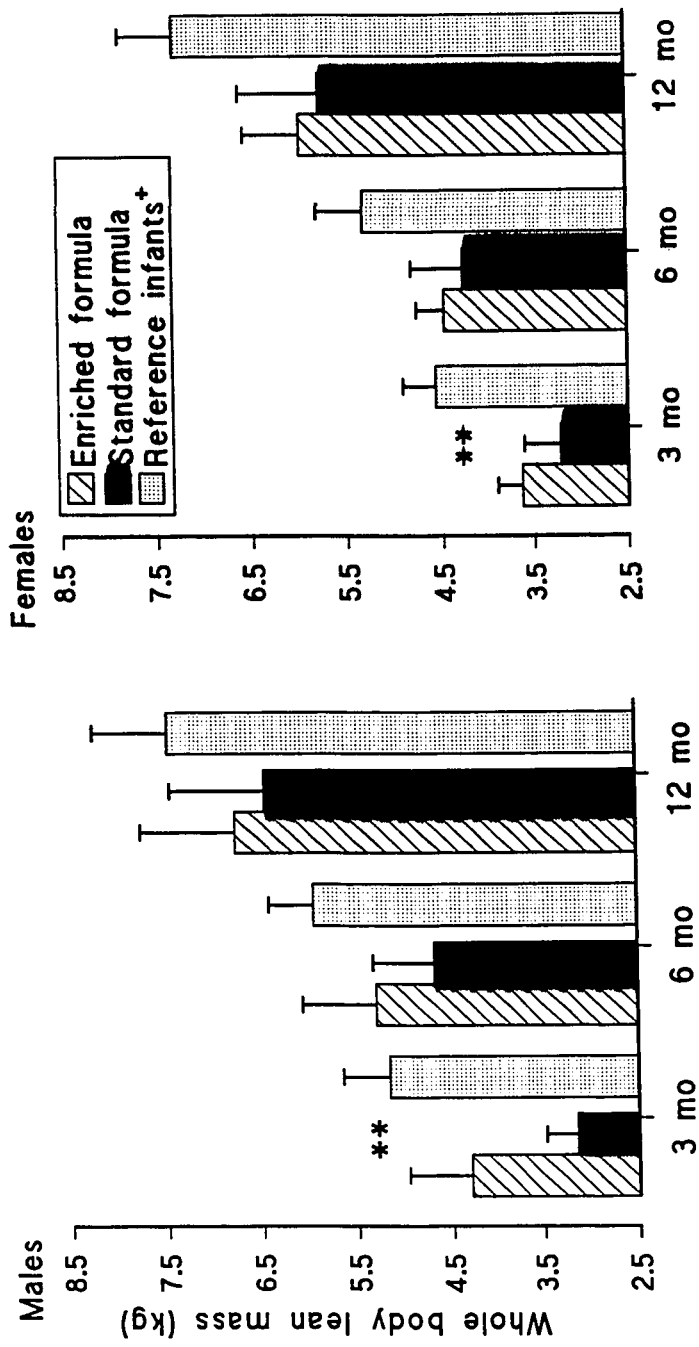
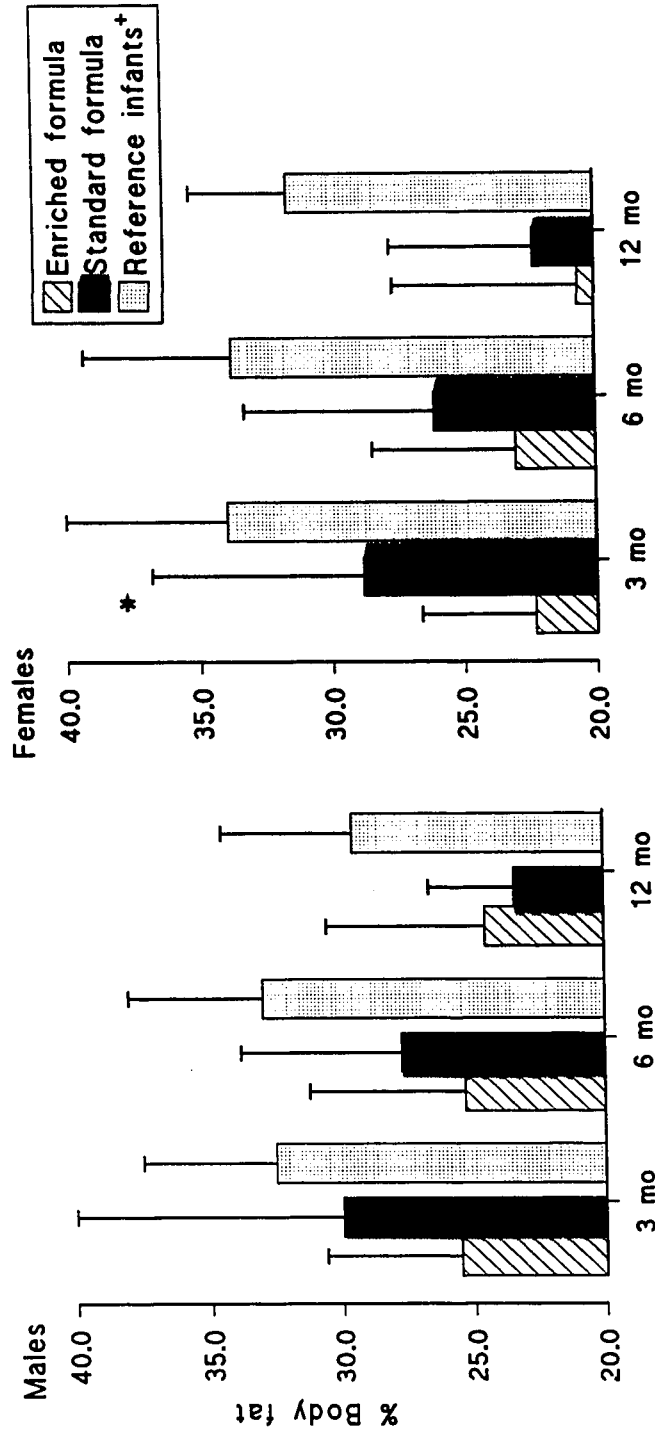


Figure 4A Whole body bone mineral content of infants fed the EF or the SF to 3 mo CA, measured at 3, 6 and 12 mo CA and compared to a term born reference group of infants (+) who were formula fed to 1 year (23). The difference between treatment groups (EF and SF) is identified by an asterisk (\* $p < 0.05$ ).



**Figure 4B** Whole body lean mass of infants fed the EF or the SF to 3 mo CA, measured at 3, 6 and 12 mo CA and compared to a term born reference group of infants (+) who were formula fed to 1 year (23). Differences between treatment groups (EF and SF) are identified by asterisks (\*\* $p < 0.01$ ).



**Figure 4C** Percent body fat of infants fed the EF or the SF to 3 mo CA, measured at 3, 6 and 12 mo CA and compared to a term born reference group of infants (+) who were formula fed to 1 year (23). Difference between treatment groups (EF and SF) is identified by an asterisk (\* $p < 0.05$ ).



*Nutrient intake*

There were no differences in intake between groups for any nutrient assessed (Table 1). Compared to current Canadian nutrient recommendations for premature infants (P-RNI) during the first year post-hospital discharge, both groups of infants had intakes of zinc and vitamin D (from food sources alone) that were low (24). At 6 mo CA, Vitamin D supplements were still being given to almost 50% of all study infants, but supplementation declined to 18% at 9 mo CA, and less than 10% at 12 mo CA. Mean energy intakes were within the recommended range, but the proportion of infants consuming less than the P-RNI for energy of 420 kJ/d (24) at 6, 9 and 12 mo CA was 52%, 49% and 38%, respectively.

