LONGITUDINAL CHANGES IN ENERGY EXPENDITURE AND BODY COMPOSITION OF PRETERM INFANTS

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ABSTRACT

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The objective of this research was to examine the relationship between energy intake, energy expenditure and body composition in healthy, formula-fed preterm infants at term and 3 months corrected age (CA). Energy expenditure (EE) of 9 preterm infants (5 males, 4 females; birthweight: 1186±295 g; gestational age: 29±3 wk), determined by the doubly labeled water method, at term CA (EE=233±93 $kJ.kg^{-1}.day^{-1}$) and 3 months CA (EE=224±47 $kJ.kg^{-1}.day^{-1}$) was similar. The percentage of energy intake which was expended increased in 7 of the 9 subjects between term (mean = 42.8±16.2%) and 3 months corrected age (mean = 59.1±13.5%). Body composition as determined by dual-energy x-ray absorptiometry (DXA) showed percentage fat free mass decreased in 9 of the 10 subjects between term (mean = 80.3±6.4%) and 3 months corrected age (mean = 70.4±6.8%). These values were comparable to term infants. The mean weight well above the 25th centile of the NCHS growth curves indicating catch-up in weight by 3 months CA but the subjects remained short for their weight based on Z-scores. These new, preliminary findings provide longitudinal estimates of energy intake, energy expenditure and body composition during early catch-up growth in healthy, free-living premature infants.

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LIST OF ABBREVIATIONS
(in alphabetical order)

AGA	appropriate for gestational age
APE	atomic percent excess
BMC	bone mineral content
BPD	bronchopulmonary dysplasia
CPS	Canadian Pediatric Society
DDI	double distilled water
δ	delta per millilitre, unit for isotopic enrichment
DLW	doubly labeled water
DXA	dual-energy x-ray absorptiometry
FFM	fat free mass
GIRMS	gas isotope ratio mas spectrometry
IDECG	International Dietary Energy Consultancy Workshop
IUGR	intrauterine growth retardation
k	elimination constant for stable isotopes
N	isotopic dilution space
NCHS	National Centre for Health Statistics
NICU	neonatal intensive care unit
P-RNI	recommended nutrient intake for premature infants
PMA	post menstrual age

rCO2	rate of carbon dioxide production
RNI	recommended nutrient intake
SAB	size-at-birth
SGA	small for gestational age
SLAP	Standard Antarctic Precipitation
SMOW	or V-SMOW, Vienna Standard Mean Ocean Water
TBW	total body water
TEF	thermic effect of feeding

FREQUENTLY USED TERMINOLOGY PERTAINING TO PRETERM INFANTS

Gestational age The estimated number of weeks between conception and birth of an infant. Since the exact date of conception cannot be determined, the date of the first day of the last menstrual period is commonly used to estimate the due date of the infant, which is estimated to be 40 weeks from the first day of the last menstrual period.

AGA Abbreviation for appropriate for gestational age. At birth, the weight of an infant is assessed for appropriateness according to the corresponding gestational age, using intrauterine growth curves.

PMA Abbreviation for postmenstrual age. The PMA of a preterm infant is equal to the gestational age plus the postnatal age in weeks. PMA is used until the infant reaches 40 weeks PMA, which is also known as term corrected age.

CA Abbreviation for corrected age. The postnatal age of an infant born prematurely is corrected for prematurity. Therefore, for an infant born at 31 weeks gestational age reaches 5 months postnatal age, the corrected age of infant is 3 months.

CHAPTER 1 LITERATURE REVIEW

1.1 Introduction: Rationale and Global Objectives

The Canadian Pediatric Society's (CPS) 1995 recommendations on feeding the preterm infant were the first in history to address the differences in nutritional needs due to the differences in birthweight and stages of development. The recommended nutrient intakes for premature infants (P-RNIs) during the "stable-growing" period, i.e., from establishing medical stabilization to discharge from the neonatal intensive care unit, were established based on a large body of research conducted in the past three decades. However, such is not the case for the P-RNIs of the "post-discharge" period, which is the 12 month period beginning at the time of discharge from hospital, usually at approximately 36 to 38 weeks post menstrual age (PMA). In fact, the Nutrition Committee (CPS, 1995) states that "establishing recommendations for this period (post-discharge period) was hampered by a marked lack of research".

The small body of research focused on nutrition of free-living premature infants is outlined in section 1.3.3 of this thesis. Two of these studies have examined the relationship between mineral-enriched formulas and bone mineralization (Chan, 1993; Bishop et al., 1993) in formula fed, healthy preterm infants up to 2 months corrected (Chan, 1993) and 9 months corrected age (Bishop et al., 1993). The effects of feeding protein-enriched (Chan et al., 1994) or protein and energy-enriched formulas (Lucas et al., 1992) beyond term corrected age on the weight, length and head circumference of preterm infants have also been studied. No attempt to monitor body composition or energy metabolism beyond term corrected age has been reported in any of these studies. The absence of research in

energy metabolism in free-living premature infants indicates that the P-RNI values for energy during the "post-discharge" period are not established based on scientific evidence. Longitudinal data on energy intake, energy expenditure and the corresponding increase in size and the changes in body compositioin during the critical stage of early catch-up growth are needed to assess the appropriateness of these published nutrient recommendations. Therefore, the global objectives of this thesis research, are to contribute to the understanding of energy metabolism of healthy, free-living premature infants, and to examine the whole body soft tissue composition associated with energy intake, energy expenditure and the unique growth patterns of healthy, formula-fed premature infants beyond term corrected age.

In practice, studying energy expenditure and body composition of premature infants in a free-living environment poses a unique set of challenges to researchers because of the limitations and the lack of feasibility of conventional methods. The rationale for using the doubly labelled water method to measure total energy expenditure and dual-energy x-ray absorptiometry to determine whole body lean and fat mass in this research, as well as the efforts in method development will be detailed in Chapter 2 of this thesis.

This longitudinal study is the first to determine total energy expenditure in healthy, formula-fed preterm infants at and beyond term corrected age. This is also the first report of longitudinal measurements of body composition corresponding to carefully documented data on growth, energy intake and energy expenditure. The methodological issues pertaining to the doubly labelled water method explored during the development of this

study and the results obtained will provide valuable information for researchers in designing future studies. More importantly, this research is an important step toward building the body of scientific evidence needed to improve and refine the recommendations on energy intake for preterm infants during their first year of life in the free-living environment.

1.2 Energy Metabolism in Infancy

1.2.1 Metabolizable energy

In order to gain insight into how dietary energy intake contributes to growth, it is crucial to understand the fate of food energy. The fraction of dietary energy intake that is not lost in urine and faeces is metabolizable energy. Metabolizable energy is less than absorbed energy since the latter does not account for energy lost in urine (Putet, 1993).

Reported percentage values of energy absorbed from feeds vary due to individual differences amongst the infants, the composition of the feeds, and the methodological approaches taken by the investigators. Full term infants reportedly absorb 92% and 93% of the energy in human milk and standard formula (Southgate, 1966), respectively, whereas metabolizable energy ranges from 88% to 92% of gross energy intake in this population (Butte, 1988). In preterm infants, energy absorbed from human milk ranges from 86% (Whyte et al., 1983) to 90% (Reichman et al., 1983), whereas metabolizable energy ranges from 84% (Putet et al., 1984) to 94% (Schulze et al., 1987). At 29 to 33 weeks post menstrual age (PMA), formula-fed infants store 38% (Freymond et al., 1986) to 52% (Reichman et al., 1983; Roberts et al., 1987) of their metabolizable energy intake.

However, not all metabolic balance studies measure energy loss in urine (Brooke, 1980) which is in the order of 10 kJ per kg per day (2.4 kcal per kg per day) for formula-fed preterm infants at approximately 33 weeks PMA (Whyte et al., 1986). Other reports (reviewed by Butte, 1988) combine energy losses through feces and urine as energy excreted. It is therefore important to review the experimental procedures to decipher whether absorbable energy or metabolizable energy is being measured.

As preterm infants approach term corrected age, their ability to absorb dietary energy is expected to increase as their gastrointestinal tract and digestive mechanisms mature (Lebenthal and Leung, 1988). Previous findings in our laboratory (Brunton et al., unpublished data) indicate that by 38 weeks PMA, formula-fed preterm infants absorb up to 97% of the energy ingested. Metabolizable energy is in turn partitioned between energy stored and energy expended in various physiological processes.

1.2.2 Total energy expenditure

Total energy expenditure is the summation of basal metabolism, physical activity, thermic effect of feeding, energy needs for thermoregulation and tissue synthesis. This section is an overview of the factors affecting each of the components. Thus the factors discussed in this section also influence the magnitude of total energy expenditure, which must be determined in order to improve the recommendations on energy intake of free-living preterm infants.

Basal metabolic rate (BMR) and resting metabolic rate (RMR) Basal metabolic rate is the rate of energy expenditure required to maintain the cellular processes fundamental to

the survival of the organism, and therefore reflects the minimal energy needs of tissues at rest. In the adult population, basal metabolic rate can be measured when the individual has fasted for 12 to 18 hours so that the thermic effect of feeding is minimal (Butte, 1988). The individual must also be at rest to minimize energy expended on physical activity (Butte, 1988). Measurement is taken under thermoneutrality to prevent energy expenditure by sweating or shivering (Butte, 1988).

Since growing infants, or any other rapidly growing young animals, experience rapid protein turnover in order to synthesize new tissue, the energy expended in the rapid synthesis and breakdown of metabolites makes estimating BMR in a rapidly growing infant impossible. Also, fasting is unethical in infants, frequent feedings impose a continuous, residual effect on energy expenditure. Thus the measured metabolic rate under thermoneutrality of an infant at rest is in fact the resting metabolic rate, which includes whatever residual thermic effect of feeding that may be present, as well as the energy cost of growth (Putet, 1993). Since these components cannot be measured separately, they will be included in the measurement of total energy expenditure.