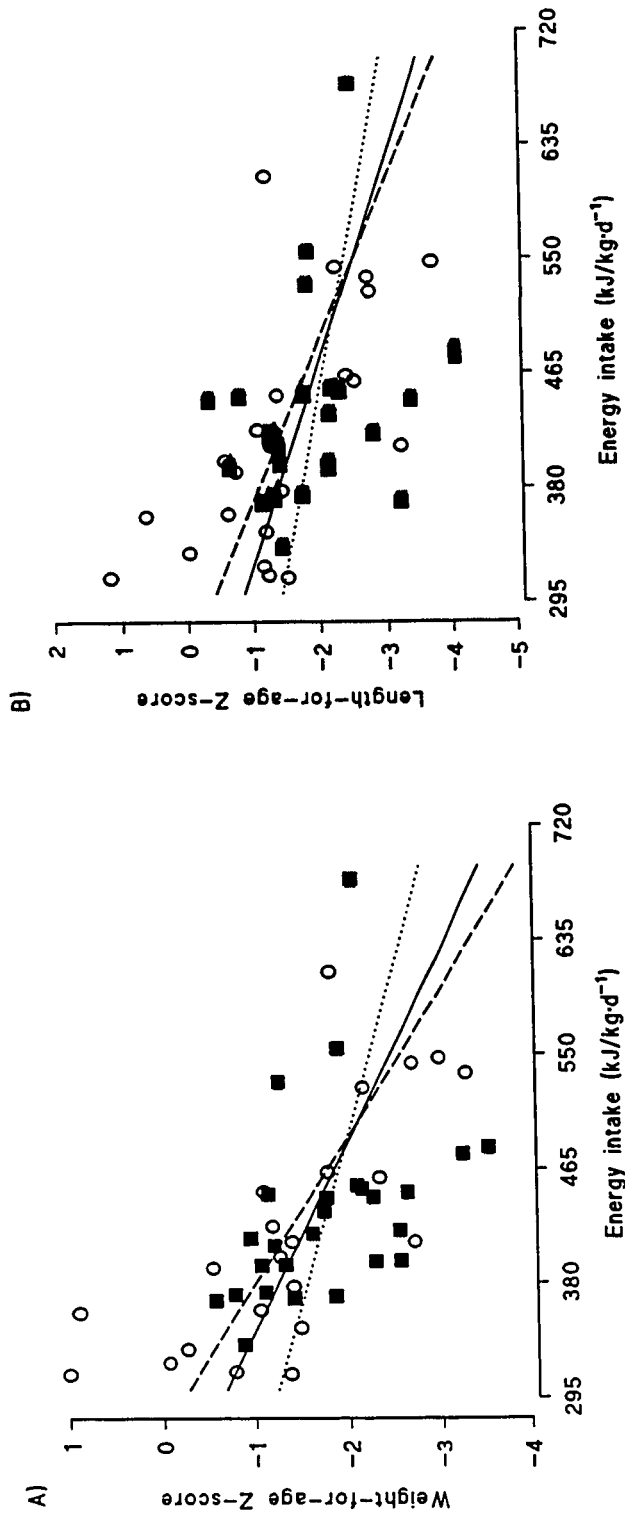
At 6 mo CA, energy intake was negatively correlated with both weight and length Z-scores when the EF and SF groups were combined (Figure 5). When regressed separately, the relationship between energy intake and weight and length was significant in only the EF fed infants. Energy intake was marginally significant when regressed against weight and length Z-scores at 12 mo CA, but the relationships were not significant when the formula groups were regressed separately (data not shown). No relationships between former treatment group, protein intake at 6 or 12 mo CA and weight or length Z-scores were apparent.

Table 1. Nutrient intakes at six, nine and 12 mo CA by infants who were fed either the enriched or the standard formula to 3 mo CA, and compared to recommended nutrient intakes for premature infants for one year post-discharge from hospital (P-RNI) (24).

Nutrient	Age	Enriched	Standard	P-RNI
Energy (kJ/kg·d <sup>-1</sup> )	6 mo	418 ± 88 <sup>†</sup>	435 ± 71	420 - 500
	9 mo	431 ± 79	456 ± 92	420 - 500
	12 mo	481 ± 121	451 ± 79	420 - 500
Protein (g/kg·d <sup>-1</sup> )	6 mo	2.5 ± 0.9	2.4 ± 0.7	2.2
	9 mo	3.1 ± 1.1	2.8 ± 0.9	2.2
	12 mo	3.9 ± 1.3	4.1 ± 1.7	2.2
Calcium (mmol/d)	6 mo	13.0 ± 5.5	13.0 ± 4.6	9.4
	9 mo	18.6 ± 8.4	17.4 ± 8.0	9.4
	12 mo	22.0 ± 9.6	23.0 ± 9.0	9.4
Phosphorus (mmol/d)	6 mo	13.2 ± 6.2	13.5 ± 5.5	8.8
	9 mo	21.0 ± 9.7	19.2 ± 9.5	8.8
	12 mo	26.4 ± 10.3	27.2 ± 10.4	8.8
Zinc (μmol/kg·d <sup>-1</sup> )	6 mo	10 ± 2.4	10 ± 1.9	15
	9 mo	9 ± 3.0	10 ± 4.1	15
	12 mo	9 ± 3.6	9 ± 2.8	15
Vitamin D (μg/d) <sup>‡</sup>	6 mo	7.8 ± 2.1 [11.2 ± 6.0]	8.2 ± 2.3 [10.7 ± 5.4]	10
	9 mo	8.0 ± 2.6 [10.5 ± 6.4]	7.9 ± 3.5 [8.0 ± 3.0]	10
	12 mo	7.3 ± 2.6 [8.4 ± 4.5]	7.4 ± 2.3 [7.8 ± 4.7]	10
Vitamin A (μg/d) <sup>‡</sup>	6 mo	1224 ± 689	1248 ± 640	400
	9 mo	1018 ± 431	1354 ± 701	400
	12 mo	1081 ± 676	1309 ± 953	400

<sup>†</sup>Values are means ± SD.

<sup>‡</sup>Values are intake from food sources only; values in parentheses [ ] include vitamin D intake from food and supplements



**Figure 5** A) Weight-for-age and B) length-for-age Z-scores (ZS) of the Enriched (○) and the Standard (■) formula groups at 6 mo CA regressed against energy intake (EI). The regression equations are as follows:

<b>Weight:</b>	Both groups (solid line)	$ZS = -0.01_{EI} + 1.47, r = -0.59, p < 0.001$
	EF (hatched line)	$ZS = -0.01_{EI} + 2.51, r = -0.72, p < 0.001$
	SF (dotted line)	$ZS = -0.01_{EI} - 0.02, r = -0.39, p = 0.052.$
<b>Length:</b>	Both groups (solid line)	$ZS = -0.01_{EI} + 1.16, r = -0.49, p < 0.001$
	EF (hatched line)	$ZS = -0.01_{EI} + 2.11, r = -0.63, p < 0.01$
	SF (dotted line)	$ZS = -0.01_{EI} - 0.26, r = -0.29, p = 0.15.$

## DISCUSSION

Aggressive nutritional intervention during the early post-hospital discharge period supported significantly improved growth; but with cessation of the nutritional intervention at 3 mo CA, there were no long term benefits to growth. Enriched formulas fed to infants post-hospital discharge had not been studied in infants with BPD or healthy infants prior to the initiation of our intervention study. The benefit of added protein and minerals was not established and we had concerns that the additional nutrients could contribute significant metabolic stress to infants recovering from chronic lung disease. Therefore it seemed prudent to end the intervention at 3 mo CA. Recently, Lucas et al (16) demonstrated improved weight and length growth in healthy preterm infants fed an enriched versus a standard formula to 9 mo CA, supporting the use of a longer intervention period.

Evidence of catch-up growth in length was seen only in the EF fed infants during the intervention (Chapter 5), which ceased after the intervention ended. In contrast, the infants formerly fed SF exhibited some evidence of length catch-up growth only during the period after the intervention ended, from 3 to 12 mo CA. In the study by Lucas et al (16), the deviation in length growth between formula groups also occurred early, but was maintained throughout the study with the continued feeding of the enriched formula. We saw no differences in body weight between our treatment groups at the end of the nutrition intervention, and both groups of infants declined on the weight growth centiles when the high energy feeding ceased. The infants studied

by Lucas et al (16) who received an energy enriched formula to 9 mo CA maintained superior weight gain for the duration of the study compared to infants fed a standard formula. These data imply that additional energy for a prolonged period of time is beneficial to growth. An important question is whether the growth advantage facilitated by enhanced nutrition up to 9 mo CA (16) will be maintained without further supplemental nutrition. The duration of nutritional support necessary to facilitate growth equal to an infant's genetic potential has not been established.

The slight catch-up in length growth by the infants formerly fed the SF occurred without any detectable differences in nutrient intake between formula groups. We employed a rigorous method of collecting nutrient intake data, providing and collecting pre-weighed foodstuffs. Therefore, the energy for growth likely was contributed by energy stores, as evidenced by the decline in the % body fat of the SF fed group.

During the intervention phase of the study, measurements of nitrogen retention and lean mass accretion demonstrated that infants fed the SF were protein deficient (Chapter 5). However, after 6 mo CA the mean protein intakes by both groups met or exceeded current recommendations (24). Protein intake was likely adequate or in excess of requirements; this is indirectly supported by the lack of a correlation between protein intake and weight or length Z-scores at 6 or 12 mo CA. Thus, energy intake, not protein, may be the major limiting factor to achieving better growth from 6 to 12 mo CA. The strong negative correlation between energy intake

and both weight and length Z-scores, particularly at 6 mo CA, may reflect compensation for elevated energy expenditure, particularly in infants with residual lung disease. In infants with BPD, elevated resting metabolic rates compared to healthy infants (19,24,25,26) have been identified. Kurzner et al (19) demonstrated that infants with BPD and growth failure had significantly greater energy expenditure than BPD infants without growth failure or healthy size matched controls. While the mean energy intakes exceeded the P-RNI (24), those recommendations were established for preterm infants without chronic lung disease. Energy expenditure of our infants at 3 mo CA (Chapter 5) was 8-18% higher compared to term born reference infants (27), so perhaps infants with BPD do have greater requirements for energy. Currently, there are no energy expenditure data available for healthy premature infants post-term age to serve as a comparison.

The optimal zinc intake by preterm infants after term age has not been established. Based on little scientific evidence the recommendation has been set at 15  $\mu\text{mol}/\text{kg}\cdot\text{d}^{-1}$  (24,29). After 3 mo CA, both groups of infants had mean zinc intakes less than this value. Healthy preterm infants with zinc intakes similar to our infants ( $9.2 \mu\text{mol}/\text{kg}\cdot\text{d}^{-1}$ ) at 6 mo CA had hair zinc values significantly lower than term-born control infants, and 37% of the infants were deemed zinc deficient (30). Long term zinc supplementation of healthy preterm infants has resulted in improved length growth velocity between 3 and 12 mo CA (31). Therefore, catch-up growth in our infants may have been impeded by sub-optimal zinc status. To definitively prove this,

a growth response to zinc supplementation in the presence of adequate protein and energy must be demonstrated in randomized trial.

Commonly, infants with BPD reach term with undermineralized bones, due to prolonged parenteral nutrition, and the mineral losing actions of diuretics (32) and glucocorticoids (33) used to treat BPD. The amount of mineral supplementation and the length of time necessary to optimize bone mineralization is unknown. The provision of minerals in concentrations above standard term formulas after hospital discharge improved bone mineral content at a peripheral bone site (Chapter 5, 17, 14). Comparing bone mineral content at the one third distal radius site, our BPD infants at 12 mo CA were similar to 12 mo old healthy term born infants (34), thus had attained "catch-up". But the BPD infants were much smaller than the healthy one year old infants, so the measurement of bone at a peripheral site may not be indicative of overall bone status.

It is likely that intakes of calcium and phosphorus were adequate in both groups of BPD infants during this follow-up study. Significant differences in bone mineral mass between EF and SF groups existed at the end of the intervention. Subsequently, by 12 mo CA the SF infants achieved comparable distal radial and whole body bone mineral content while consuming mineral intakes similar to the EF group. Furthermore, when whole body bone mineral mass is expressed per kilogram body weight, the BPD infants were similar to healthy term born formula fed infants at 12 mo (23). This is suggestive of an adequate calcium and phosphorus intake, with linear

bone growth being restricted by another factor which is limiting overall growth.

This first attempt at improving the longitudinal growth of infants with BPD by post-hospital discharge nutrition using a randomized, blinded study design ultimately did not result in any long term growth benefits to one year corrected age. These BPD infants responded with enhanced growth during aggressive nutritional management with an enriched formula post-hospital discharge. Others have demonstrated in healthy premature infants that this enhancement persisted with continued intervention (16,17). Optimal growth achievement is likely a proxy for adequate nutrition and health status. Ultimately, this could have a positive influence on neurodevelopmental outcomes (35,36,37) and morbidity (38). For these reasons, further studies of prolonged aggressive nutritional interventions with enriched formulas are essential, especially in nutritionally vulnerable populations such as premature infants recovering from chronic lung disease.

### **Acknowledgments**

We are grateful to the families and the infants who participated in this study, and to Michelle Whelan, RN, for her patience and skill in acquiring anthropometric and body composition measures. We are also appreciative of the staff of the Growth and Development Clinic at the Children's Hospital of Chedoke-McMaster for assisting in the timely follow-up of the infants in this study.



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**FINAL DISCUSSION  
AND  
FUTURE PERSPECTIVES**

## CHAPTER 8      DISCUSSION AND FUTURE PERSPECTIVES

### *Enhanced Somatic Growth and Body Composition in Infants Recovering from BPD*

Low birth weight infants with BPD will grow more rapidly, and will gain weight as lean and bone masses, if the substrates necessary for growth are available at the appropriate time. The efficiency of nutrient absorption by infants with BPD had never been investigated in a controlled study. A study such as ours was necessary to eliminate malabsorption as a cause of growth delay. We clearly established that global nutrient malabsorption does not contribute to growth failure in infants with BPD. Carefully conducted mass balance studies demonstrated similar fractional absorption of nutrients between formula treatment groups (Chapter 5).

We also demonstrated that the additional nutrients provided in an enriched formula were utilized. There was a period of time as the infants approached term, when the provision of supplemental minerals and protein facilitated a velocity of weight and length gain, protein accretion (by mass balance) and bone mineral accretion that was well in excess of rates observed in unsupplemented infants (Chapter 5). One of the key questions emanating out of these results is, "*What level of nutrient supplementation is warranted, and for how long?*". An important observation from the clinical study was the change in the rate of growth during the

intervention period for infants receiving nutrient enriched formula. The exact point (between 37 wk post-menstrual age and 3 mo CA) at which the growth rates changed is unknown, since infants were living at home; therefore, serial growth measures were not available. Also, the stimulus for the decline in growth velocity is not known. It could be developmental, triggered by an endocrine factor, or dependent upon body mass or even the respiratory disease state. This remains to be determined. This issue of the exact timing of the rapid growth is important if one concludes that the intervention was no longer effective when the growth velocity of the treatment groups appeared to become parallel (between 1 and 3 mo CA). However, it is possible that a longer intervention period may demonstrate significant but more subtle differences in growth rates.