Thermic effect of feeding (TEF) The increase in metabolic rate in response to feeding is known as the thermic effect of feeding and is primarily due to energy costs (above that of basal metabolism) of digestion, absorption, transportation and storage of the nutrients ingested (Butte, 1988). Both the quantity and composition of the diet affect the magnitude of TEF. Dietary protein stimulates the greatest postprandial increase in energy expenditure due to the various processes involved in protein metabolism such as deamination and urea synthesis. Dietary fat, however, requires little processing prior to being stored in

adipocytes, thus contributing the least to TEF on a kJ per g basis. The energy cost of converting carbohydrates to glycogen is less than that of converting carbohydrates to triglycerides, and the resulting rise in metabolic rate is intermediate between that of protein and fat (Linder, 1991). Although the composition of the diet used in this thesis research remains constant, the magnitude of TEF can still be affected if formula consumption changes longitudinally.

In both full term and preterm infants, the classical approach to determine TEF, i.e., measuring the difference in energy expenditure before and after feedings, tends to underestimate TEF. This is because the measured pre-meal metabolic rate does not reflect the basal postabsorptive metabolic rate in view of the frequent feeding schedule. Therefore, the frequency of feeding is inversely proportional to the magnitude of postprandial rise in metabolic rate (Freymond et al., 1986). Caregivers of preterm infants in the Hamilton-Wentworth region are usually instructed at discharge to feed approximately every 3 hours at discharge but feeding frequency usually decreases after reaching term corrected age. This is not a concern for this study because the objective is not to quantify TEF per se, but to determine total energy expenditure.

Energy cost of physical activity Both Bruck's activity scale (Bruck, 1961) and the simplified activity scale (Freymond et al., 1986) have been used to quantify physical activity in the premature infant. At less than 34 weeks PMA, infants that were appropriate for gestational age were found to be more active during the pre-meal period than after feedings (Freymond et al., 1986). Also, using the simplified activity scale, physical activity before and after feedings correlate linearly with measured energy expenditure. The

difference between total energy expenditure (using 24 hour indirect calorimetry) and resting energy expenditure reveals that physical activity contributes to 5.3% of total energy expenditure in this group of infants (Freymond et al., 1986). For the purpose of this thesis research, the energy cost of activity will not be estimated using any form of subjective activity records, but will be taken into account under the global measurement of total energy expenditure.

Energy cost of growth In growing infants, a fraction of metabolizable energy is expended in tissue synthesis and another fraction is deposited in new tissues. Together, energy expended in synthesis and energy deposited in new tissues contribute to the energy cost of growth. Only the fraction which is spent in tissue synthesis is included in total energy expenditure, whereas the energy deposited in the new tissues can be determined by analyzing the composition of growth.

Energy cost of growth is affected by a number of factors. The availability of energy for growth and storage is directly affected by dietary energy intake. When energy intake is limiting, protein is oxidized for energy. Also, the energy cost of depositing protein is higher than that of laying down fat (in terms of kJ used for deposition per kJ deposited) (Roberts et al., 1988), which is offset by the higher energy deposited in fat and protein on a per gram basis (Brooke, 1986). Therefore, depending on the diet composition and the types of substrate available, the composition of new tissues synthesized can vary, thus affecting the energy cost of growth (total cost of deposition and energy deposited) indirectly. Based on data derived from infants up to 36 weeks PMA fed human milk or

preterm formula, Putet (1993) concludes that an average value of 10 kcal per kg per day, or 42 kJ per kg per day is acceptable.

Although it is impractical to quantify each component of total energy expenditure in free-living premature infants, an understanding of the factors affecting each component can help explain or predict the differences in total energy expenditure measurements at different ages and associated with specific clinical characteristics. In healthy, formula-fed preterm infants, formula consumption (in litres per day) and physical activity are expected to increase from term to 3 months corrected age. Unlike term infants whose growth rate decreases over the early months of life (Guo et al., 1991), preterm infants need to maintain their rate of growth in order to achieve catch-up growth. Although the energy expended for deposition per gram of tissue deposited may not differ damatically (Roberts et al., 1988), the composition of growth in preterm infants is likely to change between term and 3 months corrected age in order to adapt to extrauterine life. Consequently, total energy expenditure may derease over this period of time.

1.2.3 Current recommended nutrient intake (RNI) for energy for term and preterm infants (P-RNI)

The factorial approach, which sums up the measured values of the various components of total energy expenditure, has been used to establish the RNI for energy for the adult population. This approach is not used for the pediatric population due to the difficulties in quantifying basal metabolic rate, thermic effect of food and physical activity separately. Instead, the factual approach is employed. The RNI for energy for term infants

is established based on the observed intake of healthy infants who are growing at an acceptable rate based on the National Centre for Health Statistics (NCHS) growth centiles (Hamill et al., 1979). The current RNI for energy for infants from birth to 5 months ranges form 95 kcal per kg per day to 120 kcal per kg per day (or 400 to 500 kJ per kg per day) (Health and Welfare Canada, 1990). Healthy term infants consuming approximately 460 kJ per kg per day based on parental reports have weight, length and head circumference at or above the 50th centile of the NCHS curves (Sauve and Geggie, 1991). However, using observed intake as the basis of the RNI involves a number of assumptions. Firstly, accuracy is in question depending on whether the caregivers were appropriately trained to complete intake records. Also, the risks of omission, or under or over-reporting intake increase as the infants are introduced to a mixed diet. Secondly, the act of monitoring intake itself may affect feeding behaviour and therefore, the results may not be representative of the infants' typical intake pattern. Thirdly, depending on the tabulated nutrient content database used to analyze intake, the calculated results may vary.

The factual approach cannot be used in preterm infants once they are free-living subjects at home because of the limited understanding of their growth rates, body composition and a lack of careful, objective monitoring of the dietary intake of these infants at present. Lucas et al. (1992) reported formula volume consumed but did not give details on weight or weight gain of the subjects. Chan et al. (1992) noted that between term and 2 months corrected age, preterm infants fed standard formula consumed between 435 to 465 kJ per kg per day and gained 22 to 25 g per day. Again, these intake values were reported by the parents and are subjected to their biases.

The inappropriateness of the factorial approach and the factual approach, pose unique challenges to research in energy metabolism in infants. In fact, when the P-RNI for energy during the post-discharge period was published in 1995, the nutrition committee of the Canadian Pediatric Society (1995) stated that "establishing recommendations for this (postdischarge) period was hampered by a marked lack of research" and "whenever possible, the evidence supporting recommendation was weighed in favour of randomized controlled trials. If such trials were unavailable, cohort studies were considered. If both types of study were unavailable, published data were reviewed and recommendations were based on consensus opinion." The existing literature focused on nutrition for free-living preterm infants (see section 1.3.3) and does not provide information on energy metabolism, indicating that the P-RNI for energy during the post-discharge period is not established based on scientific evidence, but on consensus opinion instead. Whether the recommended values of 100 to 120 kcal per kg per day (417 to 501 kJ per kg per day) throughout the 12 months following hospital discharge are appropriate for promoting and supporting catch-up growth has not been measured in clinical studies.

With the availability of the doubly labelled water method (see section 2.1), it is now feasible to measure total energy expenditure in a free-living infant population. The fraction of metabolizable energy stored in new tissues can be derived from body composition analysis. Together, the findings from longitudinal total energy expenditure measurements and body composition analysis can provide insight into the distribution of metabolizable energy, in relation to the infant's diet. Also, longitudinal data on anthropometrics and body composition can contribute to our understanding of the growth

patterns of preterm infants under current nutritional care practice. A study which addresses total energy expenditure and body composition of preterm infants will provide research based evidence noted to be unavailable by the nutrition committee of the CPS, and will provide data needed to help improve the established recommendations on energy intake for premature infants approaching term corrected age and beyond.

1.3 The Effects of Dietary Energy and Protein on Growth and Energy Metabolism

Research in preterm infant nutrition throughout the past three decades primarily focused on growth and metabolism during the stable growing period. Although the findings from the stable-growing period cannot be extrapolated to serve as recommendations for feeding preterm infants beyond term corrected age, these findings have provided valuable insight into how preterm infants respond to early nutrition intervention. These findings also provide direction for research in nutritional care for premature infants during their first year of life in the free-living environment.

1.3.1 Consensus on the goal of nutritional care during the stable growing period

In order to tailor nutritional care for preterm infants, appropriate goals in terms of growth parameters must be identified (CPS, 1995). It is debatable whether it is more desirable for a preterm infant to grow at a rate similar to that of a fetus in the third trimester or to adapt to extrauterine life by mimicking the growth rate of a term born infant (Swyer, 1987). The main concern is the rate of fat deposition, which is lower in the fetus than it is in a term infant in the extrauterine environment (Ziegler et al., 1976;

Fomon, 1967). If, in order to adapt to the extrauterine environment, a preterm infant is to deposit body fat at the rate of a term infant, it will accumulate twice the fat mass of a fetus at a similar postmenstrual age. The long term effects of early high body fat stores on health and development remain controversial (Putet et al., 1987; Swyer, 1987). The general consensus on the goal of nutritional care of the stable growing period is to mimic intrauterine growth rates during the third trimester (CPS, 1995).

Theoretically, if the rate of intrauterine growth can be achieved and maintained, when preterm infants reach term corrected age, their size and body composition may be similar to that of term infants. Micheli and Schutz (1987) conclude that a diet which provides 3.5 g protein per kilogram per day at 502 kJ per kg per day (120 kcal per kg per day), a healthy preterm infant with a birthweight less than 1500 g should attain optimal weight gain, protein gain and linear growth. In fact, these values approximate the P-RNIs for energy and protein during the stable growing period (CPS, 1995). However, in reality, the neonatal course of preterm infants is not always predictable and medical problems associated with adaptation to the extrauterine environment may result in growth setbacks. Also, under current hospital practice, preterm infants are often discharged prior to term corrected age. Infants who are formula-fed will no longer receive the specialized formulas designed for premature infants which are available only in hospitals. As a result of the unpredictable neonatal course and the discontinuation of specialized nutritional care, preterm infants do not always complete catch-up growth during hospital stay (Lucas et al., 1992; Wilson and McClure, 1994). Instead, they demonstrate unique growth patterns which will be discussed in the following section.