Alternatively, in growth delayed infants, rapid growth may be triggered again after 3 mo CA, therefore additional nutrients to support growth would be required. We had to reject our original hypothesis which stated that the growth benefits (greater rate of growth, bone mass and lean to fat ratio) would be apparent up to 12 mo CA. The recent studies which consisted of a nine month nutritional intervention period (Lucas et al 1992, Bishop et al 1993) included preterm infants who were healthy and of a birth weight which was almost double that of our study population. If growth benefits were seen in healthy preterm infants, then a longer nutritional intervention period is certainly justifiable in the more compromised infants. Our evidence of

inadequate energy intake and progressive growth failure from 6 to 12 mo CA (Chapter 7) indicates that a more aggressive approach to nutritional care should be investigated.

The finding of a period of rapid growth close to term age is of clinical importance since it coincides with hospital discharge; it is when infants are switched to standard term infant formulas in preparation for going home. Considering the impact of nutrition on the recovery from BPD (Frank 1992), and the high morbidity and frequency of re-hospitalization in this population, an improved nutritional status during this early post-hospital discharge period is likely very important. Nutritional care may be crucial in terms of both infant health and the conservation of health care resources. Therefore, another question which arises out of these data is *"Does enhanced nutritional status (as measured by improved growth) result in lower morbidity in infants with BPD?"*.

An important question that we did not answer with our clinical trial is *whether catch-up growth is indeed a realistic or attainable goal in infants of such extremely low birth weights*. Reports of growth in infants with birth weights slightly greater than our infants identified long term growth failure in all infants, regardless of the presence or absence of BPD (Davidson et al 1990). Future research may demonstrate that extremely low birth weight infants do not catch-up. It is possible that nutritional intervention is ineffective. If this is true, or alternatively, if no further effort is made



to induce catch-up growth in infants with BPD, then the question must be asked, *"What are the long term health implications of sub-optimal growth in early life?"*.

Recently, attention has been focused on the concept of "programming". This refers to events occurring in early life that can alter development during a critical period, such that long term or permanent changes ensue. Infants born prematurely are at obvious risk for altered development; commonly cited is the impact of early birth on brain development. Lucas et al (1990) demonstrated that preterm formula versus term formula fed during the first four weeks of life resulted in significantly greater developmental scores at 18 mo of age in prematurely born infants. This research group has also shown that 8 year old children who were born prematurely and fed their mothers' milk had significantly greater scores on intelligence quotient tests as compared to children who were formula fed as premature infants (Lucas and Morley 1991).

The question of programming with respect to bone growth has also been recently addressed. The impact of delayed skeletal mineralization during early life on the attainment of peak bone mass is of concern if it ultimately increases the risk for bone disease in adulthood. Controversy exists regarding the ability of prematurely born infants to spontaneously catch-up in bone mass (Rubinacci et al 1993, Chapter 2). When the bone mineral content of our BPD infants was compared to healthy reference term infants, the BPD infants were appropriate for their body weight, but

low if compared based on corrected age. Factors other than simple substrate availability may be influencing bone mineralization. A follow-up study was conducted with five year old children who were either partially or fully fed their mothers' milk as premature infants. A positive correlation was observed between the proportion of nutrient intake that was mothers' milk versus bone mass (Bishop et al 1996). It is widely recognized that solely feeding human milk to a preterm infant can result in hypophosphatemia and osteopenia due to low mineral concentrations (Greer 1985). Therefore, the authors' hypothesized that either growth factors present in human milk or the lack of substrate during a critical period programmed bone cells which lead to alterations in structure and function (Bishop et al 1996).

With respect to somatic growth, an alteration in the endocrine "set point" for body size could occur as a result of interrupted fetal development and/or impaired post-natal growth secondary to inadequate nutrition. Subsequently, the genetic potential for growth is re-programmed, and catch-up growth does not occur. Recently, a relationship between poor fetal growth and increased risk for hypertension, type II diabetes, and cardiovascular disease in adulthood was identified (Barker et al, 1993). Whether this risk extends to infants born prematurely who experience poor post-natal growth remains to be determined, but it is an important question that will require many years of prospective study to answer. Elucidating the influence of programming on long term growth and development is extremely

important to be able to fully understand the implications of a study such as our clinical trial. Feeding an enriched formula altered bone, lean and fat mass accretion. Whether these alterations in growth in early life will ultimately enhance or diminish overall health is unknown.

***Growth Failure From 3 to 12 mo CA Was Not Due to Inadequate Nutrient Intake  
(As Assessed by Current Recommendations for Nutrient Intakes)***

We have conclusively demonstrated that the intake of nutrients similar to levels recommended for healthy infants will not support optimal growth in infants recovering from BPD. Nutrient intake by the infants with BPD at 6, 9 and 12 mo CA did not identify any obvious deficiencies, as compared to current recommendations for preterm infants up to one year of age (Chapter 7). Those recommendations by the Nutrition Committee of the Canadian Pediatric Society (CPS Nutrition Committee 1995) represent the most current review of the scientific literature, compared to recommendations by American Academy of Pediatrics (AAP Committee on Nutrition 1985) or European Society for Pediatric Gastroenterology and Nutrition (ESPGN 1987). However, the CPS Nutrition Committee openly acknowledges that for many nutrients, there is a scarcity of research on post-term requirements such that many recommendations represent an "educated guess". Therefore, infants with BPD may have requirements that exceed current recommendations.

We saw no evidence of catch-up growth during the first year of life. More alarmingly, while length growth was maintained along the same percentile (or Z-score), both groups of infants had Z-scores-for-weight that became progressively more negative from 3 to 12 mo CA. Protein intakes were quite high at 9 and 12 mo CA, whereas energy intakes appeared marginal compared to current recommendations. Therefore, the question to be asked is, "*Was energy intake inadequate during the second six months CA, resulting in poor weight gain?*". The depletion of body fat in the infants who were formally in the standard formula group would certainly support this hypothesis. The changing patterns of growth that we identified throughout the study imply that nutrient needs were also changing. Ideally, nutrient and energy balance studies conducted serially from the perinatal period to late infancy would provide insight into specific nutrient requirements. This may not be practical for reasons such as the expense of studies using isotopes, or the necessity to hospitalize infants for balance studies. However, without carefully controlled studies it would be very difficult to conclusively identify which nutrients (if any) are limiting growth. Presently, the knowledge of nutrient requirements for infants with BPD after 40 wk PMA is limited to the results of our follow-up study (Chapters 5 and 7).

***The Measurement of Energy Expenditure using Doubly Labelled Water in Infants with BPD is Complicated by Methodological Considerations that May be Unique to the Disease State***

Our study was the first to attempt to describe longitudinal changes in energy expenditure in infants with BPD, and was one of the first to use the doubly labelled water method to measure energy expenditure in this population. We hypothesized that infants who received nutrient enriched formula during recovery from the disease would exhibit greater energy expenditure as a result of rapid growth and synthesis of lean tissue. Our hypothesis testing was precluded by an inadequate sample size. High variability, two to three times greater than demonstrated by studies which used respiratory gas exchange, diminished our ability to detect significant results (Chapter 6). This information will be valuable to other research groups undertaking studies of energy expenditure in infants with BPD, such that appropriate sample sizes can be calculated. Recently, two other groups have presented results similar to ours (reported in abstract form only), with inexplicably high variability (Table). This supports our explanation that the disease state, or the medical management of BPD are implicated in the high variability observed.

Table Summary of results of studies of energy expenditure using doubly labelled water in infants with BPD.

Study	Number of infants	Post-menstrual age of infants (wk)	Energy expenditure (kJ/kg·d <sup>-1</sup> )
Wilson et al, 1992	8	35	281 ± 84
Leitch et al, 1996	6	31	365 ± 164
Chapter 6 (SF infants)	12	34	237 ± 109
	7	38	270 ± 129

Imprecision in the sample preparation technique or analytical errors were eliminated as the cause of the variability by duplicate and triplicate analyses of selected samples. However, undetected errors that occurred in the isotope dosing or sample collection stages could be partially responsible.

The changing nature of the disease over the past ten years may account for some of the differences in variability reported in older studies using respiratory gas exchange compared to those using doubly labelled water in infants with BPD. In newer studies, some infants may recover quickly with the use of glucocorticoids or surfactant, while other infants experience prolonged severe disease. Therefore the disease state may account for some of the variability. *Future studies should be*

*conducted to investigate the influence of the severity of disease on energy expenditure in infants with BPD.* It is important to note from the study by Leitch et al (1996), that the infants studied were of similar age and severity of disease (all had just completed a two week course of glucocorticoid therapy for BPD), therefore the disease state is not likely completely responsible for the high standard deviations. It is probable that an unidentified factor interferes with the precision of the measurement in BPD infants. To establish this fact, further validation of the method is required in this specific population. Validation against the reference method of direct calorimetry is not feasible in chronically ill infants who require nursing care. Validation against respiratory gas exchange also has problems when infants require ambient oxygen concentration greater than room air. The lack of a suitable reference method against which doubly labelled water can be validated (for use in infants with BPD) is a problem which will not be easily resolved.

From the data we collected, it is not possible to make any deductions regarding the influence of energy expenditure on growth delays in infants with BPD. If we had included a measure of lung function or severity of disease, then we may have been able to distinguish between energy expended due to rapid growth, from energy expended due to lung inflammation and repair. However, we did not observe large differences in energy expenditure between our BPD infants and healthy term born reference infants, as was previously reported by Kurzner et al (1988). It is difficult

to assess whether methodological differences between the study which compiled the reference data and our study could partially account for the small differences noted, or alternatively could be masking a large difference. Furthermore, a more appropriate set of reference data may be healthy preterm infants measured by DLW after term age. Currently, no such reference data have been published.

The significant negative correlation of growth (weight and length) and energy intake at 6 mo CA could be interpreted as elevated requirements due to residual disease, under the assumption that growth failure represents a marker for the severity of disease. This has not been proven, but could be addressed in future studies which address the relationship between disease and energy expenditure.

## **Methods of Estimating Body Composition in Small Infants**

### ***Dual energy x-ray absorptiometry***

Ultimately, we established the strengths and limitations of the DXA technology for the measurement of body composition in small infants. Important applications of this body composition measurement tool which we investigated include the assessment of longitudinal changes within an individual or a population, and cross-sectional comparisons between two distinct groups.

Our initial validation study (Chapter 3) discounted the performance claims



made by the manufacturer's representatives and those published in the product literature. DXA was not sensitive enough to reliably measure the body fat content in small piglets, and presumably preterm infants, when total body fat is below 5%. This was not surprising since the absolute amount of body fat in piglets and preterm infants weighing 1.5 kg is probably less than 75 g. While our estimates of body fat in piglets were similar to those of other investigators, it is possible we underestimated body fat. Loss of fat during the carcass grinding and homogenizing processes is possible, if DXA was assumed to be accurate it would mean a 50% loss. This is highly unlikely. With respect to the estimation of bone mineral content, the lack of accuracy of DXA in the small animals was unexpected, considering that the technology was originally designed for this purpose.

Subsequent to the completion of our first validation study, the manufacturer revised the software (which interprets the attenuation of the x-rays and translates the information into estimates of bone, fat and lean masses). This revision was partially instigated by discussions of our results with company representatives. Re-analysis of the original piglet scans using the upgraded software provided more encouraging results (Chapter 4). The error in measurement of body fat in small piglets became a systematic over-estimation, and the accuracy of the fat measurement in large piglets improved. The only measurement which was not highly correlated to the chemical analysis was the measurement of bone in the small piglets. Our study design has been

criticized, such that the measurement of 10 small piglets represented a range of bone mineral values that was too narrow to establish a significant correlation. Recently, another group of investigators compared bone mineral content as measured by DXA to bone ash in piglets ranging in size from 1 kg to greater than 5 kg, and found a highly significant relationship (Picaud et al 1996). Therefore, if the intended application of DXA is to detect longitudinal changes in bone mineral content, starting in the preterm period and ending months later, then the poor accuracy that was identified in the 1.6 kg piglets will likely not alter the interpretation of the results. However, the use of DXA to identify short-term changes in the preterm period is not appropriate without further improvements in the technology.

The precision of DXA dictates its ability to detect longitudinal changes in body composition. We established that the high variability in the measurement of body fat limits its use to detect changes within an individual, which is an important clinical application. This is particularly true for infants during the second six months of life, when the percentage of body fat declines, and in some infants, the absolute fat mass changes very little.

Our data from the piglet model combined with new validation studies (Picaud et al 1996, Koo et al 1995) have provided evidence that DXA-estimates of bone and lean masses are precise and accurate in preterm infants if measured *after term corrected age*. For bone and lean mass, DXA is appropriate for either cross-sectional

comparisons or longitudinal studies in groups or individuals. Rapid accretion of lean mass and bone mineral during the first year of life results in changes that are easily detectable by DXA, presuming that the measurements are timed carefully.

Comparison of the precision to the anticipated change is essential in order to determine the minimum duration between measurements (Chapter 4). For future studies using DXA for the measurement of body fat, an appropriate sample size that is necessary to overcome lack of precision must also be calculated (Chapter 4).

In response to the original hypothesis regarding the validity of DXA for use in infants, we have determined that the major limitation is the lack of precision and accuracy in the estimation of total body fat and bone mineral content in piglets weighing 1.6 kg. Consequently, it is also likely not appropriate for use in preterm infants in early neonatal life. A significant correlation between total body fat and chemical analysis of piglets has facilitated the creation of equations to compensate for the over-estimation (Picaud et al 1996). However, these equations were derived from small piglets "padded" with jackets of lard, thus are not representative of fat deposition patterns in small human infants. Therefore, the question that remains is, *"Are further refinements of the software algorithms possible to improve the precision and accuracy of the DXA-estimation of total body fat?"* To address this question requires further validation studies using carcass analysis of an appropriate, but as yet unidentified animal model. The newborn piglet is similar to the human

infant in absolute body composition but not in fat deposition patterns, which is an essential criteria in order to adequately assess the performance of DXA.

Furthermore, it must be noted that there is more than one manufacturer DXA systems, and each has unique scanning features and software and algorithms to interpret attenuation data. Therefore, precision and accuracy determined in the DXA system by Hologic Inc. (Waltham, MA) do not apply to body composition by DXA in general.

### ***Methodological Issues Relating to Doubly Labelled Water for the Measurement of Body Composition***

The results of the DXA validation studies (Chapters 3 and 4) prevented its use for measuring body composition of infants prior to the first follow-up visit at 40 wk PMA. In order to assess longitudinal changes in body composition from a much earlier age than possible with DXA alone, we assessed the agreement between the DXA and isotope dilution methods. While the mean fat free mass values by the two methods were similar, we demonstrated that within an individual, the two measures were often very different. Therefore, they should not be compared or used interchangeably for clinical or research purposes.

Isotope dilution is a widely accepted method of estimating total body water and lean mass. However, for use in infants, we identified two specific methodological issues that can alter the interpretation of body composition (Chapter 6). Firstly, dietary water intake proved to be an important consideration when estimating dilution space in infants. Secondly, successful identification of the point of peak isotopic enrichment is crucial for accurate estimations of total body water. In small infants, the timing of this point is variable amongst individuals. To our knowledge, neither of these issues have been adequately addressed in the literature prior to our study. Critical review of studies using doubly labelled water to measure body composition must examine the methods to determine whether these issues have been adequately addressed.