1.3.2 Growth patterns of premature infants beyond term corrected age

Based on parental reports (Sauve and Geggie, 1991), a group of 118 Canadian preterm infants (>90% of whom were formula-fed) consumed 470 to 560 kJ per kg per day between 4 to 12 months corrected age. The male preterm infants in this group weighed less at 4, 8 and 12 months corrected age and were shorter at 4 and 8 months corrected than age and sex matched term infants. Although the length of the female preterm infants approximated that of the term female infants, the preterm females weighed less than their term born counterparts at 8 and 12 months corrected. The preterm females also tended to be light for their length. In terms of quality of growth, both male and female preterm infants had lower triceps skinfolds than term infants from 4 to 12 months corrected age. In this study, the dietary intake reported by parents may not be entirely accurate due to the reasons discussed in section 1.2.3, but the growth data reflects the unique growth pattern of preterm infants and the incomplete catch-up growth during their first year in a free-living environment. Unfortunately, the authors did not provide any information on growth or dietary intake prior to 4 months corrected age.

Growth patterns during early infancy was reported by de Gamarra et al. (1987). This longitudinal study examined body fat mass accretion in premature infants using the adiposity index, also known as the Quetelet Index. (The Quetelet Index, which is similar to the body mass index, also provides some information on weight to length proportion because it is defined as body weight in kilograms divided by the square of length in metres. The reference values are derived from the weight and height at the tenth, fiftieth and ninetieth centile values from the Swiss standards of intrauterine and postnatal growth.

However, the Quetelet Index, or the body mass index, is not commonly used for clinical purposes in the pediatric population in North American health care facilities.) Eight appropriate-for-gestational age preterm infants were followed up to 3 years corrected age. At 35 weeks PMA, the adiposity index was only 86% of the fiftieth centile standard value, however, by 6 months corrected, adiposity index reached 103% of the fiftieth centile standard value. Although the adiposity index correlates well with skinfold measurements, it is still an indirect measurement of total body fat and body composition. When the growth data are compared with centile standards at 6 months of age, there was evidence of disproportionate growth because only weight reached the fiftieth centile while length remained between the tenth and fiftieth centiles. At three years corrected, weight and height remain between the tenth and the fiftieth centiles but no longer appeared disproportionate. Unfortunately, neither in hospital feedings nor postdischarge diet of these infants were specified.

Since diet is an important factor in determining the composition of growth (Bell, 1994), growth data which do not specify dietary patterns is of limited use to the assessment of the appropriateness of the P-RNIs. This is especially true when the growth data reveal growth deficits such as in the case of preterm infants in the Hamilton-Wentworth area because according to the data collected by our laboratory, after hospital discharge, preterm infants who receive standard formula designed for term infants, experience disproportionate growth by term corrected age. Their weight tends to fall below the fiftieth centile of the National Centre of Health Statistics (NCHS) growth

standards (Hamill et al., 1979) and their length is only at the fifth centile of the same reference curves.

The Canadian Pediatric Society (CPS, 1995) states that the aim of nutritional care during the postdischarge period is to promote and support catch-up growth in preterm infants. In view of the lack of nutrition research during this period, studies involving careful examination of the increase in size and the changes in body compositioin as well as dietary patterns of free-living preterm infants are needed. The few published studies with this focus will be discussed in the following section.

1.3.3 Research in nutritional needs and growth of preterm infants beyond term corrected age

Although only a few studies have examined the effects of postdischarge nutrition on growth of preterm infants, the results are promising (table 1.3.3.1). Amongst these studies, the longest intervention period was up to 9 months corrected age (Lucas et al., 1992 and Bishop et al., 1993). In the rest of the studies, intervention only lasted 8 weeks (Chan, 1993; Chan et al., 1993). At present, little is known about the duration of the "critical epoch", which complicates decision making in experimental design because of the costliness of conducting long term intervention trials. If the critical epoch for catch-up growth indeed extends throughout or even beyond the postdischarge period, it will allow more time for the intervention to take effect and thus improving the infants' opportunity to fulfill their genetic potential.

Table 1.3.3.1. Published studies on nutrition of free-living premature infants. RCT: randomized controlled trial; GA: gestational age; BW: borthweight; SF: standard formula designed for term infants; EF: experimental formula; PMA: post-menstrual age; CA: corrected age; BMC: bone mineral content; RE: retinol equivalent.

Author(s)	Study Design	Subjects	Intervention	Key Findings
Lucas et al. (1992)	RCT	n=32 GA: 31 wk BW<1850g	Energy Protein SF 2850 kJ/L 15 g/L EF 3010 kJ/L 18 g/L duration: 37 wk PMA to 9 mo CA	- similar formula consumption between groups - weight & length of EF group tracked close to 50th centile but SF group tracked along 25th of centile of Gairdner-Pearson charts (1971) - tricep skinfold thickness similar between groups
Bishop et al. (1992)	RCT	n=32 GA: 31 wk BW<1850g	Energy & protein concentrations: same as Lucas et al. (1992) Ca P SF 350 mg/L 290 mg/L EF 700 mg/L 350 mg/L duration: 37 wk PMA to 9 mo CA	- improved BMC in EF group - BMC improvement independent of body size & bone width

Table 1. (continued)

Author(s)	Study Design	Subjects	Intervention			Key Findings
Chan	RCT	n=59		Ca	Protein	- BMC of PF group greater than other formula
(1993)		GA:30wk	SF	545 mg/L	15 g/L	
		BW<1650g	EF	660 mg/L	17 g/L	
			PF	1290 mg/L	19 g/L	
			duration: 37 wk PMA to 8 wk CA,			
			then SF from 8wk CA to 16 wk CA			
Chan et al.	RCT	n=59		Ca	Protein	- no difference in weight, length & head
(1994)		GA:30wk	SF	545 mg/L	15 g/L	circumference amongst groups in during the 16
		BW<1650g	EF	660 mg/L	17 g/L	weeks of the study
			PF	1290 mg/L	19 g/L	
			Formulas were isoenergetic: 2790 kJ/L			
			duration: same as Chan (1993)			

Table 1. (continued)

Author(s)	Study Design	Subjects	Intervention	Key Findings
Carlson et al. (1995)	cohort	n=63 GA: 28 wk BW: 1034 ± 148 g	PF (204 RE/100 kcal) + 225 RE/d* duration: 3 d postnatal until 2 mo CA (* 225 RE/d until discharged from hospital)	- all infants had normal plasma retinol concentrations - more rapid return of plasma retinol concentration to normal and an increase in retinol binding protein concentration compared with previous clinical experience

In all of the studies mentioned, one or more of protein, energy or mineral was added to formulas to achieve concentrations greater than standard term formula. One concern of feeding nutrient-enriched formulas after hospital discharge is that the infants may consume less formula thus negating the effects of the increased nutrient density of the feeds. Such concern originated from studies involving normal infants (Fomon et al., 1975) and infants who were small-for-gestational-age (SGA) (Brook et al., 1985). However, healthy preterm infants who received an energy and protein-enriched formula from 37 weeks PMA to 9 months corrected age, did not consume less formula than those who received a standard commercial formula designed for term infants (Lucas et al., 1992).

Bone mineral content (BMC) responds positively to continual nutrition intervention beyond term corrected age as demonstrated in a randomized controlled trial by Bishop et al. (1993). This finding was supported by another randomized controlled trial (Chan, 1993; Chan et al., 1994) which examined the effects of isoenergetic (2790 kJ per litre) formulas with different calcium and protein concentrations on growth, bone mineral status and protein status of preterm infants.

Unlike the reports on BMC, the effect of protein and energy-enriched formulas on growth was found to be positive in one report (Lucas et al., 1992a) but insignificant in another (Chan et al., 1994). Although infants who received the preterm formula (Chan et al., 1994) took in significantly higher amounts of energy and protein than those on the standard formula at week 2 postdischarge, the difference in nutrient intake did not persist after week 2 and the investigators reported no differences in weight, length and head circumference among the three formula groups during the 16 week study period. The rate

of weight gain between feeding groups cannot be compared because they were reported in grams per day rather than grams per kilogram per day. The authors did not show detailed growth data and therefore the presence or absence of disproportionate growth at term corrected age (about week 2 of the study) cannot be determined. Contrary to these findings, positive growth responses were reported by Lucas et al. (1992a), who monitored the trends of weight and length gain of preterm infants up to 9 months corrected age.

The difference in growth in response to postdischarge nutritional intervention between the studies may be partially attributed to the sample characteristics. In the studies by Lucas et al. (1992), the inclusion criteria allowed for infants with higher birthweight (1850 g) than in the study by Chan (1993) (less than 1540 g). Also, the subjects of Lucas et al. (1992) tend to be slightly more mature at birth than the subjects of Chan (1993). The more premature a preterm infant is at birth, the longer the infant has to survive the extrauterine environment prior to term corrected age and the more physiological stress the infant experiences during the early neonatal course. Also, energy reserves decrease with increasing prematurity (Ziegler et al., 1981), and the problem is compounded by inadequate nutrient intake during early neonatal life (Wilson and McClure, 1994). Consequently, the infant is likely to be at increased risks for various medical problems and growth setback. These factors are likely to increase the infant's nutritional needs (Wilson and McClure, 1994), and may even affect his or her response to postdischarge nutrition intervention.