### *Summary*

This thesis investigated the impact of a short term nutritional intervention on growth, body composition and energy expenditure of infants with BPD. Enhanced growth was identified during the nutritional intervention, but no sustained benefits remained at 12 mo CA. Sub-optimal growth was identified in both groups of infants throughout the study period; no evidence of catch-up growth was detected. Our results, combined with recent reports of improved growth in healthy preterm infants at 9 mo CA with enriched formula feeding, provides convincing evidence that further

studies are warranted. Studies of enhanced nutrition for at least 9 mo after hospital discharge, with attention to energy intake, energy expenditure, and composition of growth, would help to further elucidate the etiology of growth failure in these compromised infants.

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## APPENDIX I

The descriptive characteristics (Table 1), nutrient intake (Table 2) and growth performance (Table 3) of the infants studied retrospectively to quantify growth failure in BPD infants discharged from the Children's Hospital of Chedoke-McMaster.

**Table 1** Infant characteristics

n	12
Gestational Age (wk)	25.2 (1.8) <sup>1</sup>
Birth Weight (g)	808 (273.7)
Ventilated (d)	43 (21.6)
Regain Birth Weight (d)	19 (5.9)
Full Oral Feeds (d)	32 (15.4)

<sup>1</sup>Mean(SD)

**Table 2** Nutrient intake post-hospital discharge

Corrected Age	Nutrient	Intake
Term (n=8)	Fluid (ml/kg/d)	146 (34.4) <sup>1</sup>
	Energy (kcal/kg/d)	114 (14.5)
	Protein (g/kg/d)	2.5 (0.31)
3 mo (n=8)	Fluid (ml/kg/d)	154 (20.1)
	Energy (kcal/kg/d)	113 (16.6)
	Protein (g/kg/d)	2.4 (0.30)

<sup>1</sup>mean (SD)

**Table 3** Long term growth performance

<b>Corrected Age</b>	<b>n</b>	<b>Mean Weight (kg)</b>	<b>Mean %ile<sup>1</sup></b>	<b>Mean Length (cm)</b>	<b>Mean %ile<sup>1</sup></b>
Term	12	2.82 (0.67)	3-10	48.9 (3.34)	3
3 mo	11	4.8 (0.84)	3-10	56.5 (3.68)	3-10
6 mo	9	6.3 (1.03)	3-10	63.6 (3.70)	3-10
12 mo	9	8.6 (0.73)	3-10	72.6 (3.82)	3-25

<sup>1</sup>Tanner Growth Standards (J.M. Tanner et al., 1966)

<sup>2</sup>Mean (SD)

## APPENDIX 2

### The Doubly Labelled Water Method: Description and Calculations

#### *Introduction*

The indirect calorimetry method of estimating energy expenditure, known as doubly labelled water (DLW), was first described in the 1950s by Lifson et al (1955). The method uses two separate isotopically labelled waters,  $^2\text{H}_2\text{O}$  and  $\text{H}_2^{18}\text{O}$ , which are introduced into the body and subsequently equilibrate with the body water pool. The discovery that the oxygen in expired  $\text{CO}_2$  was also in isotopic equilibrium with the body water pool provided the key to estimating carbon dioxide production via the determination of isotopic enrichment of body water (Nagy 1990).

The basic principles of the DLW method have been recently reviewed (Prentice 1990, Coward 1991, Ritz and Coward 1993). In brief, the method involves isotopically enriching body water with deuterium ( $^2\text{H}$ ) and  $^{18}\text{O}$  oxygen ( $^{18}\text{O}$ ), and the relative decline in isotope enrichment is plotted over time. The  $^2\text{H}$  remains almost totally associated with water molecules, therefore the decline in enrichment over time occurs due to dilution with new water intake, and due to excretory and evaporative water losses. Most of the  $^{18}\text{O}$  is also found in the body water pool. But due to the action of carbonic anhydrase in red blood cells, the isotopically labelled water equilibrates with  $\text{CO}_2$ . Therefore, the decline in  $^{18}\text{O}$  enrichment is due to water and vapour losses, but also due to expiration of  $\text{CO}_2$ . When the exponential decline of the two isotopes is plotted as natural log enrichment against time (Figure 1), the slope of

decline in enrichment for  $^{18}\text{O}$  is steeper than for  $^2\text{H}$ . The difference between slopes represents an estimate of  $\text{CO}_2$  production.

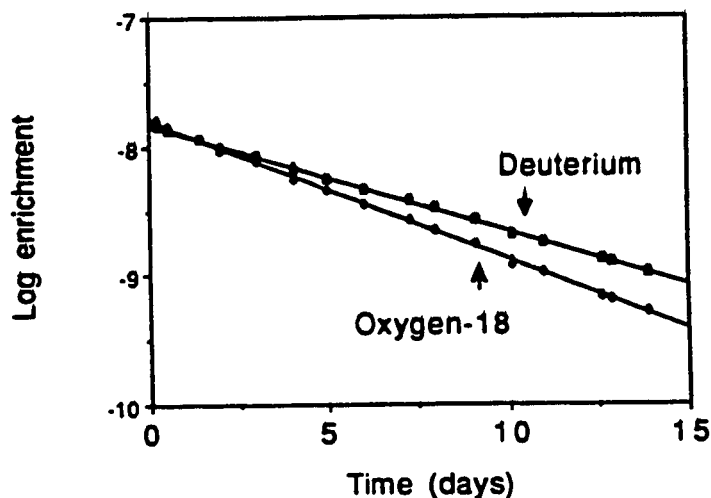


Figure 1 Natural log of isotope enrichment of body water versus time (Nagy 1990).

As the DLW was modified and validated for use in humans, the basic assumptions of the initial model as described by Lifson et al (1955) have been studied to assess the magnitude of potential errors which could evolve from these assumptions (Ritz and Coward 1995). Furthermore, many investigators have devised correction factors to adjust energy expenditure measures when it was determined that the assumptions were violated (Roberts et al 1986, Schoeller et al 1986, Jones et al 1987, Jones et al 1988, Jensen et al 1992). The following are assumptions of the method

described by Lifson et al (1955), as reviewed by Ritz and Coward (1995), which may have impact of the measurement of energy expenditure in growing premature infants.

*Total body water and the composition of the body remain constant throughout the measurement period.* It is obvious that this assumption is violated in growing infants. However, Jensen et al (1992) assessed the change in body water as a proportion of weight over a 5 day study period, and found a difference of less than 0.5%. This had negligible impact on the calculation of carbon dioxide production.

*The dilution spaces of  $^{18}\text{O}$  and  $^2\text{H}$  are equal to body water, and  $^2\text{H}$  only labels body water, while  $^{18}\text{O}$  labels body water and  $\text{CO}_2$ .* Isotope sequestration into non-aqueous body pools occurs at a different rate for the two isotopes, which may alter the estimation of the rate of decline of isotope enrichment. Schoeller et al (1986) suggested that the factors of 1.04 for  $^2\text{H}$  and 1.01 for  $^{18}\text{O}$ , or a ratio of 1.03, would appropriately correct for isotope sequestration. Recently, this has been challenged in the literature, since isotope sequestration is dependent upon the physiological state, growth rate etc. (Ritz and Coward 1995). Validation studies with preterm infants which compared DLW to respiratory gas exchange have demonstrated accurate results when the ratio calculated from their own body water measurements was employed (Jensen et al 1992), or when the factors suggested by Schoeller et al (1986) were used (Westerterp et al 1991)

*The losses of  $^2\text{H}$  and  $^{18}\text{O}$  from the body water pool occur at the same level of enrichment as exists in the body water pool at that time.* This assumption addresses



the issue of fractionation of isotopes. More specifically, do lighter isotopes evaporate more readily than heavier isotopes? The implication is a progressive concentration of isotopes in the body water pool as evaporative losses from breath and insensible water loss occurs. Roberts et al (1986) conducted simultaneous water balance studies with a DLW measurement period in preterm infants, and estimated that 16% of water turnover was attributed to breath vapour and insensible water losses. Schoeller et al (1986) demonstrated that these losses are subjected to isotope fractionation. To address this problem, in validation studies with infants, Jones et al (1987) employed a calculation to account for isotope fractionation, which proved satisfactory in estimating the impact of isotope loss. The correction factors will be valid for use in other studies only when the environmental conditions such as temperature, ambient humidity, and proportion of skin exposed are the same as the conditions assessed by Jones et al (1986)

### ***Methods***

Details of the dosing procedures and urine sampling times are described in Chapter 6. All urine samples and isotope dose waters were frozen at -20 °C until analysis. Sample preparation for isotope ratio mass spectrometry analysis of  $^2\text{H}$  and  $^{18}\text{O}$  occurred separately, and are described below.