Two studies assessed triceps skinfolds in free-living preterm infants (Sauve and Geggie, 1991; Lucas et al., 1992). Sauve and Geggie (1991) found both triceps skinfolds and arm muscle area to be lower in preterm infants than term infants. These findings suggest that preterm infants do not catch up in fat and lean mass during the first year of life. Lucas et al. (1992) also compared the skinfold thickness of the infants on the protein and energy-enriched formula with those on the standard formula. The absence of significant differences up to 9 months corrected age shows that feeding the enriched formula does not result in greater fat mass. Although convenient to perform, skinfold measurement is only an indirect measurement of body fatness. This approach assumes that the thickness of subcutaneous fat is at a constant ratio to total body fat and that specific measurement sites represent the average thickness of subcutaneous adipose tissue (Lukaski, 1987). However, little is known about individual differences in the pattern of fat deposition (Jensen, 1992) and differences in tricep skinfold thickness between two groups of infants may or may not reflect differences in whole body fatness. In fact, no direct, longitudinal measurements of body composition, namely whole body fat and lean mass, at and beyond term corrected age has been reported, even though quality of growth is a crucial aspect in our attempt to understand the effect of nutrition on the pattern of catch-up growth. Based on the reported decrease in percentage fat free mass and increase in percentage total body fat as term infants adjust to the extrauterine environment during the early months of life (Fomon et al., 1982), it is conceivable that preterm infants also demonstrate similar trends of changes in whole body soft tissue composition during catch-up growth.

No data on the relationship between body composition and energy expenditure using the doubly labelled water method in healthy, formula-fed preterm infants during the early months in the home environment have ever been published. It is possible that between term and 3 months corrected age, as preterm infants catch-up in size and achieve a body-composition similar to that of term infants, and as their energy expenditure changess for reasons discussed in section 1.2.1, their energy intake may increase to maintain a positive energy balance adequate to meet the demands of rapid growth.

An understanding of how body composition and energy metabolism respond to current nutrition practice during the early months after discharge from hospital is crucial to future studies of nutritional intervention in healthy or sick preterm infants. Therefore, a longitudinal study of energy expenditure, body composition and growth in relation to standard formula feeding in healthy preterm infants was undertaken. For the purpose of this thesis research, body composition and total energy expenditure using the doubly labeled water method will be measured at term and 3 months corrected age only.

Since the doubly labelled water method is still a relatively new technique, a number of methodological issues needed to be examined. As far as measuring whole body soft tissue composition in a free-living pediatric population is concerned, a feasible, direct method of known limitations is preferred. Dual-energy x-ray absorptiometry (DXA) not only meets these criteria but is also available for research purposes at McMaster University Medical Centre. Chapter 2 of this thesis will be devoted to the examination of the methodological approaches and method development involved in this research.

CHAPTER 2 RATIONALE FOR METHODOLOGICAL APPROACHES AND METHOD DEVELOPMENT

2.1 Total energy expenditure of preterm infants discharged from the neonatal intensive care unit: the need for a new approach

As discussed in section 1.2.3, conventional tools for studying energy metabolism in preterm infants, such as metabolic balance studies and respiratory gas exchange indirect calorimetry are not feasible for use in a free-living population. When studying energy metabolism in preterm infants discharged from hospital, the method of choice must also be safe, non-invasive and any sample collection must be simple enough for caregivers to perform. The International Dietary Energy Consultancy Group (IDECG) (Butte et al., 1996) states that "at present, the doubly labelled water technique provides the most exact quantitative measurements of total energy expenditure of free-living individuals." The doubly labelled water method (DLW) allows for a safe, non-invasive and non-restrictive approach to determine carbon dioxide production rate and total energy expenditure. It has been validated against respiratory gas exchange indirect calorimetry in infants recovering from abdominal surgery (Jones et al., 1987) and in preterm infants prior to term corrected age (Westerterp et al., 1991; Jensen et al., 1992). The doubly labelled water method has been used previously in our laboratory to study total energy expenditure in preterm infants with bronchopulmonary dysplasia (BPD) (Brunton et al., 1996). The protocol used was developed by Dr. A. Winthrop, who co-authored the validation study (Jones et al., 1987). While all research groups adopt protocols which follow the basic principles (section 2.1.1) and assumptions (section 2.1.2) of DLW, variations in details exist. For the purpose of this

present research, some methodological issues will be addressed and the efforts of our laboratory group in method development will be outlined in section 2.1.2.

2.1.1 Doubly Labelled Water Method : Basic Principle

The doubly labelled water method utilizes the rate of carbon dioxide production to estimate energy expenditure and therefore it is a modified form of indirect calorimetry. The underlying principle is that the oxygen atoms in carbon dioxide are in isotopic equilibrium with those in body water and the equilibration reaction is rapid in the presence of carbonic anhydrase (Lifson and McClintock, 1966). When two stable isotopes, deuterium and oxygen-18 (^2H_2O and $H_2{}^{18}O$) are used to label body water, deuterium is eliminated from the body water pool as ^2H_2O only through water loss, whereas oxygen-18 is not only eliminated via water loss as $H_2{}^{18}O$, but also via carbon dioxide production ($CO^{18}O$). Consequently, instead of monitoring the rate of carbon dioxide production (rCO_2) directly from expired air, rCO_2 can be derived from the differential clearance rates of two stable isotopes, as ^2H_2O and $H_2{}^{18}O$, from the body water pool (Figure 2.1.1).

The elimination rates of the deuterium and oxygen-18 can be calculated from the changes in isotopic enrichment of samples of body water over time. Isotopic enrichment of such samples are measured by mass spectrometry. In the past, limited sensitivity of mass spectrometers required high doses of stable isotopes to be used. This, together with the high cost of oxygen-18, limited the use of the doubly labelled water method to small animals of less than 10 kg. However, the use of gas isotope ratio spectrometry (GIRMS),

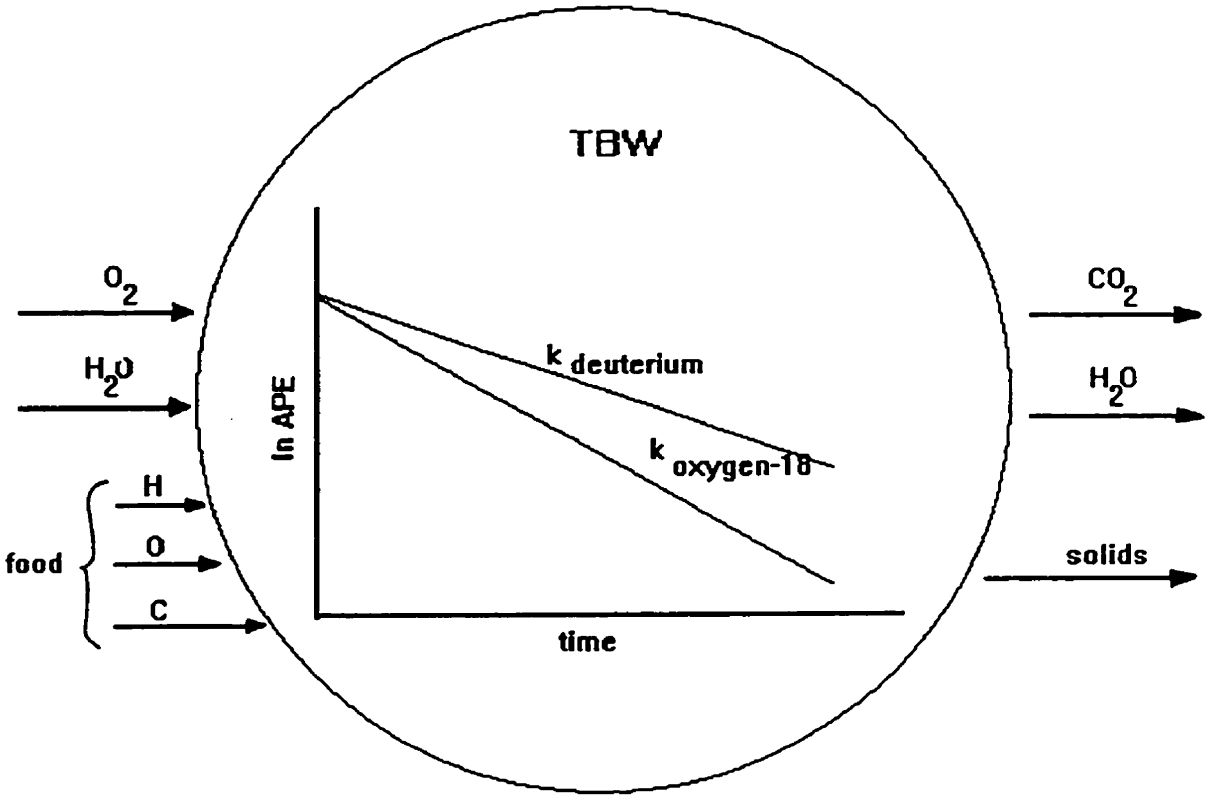


Figure 2.1.1. Basic principle of the doubly labelled water method (Adapted from Lifson and McClintock, 1966). TBW: total body water; N: dilution space; rCO_2: rate of carbon dioxide production; APE: atomic percent excess; $k_{oxygen\text{-}18}$, $k_{deuterium}$: elimination constants (day^{-1}) for oxygen-18 and deuterium, respectively.
Rate of deuterium elimination = $k_{deuterium}$ x N, which is eliminated via water loss alone; rate of elimination of oxygen-18 = $k_{oxygen\text{-}18}$ x N, and oxygen-18 is eliminated via water loss (rH_2O) and carbon dioxide production (rCO_2); therefore,

$$rH_2O = k_{deuterium} \times N$$

and $$2rCO_2 + rH_2O = k_{oxygen\text{-}18} \times N$$

Therefore
$$rCO_2 = (k_{oxygen\text{-}18} \times N - rH_2O)/2$$
$$= (k_{oxygen\text{-}18} \times N - k_{deuterium} \times N)/2$$
$$= (k_{oxygen\text{-}18} - k_{deuterium}) \times N/2$$

with its enhanced sensitivity in measuring isotopic enrichment of body water samples, requires lower doses of stable isotopes. With the lower cost of isotopes, it was feasible for Schoeller and van Santen (1982) to measure energy expenditure in adult humans (weighing 52.2 to 107.4 kg) for the first time using the doubly labelled water method.