#### **$^{18}\text{O}$ Analysis of Isotopically Enriched Urine**

The technique was originally described by Epstein and Mayeda (1953), but has been

modified to allow the use small samples. In brief, 0.2 mL of urine was placed into 9 mm pyrex tube, frozen, and the air was pumped away under a vacuum. The samples were thawed under vacuum and then frozen again. Any dissolved gases released by the thawing were again pumped away. A known quantity of CO<sub>2</sub> was added to the sample, and the pyrex tube was sealed. Once sealed, the <sup>18</sup>O in the urine sample was allowed to equilibrate with the added CO<sub>2</sub>. Ultimately, the newly labelled CO<sub>2</sub> is drawn off, and the enrichment is determined by gas isotope ratio mass spectrometry (IRMS).

*Sample Preparation: Description of the vacuum line*

The sample preparation vacuum line consists of a large CO<sub>2</sub> cylinder, a needle valve to control the delivery of CO<sub>2</sub> to the sample, a manometer to accurately measure the amount of CO<sub>2</sub> added to a sample, and a sample manifold (with six sites). To prepare the samples for <sup>18</sup>O analysis, urine samples or dose waters were thawed to room temperature. Using a long metal needle and 1 mL syringe, 0.2 mL aliquots were placed in the bottom of six 9 mm pyrex tubes (approximately 20 cm long), which were cleaned, dried and cooled to room temperature prior to use. The pyrex tubes were attached to the vacuum line manifold by ultratorrs. The sample tubes on the manifold were slowly immersed in a slush bath composed of crushed dry ice and isopropyl alcohol. Once the end of the tube containing the sample was completely immersed, a five minute waiting period was observed to ensure complete freezing. The vacuum was then opened to pump the air out of the sample tubes. Once the

vacuum had returned, hence the air was removed, the vacuum was closed, and the slush bath was removed. The samples were thawed by immersing the ends of the tubes in a room temperature water bath, and once thawed, the freezing and pumping processes were repeated. This facilitated the removal of any dissolved gases from the samples. CO<sub>2</sub> was then added to the tubes using a mercury manometer to control the quantity. The CO<sub>2</sub> in the sample tubes was subsequently frozen by immersing the end of the tubes in liquid nitrogen. While one end of the sample tubes remained immersed in liquid nitrogen, the pyrex tubes were sealed and removed from the vacuum line using a gas/oxygen torch. The tubes containing the sample and CO<sub>2</sub> were placed in a 25°C water bath for a minimum of three days, to facilitate equilibration of isotopically labelled water with the CO<sub>2</sub>.

After equilibration, the isotopically labelled CO<sub>2</sub> was transferred from the sealed 9 mm pyrex tubes into clean 6 mm pyrex tubes for gas IRMS analysis. This was achieved by attaching the sealed tube containing the sample, and the clean 6 mm tube to a vacuum line. Once the vacuum was restored in the line, the vacuum was closed, and the end of the 6 mm tube was immersed in liquid nitrogen. The sealed 9 mm tube was cracked such that the CO<sub>2</sub> moved through the evacuated line into the 6 mm tube and was frozen in the end of the tube immersed in liquid nitrogen. When the transfer was complete, the 6 mm tube was sealed using a gas/oxygen torch, and the clean gas was ready for analysis.

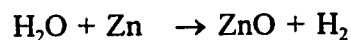
The enrichment of the CO<sub>2</sub> was determined by gas IRMS (VG-SIRA II and

VG 602D, VG Isogas, Cheshire, England). These systems used a dual gas inlet system to simultaneously measure a sample, and a reference gas of known enrichment, with a inlet system that switched back and forth between gases during the measurement period. As the gas entered the mass spectrometer, an ion source bombarded the gas and accelerated the particles. The particles were propelled past a magnet which separated the CO<sub>2</sub> particles according to mass. This resulted in three distinct beams (masses of 44, 45 and 46 for <sup>12</sup>C<sup>16</sup>O<sub>2</sub>, <sup>13</sup>C<sup>16</sup>O<sub>2</sub>, and <sup>12</sup>C<sup>18</sup>O<sup>16</sup>O, respectively) which created an electrical current they struck collectors specific for that mass, which is translated into an estimate of particles at that mass.. Ultimately, enrichment of the sample is calculated by comparison of the ratio of 46:44 masses to that of the reference gas. The reference gas is derived from Standard Mean Ocean Water (SMOW) (International Atomic Energy Agency, Vienna). The isotopic enrichment of a sample relative to SMOW is described as delta (δ).

### **<sup>2</sup>H Analysis of Isotopically Enriched Urine**

Urine samples labelled with <sup>2</sup>H must also have the hydrogen released as a gas prior to mass spectrometer analysis, through a process called zinc reduction (Prentice et al 1990). Prior to the induction of this reaction, the urine was distilled to separate the water from the salts. This was accomplished by connecting an open ended 6 mm pyrex tube (20 cm long) to the vacuum line, and attaching it with an ultratorr to a short 6 mm culture tube (5 cm in length) in which 4 μL of sample had been pipetted.

The sample was frozen in a slush bath as described above, a vacuum was achieved, and the open ended tube was removed from the vacuum line by sealing it with a gas/oxygen torch. The tubes were inverted in the slush bath, and the end containing the urine sample was gently heated with warm air. The water from the urine evaporated and condensed in the clean end of the tube immersed in the slush bath. The small tube with the residual salt was removed, and 125 mg of zinc shot was added to the frozen water sample. The tube was replaced onto the vacuum line, the water was frozen with a slush bath, the vacuum was re-instated, and the tube that contained the enriched water and the zinc shot was sealed and removed from the line using a gas/oxygen torch. The samples were reacted with zinc to release the hydrogen gas by heating in a block at 480°C for 60 minutes, such that the following reaction occurred:



The gas IRMS analysis of the hydrogen gas occurred similarly as described above for CO<sub>2</sub>, however the enrichment of hydrogen gas was corrected relative to SMOW, and Standard Light Arctic Precipitation (SLAP) (International Atomic Energy Agency, Vienna). The <sup>2</sup>H enrichment of the samples was also expressed as δ.

### *Calculations of Total Body Water and Energy Expenditure*

#### **Total Body Water:**

The calculation for total body water (TBW) used in Chapter 6 was:

$$\text{TBW (kg)} = \frac{d \times \text{APE}_d}{\text{MW}_d} \times \frac{18.02}{\text{APE}_{\text{BW}}} \times 10^{-3}$$

where d = dose water  
 APE = atom percent enrichment  
 MW = molecular weight  
 APE<sub>BW</sub> = peak APE of body water after dosing  
 18.02 = molecular weight of body water (prior to dosing)

The APE of dose water or body water is calculated from the  $\delta$  analyzed for <sup>18</sup>O and <sup>2</sup>H by the equation:

$$\text{APE} = \frac{R \times 100}{(R + 1)}$$

where R = ratio of excess isotope

$$R = \frac{(\delta_{\text{sample}} - \delta_{\text{baseline}}) \times R_{\text{std}}}{1000}$$

$$R_{\text{std}} = 2.005 \times 10^{-3} \text{ (for } ^{18}\text{O)} \text{ or } 1.5576 \times 10^{-4} \text{ (for } ^2\text{H)}.$$

The dilution of both isotopes was calculated as described above, to have two separate determinations of TBW.