2.1.2 Doubly labelled water method: methodological issues

The doubly labelled water method is based on a number of assumptions. It is assumed that 1) no extra stable isotopes enter the body water pool during the metabolic period, which is defined as the period (in days) between dosing and the collection of the final sample; 2) total body water (TBW) remains constant during the metabolic period; 3) daily carbon dioxide production rate is constant; 4) deuterium is eliminated via water losses only and ^{18}O is eliminated via water losses and carbon dioxide only; 5) chemical and physical properties of the stable isotopes are not different from the most abundant form of water in the body.

Such assumptions do not always hold true. In practice, a portion of the isotopes can be lost in urine prior to equilibration. Deuterium may be sequestered into adipose tissues. During the metabolic period, isotopes may be eliminated from the body water pool through insensible water losses and fecal material. Also, additional fluid intake between dosing and sample collection may further dilute the isotopes in the body water pool. In growing infants, body water pool size changes as weight and body composition change. The extent to which the percentage of total body water changes during the metabolic period will be addressed under issue number 3 in this section.

2.1.2.1. Dosing protocol and body water sampling protocol

The doubly labeled water method is a relatively new technique in the study of energy expenditure in preterm infants. Much effort has been made to fine tune the mathematical model to minimize error such as correcting for sequestration of ^{2}H and ^{18}O into non-aqueous body compartments. These have been reviewed by Schoeller (1988), Coward (1988), Roberts (1989) and Goran (1994). A review of the literature reveals considerable variations between studies in terms of dosage of isotopes, length of metabolic period, safety precautions and body water sampling protocol. For the purpose of this present study, these factors need to be taken into consideration and documented for future reference, since DLW is still new to our laboratory.

Dosages of deuterium oxide and $H_2{}^{18}O$

The dosage of isotopes used in preterm infants ranges from 0.3 $g.kg^{-1}$ to 1.42 $g.kg^{-1}$ body weight of ^{18}O and 0.1 $g.kg^{-1}$ to 0.24 $g.kg^{-1}$ of deuterium oxide(Wong et al., 1990; Jones et al., 1987; Roberts et al., 1986). Dosages used in our laboratory previously were similar to those of Wong et al. (1990), and were found to be sufficient for generating high initial enrichment in body water, which in turn helps to minimize the impact of analytical error (Schoeller and Taylor, 1987). For this reason, the protocol for this study adhered to 0.3 $g.kg^{-1}$ of ^{18}O and 0.1$g.kg^{-1}$ of deuterium. Using stock solutions of oxygen-18 normalized water and deuterated water at approximately 10 atomic % and 99.9 atomic %, respectively, means that approximately 3 g of oxygen-18 normalized water and 0.1 g of deuterated water are needed for each kilogram body weight. Also, while conserving research funds (since ^{18}O costs $4.00 per gram), conforming to dosages used in

a previous study in our laboratory also helps when the data collected are compared to the energy expenditure of the infants in the BPD study.

Safety precautions

For ethical reasons, it is recommended that dose water be tested for microbial contamination and the presence of pyrogens prior to administration to human subjects (Wong and Schoeller, 1990). Wong et al. (1990) reported sterilization of the isotope solutions using 0.2 micrometre pore size sterile filters. In our laboratory, once the vacutainer is opened, dose water samples are drawn up directly and samples which are filtered through a 0.2 micrometre pore size sterile filter are sent for culture. Culture results demonstrate that the filtered water samples show no microbial growth after ten days in culture.

Two point method versus multiple point method

In most studies conducted in a clinical setting (Wong et al. 1990, Jones et al. 1987, Jensen et al. 1992 and Roberts et al., 1986), urine samples were collected at more than two points. In studies where samples are collected by caregivers in a home setting, such as in this study, it is important to consider a simple collection schedule to maximize compliance. For this reason, the two point method which requires only two samples to calculate the rate of elimination is an attractive alternative.

The precision of the two point method versus the multiple point method is a concern. Welle (1990) indicated that the impact of technical errors on precision by analyzing replicates of two samples was less than that from single samples from multiple points during the metabolic period. Intra-subject precision decreases with decreasing initial

isotope enrichment (Schoeller and Taylor, 1987). When using the two point method, initial isotope enrichment should be high enough to minimize the impact of analytical errors (Schoeller and Taylor, 1987). As mentioned previously, the dosages chosen for this study are sufficient for generating sufficiently enriched initial body water samples (Brunton et al., 1996).

Length of metabolic period

The length of the metabolic period for the various studies in infants ranges from 5 to 14 days (Wong et al. 1990, Jones et al. 1987, Jensen et al. 1992 and Roberts et al., 1986). When using the two point method, the appropriate duration of the metabolic period should be between 1 to 3 biological half-lives of the isotopes (Schoeller and Taylor, 1987). For this study, a seven-day metabolic period was chosen in accordance with the protocol adopted by Brunton et al. (1996), who found this length of time sufficient for considerable clearance of isotopes from the body water pool, while still resulting in final body water samples more noticeably enriched than the pre-dose state.

Sampling of body water

In studies which involve human subjects, plasma, serum, urine and saliva (Schoeller et al., 1982; Salazar et al., 1994) have been used to determine isotopic enrichment in the body water pool. Collection of plasma or serum is the most invasive among the options and serial samples are not feasible in specific populations (such as preterm infants) due to ethical concerns. In adult subjects, urine collection is noninvasive. Urine extraction from either diapers (Roberts et al., 1988), or cotton balls in the diaper (Davies et al., 1991) or even adhesive urine collection bags (Jensen et al., 1992; Brunton

et al., 1996) are much less invasive than blood collection, and the volume of sample collected is considerable thus allowing for repeated analysis. However, the time of void may not always coincide with the time of isotopic equilibration and may in turn affect the calculated value of total body water, which is required in the derivation of rCO_2. Adhesive urine collection bags were chosen for this study for the convenience of home collection. Although the time of void cannot be controlled, the enrichment at equilibration time can be determined using the back extrapolation method (see method development).

The use of saliva has been validated in a group of nine adolescent subjects (Schoeller et al., 1982). One to three grams of sample can be collected by placing dry cotton rolls under the tongue for 2 to 5 minutes (Schoeller et al., 1982). Unfortunately, saliva collected in infants by this approach may be contaminated by milk or formula residues in the oral cavity. Also, in infants, the volume of saliva that can be collected by this approach has not been reported.

2.1.2.2. The calculation of total body water (TBW)

The calculation of rCO2 is also dependent on the knowledge of TBW and is therefore influenced by the method used to determine the value of TBW. In studies which employ DLW (Wolfe, 1992), isotopic tracer dilution is the preferred method for the determination of TBW, which approximates the volume of isotopic dilution space (in litres or kilograms). Isotopic dilution space is in turn equal to the amount of isotope administered divided by the concentration of isotope in body water at the time at which the isotopic equilibration is complete. Complete equilibration is the time point where analysis of serial samples reveal maximal elevation of isotopic enrichment above

background. Salazar et al. (1994) defined equilibration as a time interval during which isotopic enrichment of consecutive samples vary by less than 2%.

The measurement of TBW in infants requires safe and non-toxic tracers. Instead of radioactive tracers, stable isotopes have been used in infants so that these infants are not subjected to unnecessary radiation exposure. The newborn piglet model has been used to evaluate the accuracy and precision of isotopic dilution using deuterium and ^{18}O as tracers to be compared with TBW determined by carcass analysis. This particular animal model is chosen due to its similarities in body weight and body composition to the human infant. (Whyte et al., 1985). It is established that deuterium overestimates TBW as a result of sequestration into and exchange of deuterium with non-aqueous hydrogen in the body, such as those in adipose tissues. Thus, the concentration of isotopes at equilibration reflects only the dispersion of deuterium remaining in the aqueous compartment (Westerterp et al., 1991). However, when oxygen-18 is used as the tracer to estimate TBW, the calculated dilution space has been found to be 3% less than the value based on deuterium dilution and is thought to be a more suitable tracer than deuterium (Schoeller et al., 1980). Using the piglet model, oxygen-18 is shown to overestimate TBW by 2% when compared to the TBW value established by carcass analysis (Whyte et al., 1985).

When sampling physiological fluid for the calculation of TBW, practical considerations similar to those under the doubly labelled water method, as mentioned previously, also holds true. Using a newborn piglet model, serial sampling of plasma and urine reveal that after a dose of $H_2{}^{18}O$, analysis of both plasma and urine samples yield isotopic enrichment that results in similar calculated dilution volumes (Whyte et al., 1985).

Method development

The time required for ingested isotopes to equilibrate with the oxygen and hydrogen in the body water pool affects the urine sampling schedule. The isotopes ingested, deuterium and oxygen-18, have been reported to equilibrate with the hydrogen and oxygen in body water in approximately 3 hours post-dosing in infants (Jones et al. 1987); other investigators indicate that this occurs at 6 hours post dose (Wong et al., 1990). When DLW was used in our laboratory to measure energy expenditure in preterm infants with BPD, equilibration was estimated to occur at approximately 5 hours post dose, according to the protocol developed by Dr. Winthrop. However, the isotopic data showed that the isotopic equilibration time varied considerably from literature values in a number of cases. It was unclear whether the compromised lung function and the resultant variation in fluid spaces of these infants with residual lung disease accounts for the discrepancy.

In view of the equivocal reports in the literature, our laboratory group decided to conduct our own investigation. This research was set up as the undergraduate biochemistry student thesis of Shannon Brady (1996), and co-supervised by Dr. S.A. Atkinson and the M.Sc. candidate. The proposed study was approved by the Research Advisory Group (Ethics Board) of the Faculty of Health Sciences, McMaster University (see Appendix 3). Written, informed parental consent was obtained for all participants.

Briefly, after the collection of a baseline urine sample, healthy, appropriate-for -gestational-age, formula-fed preterm infants between 33 to 36 weeks PMA were given a single oral dose of ^{18}O and deuterium via a gavage tube. This age bracket was chosen to

conform to the BPD study design. Also, at this age, the infants were still staying in the neonatal intensive care unit and the setting allowed serial sample collection to proceed smoothly. 24 hour serial urine samples were collected and each sample was labeled with the time of the void. In order to protect the integrity of the infant's skin, instead of disposable adhesive urine specimen bags (U-Bag, Hollister; Libertyville, IL), a sterile condom made from a surgical glove finger was attached to the infant's genitals using medical adhesive (no.7730, Hollister; Libertyville, IL) and waterproof medical tape (hy-tape). A small opening was made at distal end of the condom and the condom was taped to sterile, clear tubing, which drained into a disposable specimen container.

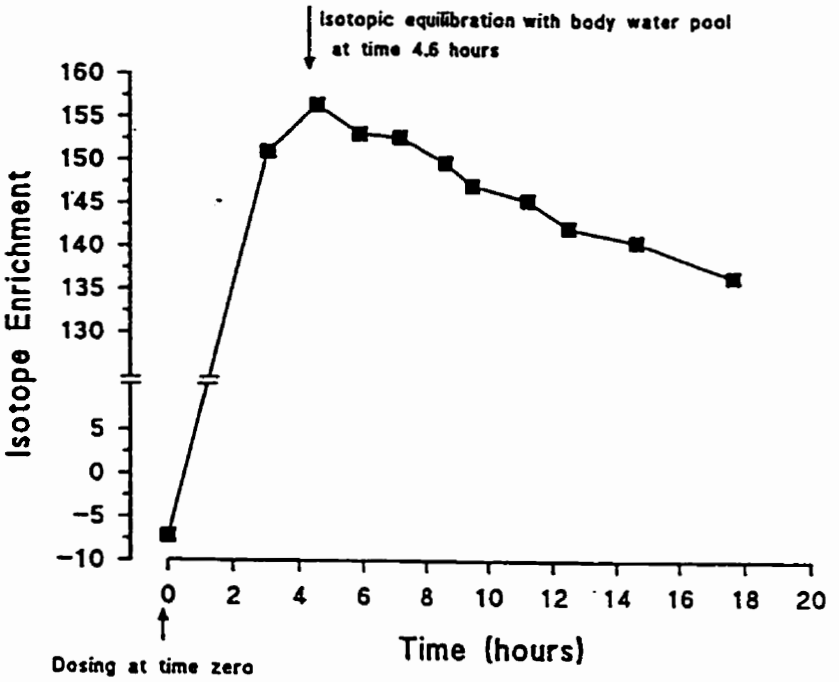
Between October, 1995 and April, 1996, one male infant and one female infant who fit the inclusion criteria participated in the 24 hour serial urine collection. The subject characteristics are described in Table 2.1.2.1.

The urine samples were analyzed for ^{18}O enrichment using Gas Isotope Ratio Mass Spectrometry (GIRMS) and mass spectrometry results of serial samples were plotted against time. From the shape of the curves, isotopic equilibration time was determined to be 4.2 hours and 4.6 hours post dose for subjects #1 and #2 respectively (Figure 2.1.2).

Table 2.1.2.1 Subject characteristics of 24 hour serial urine collection. PMA: post-menstrual age

	Gestational Age (wk)	Birthweight (g)	PMA (wk)	Postnatal Age (wk)	Weight on day of dosing (g)
Subject #1 (male)	29	1510	33.5	13	1875
Subject #2 (female)	30	1503	33	28	1450

A.



B.

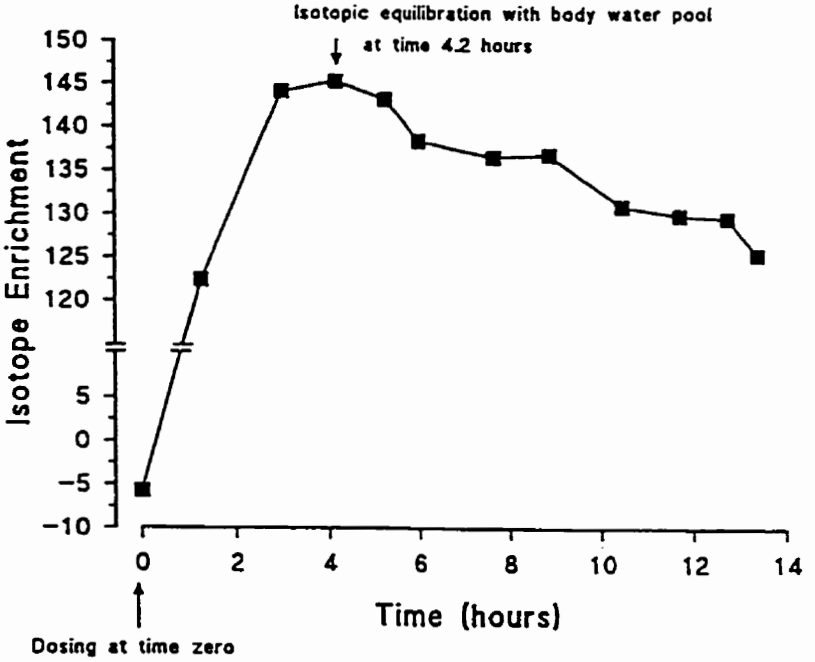


Figure 2.1.2. Graphical analysis of mass spectrometric results for oxygen-18 enrichment versus time in A) subject #1 and B) subject # 2. (Brady, 1996)

These results justify the attempt to collect a urine specimen between 4 to 5 hours post dose. However, in reality, only preterm infants less than 36 weeks post menstrual age void almost hourly. By term corrected age, these infants do not void hourly and they do not void on demand. Using a urine specimen collected 2 hours before or after the equilibration time can result in an overestimation of TBW of up to 18% (Brady, 1996). Therefore, the attempt to collect a specimen at equilibration time for every single subject may not be a practical approach to secure the information needed to determine TBW.

The back extrapolation method (Davies and Wells, 1994) for calculating TBW was recommended by Dr. A. Winthrop. This approach does not require a specimen at equilibration time. Rather, the isotopic enrichment at equilibration is determined by back extrapolating to the equilibration time using the following equation (Salazar et al., 1994):

$$\delta_{day1\ sample} = \delta_{plateau} \times e^{-kt}$$

where δ per millilitre is the measured enrichment of a sample; $\delta_{day\ 1\ sample}$ is the corrected δ of the Day 1 sample, i.e. corrected for the mass spectrometer and normalized against two international water standards: Vienna Standard Mean Ocean Water (V-SMOW) and Standard Antarctic Precipitation (SLAP); $\delta_{plateau}$ is the corrected d of the 5 hours post-dose sample (plateau sample); t is the time elapsed between the plateau sample and the Day 1 sample in days; k is the elimination constant of ^{18}O. (The calculation of k is detailed in section 3.2.5). This equation can be used with either ^{18}O or deuterium but for the purpose

of this thesis research, only ^{18}O was used because it was a more suitable tracer than deuterium (Schoeller et al., 1980).

The protocol developed by Dr. Winthrop also calls for correction of dietary water intake between dosing and urine sampling when calculating TBW volume. Brunton et al. (1996) found dietary water intake (less than 5% of TBW) accounted for an overestimation of TBW . In this study, in order to avoid overestimation of TBW due to additional water intake caused by frequent feeding, the amount of free water in the study formula ingested between dosing and urine sampling will be subtracted from the calculated TBW value.

2.1.2.3. Changes in % total body water (TBW) volume during the metabolic period

Due to the rapid growth experienced by preterm infants in early infancy, the volume of TBW increases by the end of the metabolic period. Jones et al. (1987) used the mean value of TBW volume at the start and at the end of the metabolic period to calculate the rate of carbon dioxide production. The final TBW was determined by repeating a dose of ^{18}O at the end of the metabolic period. This approach was also taken by Jones et al. (1987) and Jensen et al. (1992).

For this present study, it is important to consider the cost of repeated dosing, since ^{18}O is costly. Also, due to the labour intensive nature of the procedures in sample preparation, repeated dosing translates into an increase in the number of samples and consequently, the analytical costs involved. Jensen et al. (1992) compared the change in percentage TBW at the start of the metabolic period with that at the end and found a less than 0.5 % change in percentage TBW. This means that the final TBW volume can be

calculated from the weight at the end of the period and repeat dosing is therefore not necessary.

2.1.2.4. Respiratory quotient

The equation of De Weir (1949), which is used to calculate TEE, involves the knowledge of the rate of carbon dioxide production and the respiratory quotient (RQ). As mentioned previously, respiratory gas exchange monitoring is not feasible for a free-living infant population. The alternative approach is to determine RQ based on the composition of the diet, also known as the dietary respiratory quotient (Jones et al., 1987). This alternative is chosen for our population of interest because the study formula remains the sole feeding of the infants throughout the study and thus the composition of the diet remains constant.

2.1.3 Analysis of stable isotope enrichment by gas isotope ratio mass spectrometry

Gas Isotope Ratio Mass Spectrometry (GIRMS) technology is available and accessible at the stable isotope laboratory of the Department of Geology at McMaster University.

Basic principle of GIRMS

A sample in gaseous form is emitted through an inlet into vacuum and is bombarded by electrons emitted by the source. Sample molecules became positively charged and gain kinetic energy as a result of the bombardment, and are focused into a beam which enters the mass analyzer. The mass analyzer consists of an electromagnetic field which separates the beam of positively charged molecules into separate beams based

on differences in mass, i.e., carbon dioxide molecules with an ^{18}O and an ^{16}O atom will be separated form those with two ^{16}O atoms. When these beams hit separate detection plates, the ions discharge and generate electric currents. Since the number of ions discharged is directly proportional to the magnitude of the current, the ratio of the two currents reflect the isotope ratio. (Wolfe, 1992, chapter 3)

Sample preparation

Body water samples must be in a gaseous state in order to be analyzed with GIRMS. Oxygen-18 in urine samples must be completely equilibrated with carbon dioxide gas so that isotopic enrichment of CO_2 represents that in the urine sample. This is achieved by allowing dissolved gases in urine samples to be pumped out under vacuum and introducing a known amount of a standard CO_2 gas into the vacuum. The volume of standard CO2 introduced is measured to ensure an excess of water, so that the oxygen in CO_2 can be completely replaced by the oxygen from water.