Since some measurements were conducted on ambulatory subjects, a urine sample was not collected when peak isotopic enrichment was believed to occur. In these subjects, the  $\delta$  which represented peak enrichment in the calculations was determined by the equation (Davies and Wells 1994, Ritz and Coward 1995):

$$E_t = E_p e^{(-kt)}$$

Where:

$E_p$  = enrichment at plateau (5 hours post-isotope dose)( $\delta_{\text{peak enrichment}}$ )  
 $k$  = the water turnover constant  
 $t$  = the difference in time from theoretical plateau to the time of the first sample

#### **Water turnover constant (k):**

For infants in hospital, the multipoint method of determining water turnover constant was used (Davies and Wells 1994). This is the slope of the natural log of the isotope enrichment plotted against time in days (see Figure 1). The two point system was used for ambulatory measures (Welle 1990), in which:

$$k = \frac{\delta_{\text{final}} - \delta_{\text{initial}}}{\text{time (days)}}$$

The TBW estimate was corrected for dietary water intake. This was accomplished by subtracting the weight of dietary water which was consumed during the period between isotope dosing and the acquisition of first urine sample from the TBW estimate. Dietary water was estimated from weighed formula intake which was corrected for solids content. For standard premature infant formula, a factor of 0.81 was used to correct for solids content. The solids content of the two study formulas varied with each production batch, so a value of 0.83 was chosen. The difference between study formulas in solids content was negligible (approximately 0.5%). Therefore, using the measured value for each calculation as opposed to the estimated value would ultimately alter the estimate in total body water by less than 2 grams.

The value of TBW employed in the equations to calculate energy expenditure was a mean of the values determined by the two isotopes which were corrected for dietary water intake.

### Energy Expenditure:

The equations used to calculate an estimate of CO<sub>2</sub> production (rCO<sub>2</sub>) from which energy expended could be calculated were as follows are described below.

In-hospital measures:

The equation was derived from validation work by Jones et al (1987) on hospitalized term and preterm infants, which presumed 75% of the skin was exposed.

$$rCO_2 = 0.445N(1.01k_O) - (1.04k_H)$$

where:

N = TBW (moles)

k = water turnover constants for oxygen and hydrogen

and where 1.01 and 1.04 represent factors to correct for isotope sequestration into tissues.

The equation used to calculate rCO<sub>2</sub> for infants at home was derived from Schoeller et al (1986), which presumes that 50% of the skin was exposed.

$$rCO_2 = \frac{N(k_O - k_H)}{2.08 - 0.015Nk_H}$$

The estimation of energy expended was calculated from the rCO<sub>2</sub>, using the Weir equation (Weir 1949) as follows:

$$EE \text{ (kcal/d)} = 22.4(1.106 \times rCO_2 + 3.941 \times rO_2)$$

Since rO<sub>2</sub> was not measured, it was estimated from the relationship between respiratory quotient (RQ), rCO<sub>2</sub> and rO<sub>2</sub>, such that:

$$RQ = rCO_2/rO_2$$



RQ was estimated from the food quotient (FQ), as described by Black et al (1986) using the equation:

$$FQ = (p \times 0.81) + (f \times 0.71) + (c \times 1.00)$$

where p = the energy contributed by protein in the diet  
f = the energy contributed by fat in the diet  
c = the energy contributed by carbohydrate in the diet

and using the factors of 4.4, 9.4 and 3.75 kcal/g for protein, fat and carbohydrate, respectively (Black et al 1986).

Jones et al (1987) substituted the calculated FQ for the measured RQ into equations when validating DLW in infants, and found no significant error was introduced.

All energy values reported in Chapter 6 were as kilojoules (kJ), and 1 kcal = 4.18 kJ.

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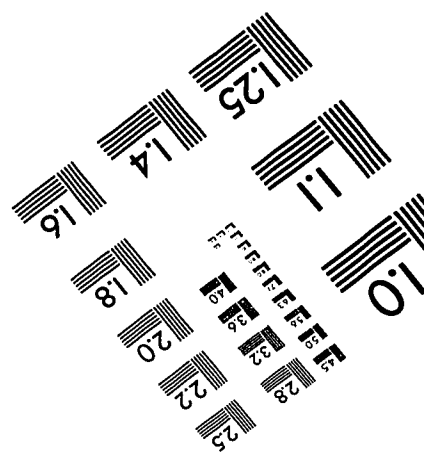
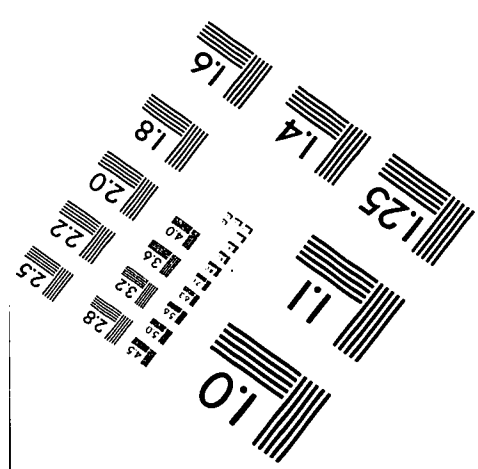
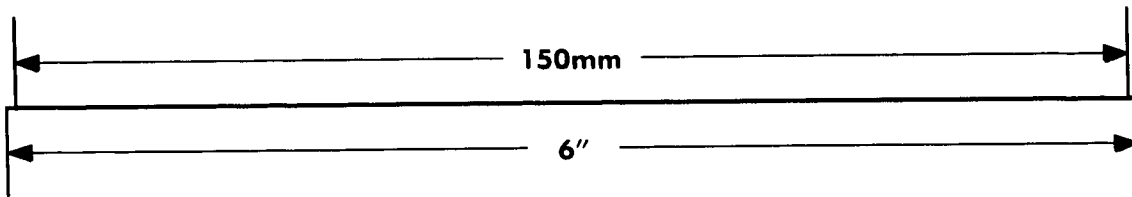
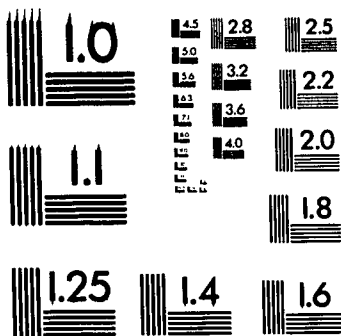
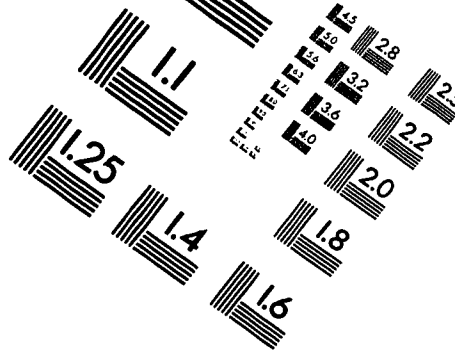
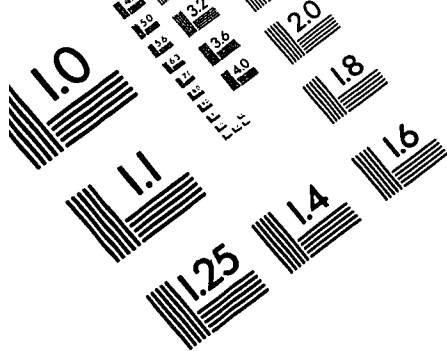
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 MAR 06 1997



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