Deuterium in urine samples can be released as hydrogen gas by two methods. Hydrogen gas can be released by heating uranium turnings and water (from the distillation of urine samples) at 600 degrees Celsius. The alternative method does not require the use of uranium but four times the volume of sample (4 microlitres). Briefly, the sample fluid is distilled and metallic zinc is added to the water, which is then sealed in a vacuum pyrex tube. The contents of the sealed tube are heated at approximately 480 degrees Celsius for 30 minutes to produce zinc oxide and hydrogen gas. At McMaster University, the stable isotope laboratory is equipped for the latter approach.

2.1.4 Intended contribution to research in post-discharge nutrition for preterm infants

This study employs DLW and meets the challenges of measuring total energy expenditure in preterm infants during the post-discharge period. This research, with the careful documentation of methodological details, will contribute to the application of DLW for rapidly growing infants in the free-living environment, and to the understanding of energy metabolism in healthy preterm infants in response to current nutritional care practice. These energy expenditure data will provide background information and reference for future nutrition intervention studies, as well as for future research in energy metabolism in sick preterm infants.

2.2 Rationale for using Dual-Energy X-ray Absorptiometry (DXA) to study body composition

A number of methods have been used in the study of body composition in the past (Lukaski, 1987). Some methods measure body composition directly whereas others are indirect methods. Although all existing methods are dependent upon a number of assumptions and have technical limitations, indirect methods are further confounded by the problems of the methods used in cross-validation (Jensen, 1992). For example, the method of whole body potassium counting to estimate fat free mass is dependent on an accurate knowledge of potassium content of fat free mass, while the composition of fat free mass is assumed to be relatively constant in densiometry (Lukaski, 1987). Therefore direct methods are preferred if available.

Equally important are a number of practical considerations pertaining to a free-living pediatric population. The method used must be safe, unrestrictive and feasible. Traditional methods such as densiometry are not appropriate because submersion in water and the measurement of lung volume are not feasible for our population of interest. One direct method which meets the criteria is Dual-Energy X-ray Absorptiometry (DXA). This technology is available at McMaster University Medical Centre, where prospective subjects visit the Growth and Development Clinic on a regular basis up to 12 months corrected age. The low radiation exposure (less than 1 mrem) makes this method safe to use in the pediatric population. DXA does not cause serious disruption to the infants' daily routine becasue an infant whole body scan can be completed in approximately 8 to 10 minutes per whole body scan. Although it is required that the subject remains stationary during the procedure, DXA is still feasible in infants without using sedatives (Brunton et al. 1993). Furthermore, validation studies (Brunton et al., 1993, 1997; Picaud et al., 1995) have been conducted so that the limitations of the method are known, and will be discussed in section 2.3.2.

In this study, DXA is chosen as the principle method of determining body composition. However, an indirect method is also involved because the dilution space of oxygen-18 is also determined as part of the DLW protocol. Since the dilution space of oxygen-18 exceeds TBW volume by approximately 1 % (Davies et al., 1994), TBW and thus fat free body mass and fat mass can be calcaluted. When the body composition measurements using these two methods were compared in preterm infants with BPD (Brunton et al., 1996), the results did not agree. In order to determine whether this

disagreement is due to the etiology of BPD or the incompatibility between the two methods, body composition measurements of the healthy, preterm subjects of this study using the two methods will also be examined.

2.2.1 Principles of DXA

DXA is independent of assumptions about the constancies of body tissue densities (Mazess et al., 1984). Direct measurement of body composition is made based on the differential attenuation of two x-ray beams of different energy intensities by bone and soft tissues. In areas of the rectilinear scan where there is a minimal amount of soft tissue but no significant bone, relative composition of lean and fat tissues can be measured (Gotfredsen et al., 1986; Mazess et al., 1990) using the attenuation coefficients for soft tissues of the two x-ray beams. For each picture element of a scan, known as a pixel (Mazess et al., 1984), the ratio of attenuation coefficients for soft tissues of the lower energy x-ray beam to that of the higher energy beam is calculated and expressed as R_{st} (Gotfredsen et al., 1986). R_{st} value is highest in 100% lean, whereas pure fat has the lowest R_{st}. Since R_{st} has a linear relationship with the amount of lean present, the knowledge of R_{st} of pure fat and pure lean will therefore allow the calculation of the fat fraction as well as the lean fraction (Gotfredsen et al., 1986). The water content of lean tissues, known as the lean tissue hydration factor, affects the R_{st} value of pure lean and is programmed into the software used (Brunton et al., 1993). In a whole body scan, areas where there is a minimal amount of soft tissue but no significant bone account for approximately 40 to 45% of whole body soft tissue mass and the composition of these

pixels are extrapolated to the remaining 55 to 60% (Mazess et al., 1984) of soft tissues. R values exceeding that of R_{st} of pure lean tissue indicate that the pixel in question contains bone (Gotfredsen et al., 1986) and total body bone mineral is calculated from these bone-containing pixels (Mazess et al., 1984).

2.2.2 Validation Studies of DXA in the Neonatal and the Pediatric Populations

The performance of DXA in analyzing body composition of neonates and infants has been assessed in a number of studies. However, the types of software used were not always specified (Braillon et al., 1992; Chan, 1992; Venkataraman et al., 1992). DXA was validated against chemical analysis for measuring regional bone mineral content (BMC) using femurs excised from preterm stillborns (Braillon et al., 1992). Regional BMC, fat and fat free mass measurements of fresh chicken parts have been compared to chemical analysis (Chan, 1992). Venkataraman et al. (1992) conducted whole body BMC, total body fat and fat free mass of term born infants using DXA technology. The results were compared with literature values derived from cadaver analysis. Comparison between DXA measured body composition with literature chemical analysis can provide a crude assessment of DXA performance but does not establish the accuracy of the method. In addition, DXA measured BMC and total body fat values were compared to skinfold measurements (Venkataraman and Ahluwalia, 1992), which is not the ideal reference method. Validation studies employing whole carcass analysis and careful documentation of methodology have been conducted in our laboratory in order to establish the accuracy and

precision of DXA as a tool for body composition assessment in the neonatal and pediatric populations (Brunton et al., 1993; Brunton et al., 1997).

Pediatric Whole Body Software version 6.01 (PedWB) (Hologic Inc.) used with a Hologic QDR-1000/W (Hologic Inc., Waltham, MA) was first validated against piglet carcass analysis by Brunton et al. (1993) in our laboratory. The newborn piglet was chosen as a model for the human preterm infant due to similarities in weight and body composition (Whyte et al., 1985). Brunton et al. (1993) performed triplicate DXA scans on ten 1.6 kg piglets and ten 6 kg piglets. DXA was found to underestimate bone mineral content (BMC) and fat free mass, while overestimating fat in 1.6 kg piglets. One desirable feature worth mentioning is that DXA scans can be saved on optical disks for re-analysis when improved software becomes available. When, Infant Whole Body (InfWB) software became available, the scans of the piglets were reanalyzed using this new software (Brunton et al., 1997) and the results compared with those of PedWB as well as chemical analysis. InfWB overestimated total body fat to a lesser extent than PedWB (125% instead of 244%), especially in the 1.6 kg group. Precision of fat measurement, however, did not improve with the use of InfWB with CVs amounting to 8% and 7% for the 6 and the 1.6 kg groups respectively. InfWB yielded total fat free mass measurements and weight within 2.5% of those from carcass analysis in both groups of pigs, while preserving the precision of PedWB (CV less than 1%). Both software programs determined BMC in large pigs with accuracy and precision but neither of the software yielded accurate BMC measurements in the 1.6 kg group, with PedWB and InfWB underestimating BMC by 29.7% and 15.8% respectively. Although the accuracy and precision of the 6 kg piglet

were acceptable, when piglets approach 6 kg, their body fat content and regional fat distribution no longer approximate that of infants of a similar weight. The results from the samll piglets showed a lack of accuracy in BMC and fat measurements and low precision in fat estimation at this weight thus likely limiting the usefulness of DXA in small infants.

Piglets between 2.5 and 4 kg were not represented in the study by Brunton et al. (1993). Since infants born prematurely often weigh between 2.5 and 4 kg at term corrected age, in order to study these disproportinately growth restricted infants, DXA technology must be able to measure body composition within this weight range with acceptable accuracy and precision. A new pediatric software was validated against chemical analysis using 13 piglets weighing 1.5 to 5.5 kg (Picaud et al., 1995). Four of these piglets from 1.7 to 3.7 kg were scanned with various added lard pads around the abdomen to simulate 17 different fat contents to help evaluate the accuracy precision of the software in measuring a wide range of fat content. Results of DXA scans and chemical analysis were compared and equations were generated to transform DXA measurement to allow the deviation of the slope of the regression line of DXA values from the identity line to be expressed as accuracy, and the dispersion of DXA values as precision. Accuracy in fat measurement improved when lard pads were added but fat content was still overestimated in these higher percentages of fat. The precision in fat measurement improved dramatically when only pigs with more than 250g of fat were included. Therefore, total body fat measurements remain the most limited is precision, especially when the fat content is low. Also, the added lard pads do not mimic fat distribution in infants accurately since infants gain not only subcutaneous fat but visceral fat as well.

Both studies (Brunton et al., 1993; Picaud et al., 1995) concluded that there is room for further refinement for DXA technology in terms of accuracy and precision. In the meantime, limitations in accuracy can be overcome by expressing body composition as percentage fat free mass. Any error in differentiating between fat and lean will cause a smaller percentage error in fat free mass than in total body fat mass. Similarly, limitations in precision can be overcome by spacing serial measurement points far apart within the rapid growing period, so that the actual change in body composition far exceeds the limitations of precision (Brunton et al., 1997). Currently, DXA remains the only feasible direct method for studying body composition in a free-living pediatric population.

2.2.3 Test of agreement between DXA and ^{18}O dilution

As discussed in section 2.1.2.2., the doubly labelled water method involves the determination of total body water, hence also allows for the estimation of whole body lean and fat mass. When both DXA and ^{18}O dilution measurements were conducted in a group of preterm infants with BPD, the results obtained from the two methods did not agree (Brunton et al., 1996). It remains unclear if this limitation is unique to infants with BPD who suffer from fluid reterntion and require diuresis as part of their therapy, or if the two methods yield different results in preterm infants in general. This study allows for the two methods to be compared in a group of formula-fed, healthy, preterm infants.

CHAPTER 3 CLINICAL STUDY

3.1 Objectives and hypotheses

3.1.1 Objectives

The objectives of the research reported in this thesis were:

1. To determine total energy expenditure in healthy premature infants at term and 3 months corrected age;

2. To investigate the methodological issues pertinent to the use of the doubly labeled water method to measure energy expenditure and soft tissue composition in rapidly growing, healthy, premature infants;

3. To determine body composition using dual-energy x-ray absorptiometry at term and 3 months corrected age, and to examine the relationship between body composition and energy intake, energy expenditure and growth;

4. To examine the agreement between body composition determined by the dual-energy x-ray absorptiometry and oxygen-18 dilution methods.

3.1.2 Hypotheses

Hypothesis 1 Energy expenditure (kJ kg^{-1} day^{-1}) of healthy preterm infants at term corrected age is greater than at 3 months corrected age.

Hypothesis 2 Percentage of fat free mass of healthy preterm infants decreases while the percentage of total body fat increases from term to 3 months corrected age.

Hypothesis 3 The percentages of whole body lean and fat measured by oxygen-18 dilution and dual-energy x-ray absorptiometry will provide similar values.

3.2 Materials and Methods

3.2.1 Study design

A descriptive, longitudinal study was designed to investigate changes in total energy expenditure and body composition of preterm infants from term corrected age to three months corrected age. All participating infants were exclusively formula fed from the time of entry into the study and in accordance with current nutritional care practice, received a standard term formula (Similac 20 with iron; Ross Laboratories, Colombus, Ohio) throughout the duration of the study. The study was approved by the Research Advisory Group (Ethics Board) of the Faculty of Health Sciences, McMaster University.

3.2.2 Subjects

Preterm infants admitted to the neonatal intensive care units at McMaster University Medical Centre and St. Joseph's Medical Centre in Hamilton, Ontario, and at Kitchener-Waterloo Hospital in Kitchener, Ontario were screened by attending neonatologists. The criteria for inclusion were that the infant was under 1800 grams at birth and was appropriate for gestational age, was solely formula fed at the time of recruitment and was free from bronchopulmonary dysplasia, genetic disorders, severe cardiac or gastrointestinal diseases or intraventricular hemorrhage or HIV infection. The mother of the infant had to be free from drug or alcohol dependence. The infant was also under 2500 grams, between 37 to 42 weeks post menstrual age at entry to the study. Written, informed parental consent was obtained for all participants.

The infants entered the study approximately 7 days prior to hospital discharge. Upon entering the study, participating infants began to receive the study formula at alternate feeds for 24 hours. This was done so that signs and symptoms of intolerance to the study formula, such as diarrhea or vomitting, could be monitored and treated. Once tolerance was established based on the clinical judgement of the attending physician, participating infants were fed the study formula exclusively.

3.2.3 Dietary intake

The Canadian Pediatric Society recommends that solid foods should not be introduced to preterm infants until 4 to 6 months corrected age (CPS, 1995). Therefore, within the time frame of this research, i.e., from hospital discharge to 3 months corrected age, the study formula was to be the sole source of food intake for infants.

A five-day dietary intake record was kept by the caregivers at term corrected age and at three months corrected age, beginning on the day of the follow-up visit. During the 5-day intake recording period, biases in recording formula intake were overcome by using an objective approach used in a previous clinical trial conducted in our laboratory. At the follow-up visit, caregivers were given and instructed to use 120 ml, ready-to-feed jars of study formula exclusively (instead of the commonly used 1 L cans) during the 5-day period. The jars were pre-weighed with an electronic balance (EP-40KB, A & D Engineering Inc., CA) to the nearest 0.5g. Each jar was numbered on the lid as well as the bottle and the weight of each numbered jar was recorded prior to distribution to participating families. Caregivers were instructed to record the time and the number of the

jars used for each feed for 5 days. The jars were recapped and the residuals were collected to be re-weighed with the same electronic balance. The caregivers did not have to estimate the infant's intake at each feed. Instead, the difference between the pre-weight and the post-weight was estimated to be the actual formula intake. This was a valid assumption in our population of interest since regurgitation was a concern in only 3 of the 11 subjects, and reflux was managed by gastric motility drugs in these cases. Compliance was not a problem in diet record keeping. However, one male infant was given infant cereal with the study formula by the caregiver by three months corrected age (whereas the Canadian Pediatric Society recommends that no solid foods be given until the preterm infant reaches 4 months corrected age).

Mean formula intake was calculated as the mean intake of the five days. Energy intake was calculated as the product of mean formula intake and the energy content of the study formula, as determined by bomb calorimetry (Miller and Payne, 1965) with a coefficient of variation (CV) of 1.5%. A formula sample of known energy content (SMA 20, Wyeth-Ayerst Ltd., ON) was used as a standard.. Protein intake was calculated as the product of mean formula intake and the protein content of the study formula, which was determined by the micro-Kjeldahl technique (Association of Official Analytical Chemists, 1965) using a urea standard (enzyme grade, Bethesda Research Laboratories, MD). The CV of the standard formula was 1.4%.

3.2.4 Anthropometric measures

Participating infants were weighed at entry to the study, term corrected age and 3 months corrected age using a digital scale (Sartorius, Gottingen, Germany) to the nearest gram. At the same time points, crown to heel length and occipitofrontal circumference were obtained using an infant length board (Ellard Instrumentation Inc., Seattle, Washington) and a non-stretchable tape measure supplied by Wyeth-Ayerst Ltd., ON and Ross Laboratories (Columbus, OH).

Prior to term corrected age, the mean weight, length and head circumference of the 7 male subjects and the 4 female subjects were assessed using the size-at-birth (SAB) standards for Hamilton, ON (Blinder et al., 1984). The SAB standards were based on over 1200 singleton, live births, which occurred in Hamilton, ON, over a 18-month period between 1974 and 1975. SAB centiles were calculated for 31 weeks of gestation up to 44 weeks of gestation, and sex-specific values were calculated for 32 to 43 weeks of gestation (Blinder et al., 1984). After reaching term corrected age, the mean weight, length and head circumference of the male and the female subjects were assessed using the longitudinal data of the National Centre for Health Statistics centile standards for healthy term infants from birth to 3 years (Hamill et al., 1979). The weight-for-age and the length-for-age Z-scores were calculated using the Centers for Disease Control Anthropometric Software Package, Version 3.0 (Statistics Branch, CDC; GA).

3.2.5 Body composition: dual-energy x-ray absorptiometry (DXA)

Dual-energy x-ray absorptiometry scans were performed with the infants swaddled with a cotton blanket, placed next to the tissue bar and covered with a single layer of

flannel blanket. They were allowed to fall asleep naturally and no sedatives were used. One subject was not scanned due to refusal to sleep at the term corrected visit and unavailability of equipment at the 3 months corrected visit. All other subjects, 6 males and 4 females, were scanned once at the term corrected visit and once at the 3 months corrected visit. Infant whole body scans were performed by a dual-energy x-ray absorptiometer (Hologic QDR-1000/W; Hologic Inc., Waltham, MA). The scans were analyzed with Infant Whole Body Option version 5.56 (Hologic Inc.,Waltham, MA). Quality control check of the absorptiometer for accuracy was performed daily against the spine phantom (Hologic Inc.,Waltham, MA).

3.2.6 Total energy expenditure: doubly labeled water method

Dosing procedure and sample collection

Participating infants received a dose of $^{2}H_2O$ and $H_2{}^{18}O$ at term corrected age and at 3 months corrected age. The dosages were 0.3 gram $H_2{}^{18}O$ (9.9 atomic percent enrichment, Isotec Inc., Miamisburg, Ohio) per kilogram body weight and 0.1 gram $^{2}H_2O$ (99.9 atomic percent enrichment, Isotec Inc., Miamisburg, Ohio) per kilogram body weight. Both $^{2}H_2O$ and $H_2{}^{18}O$ were tested regularly for bacterial growth and $H_2{}^{18}O$ was passed through 0.2 micron sterile filters (Acrodisc: Gelman Scienes, Ann Arbor, Michigan) twice before administrating to the infants. The isotopes were mixed in an accufeed graduated nurser (Wyeth Laboratories Inc., Philadelphia, PA) and 0.2 ml of the mixture was retained for isotope enrichment analysis. The remaining water was mixed with an equal volume of study formula and was given to the infant by mouth. Any regurgitation

was collected with a pre-weighed disposable napkin which was kept in a plastic bag to prevent it from dessication. The napkin was then re-weighed as soon as possible to determine the amount of regurgitation.

A urine sample was collected using a disposable specimen collection bag (U-Bag; Hollister, Libertyville, Illinois) prior to dosing to establish the background isotope enrichment. A urine sample was collected approximately 4 hours post-dose (Day 0). The amount of fluid intake between dosing and the first post-dose urine sample was calculated from the weighed formula intake. Caregivers were instructed to collect post-dose urine samples from the first void on days 1, 7 and 8 using U-bags. The caregivers were also instructed to record the time of each urine collection. The samples were frozen at -20 degrees Celsius until they were returned to the our laboratory and then at -70 degrees Celsius in tightly capped scintillation vials until they were analyzed by gas-isotope-ratio mass spectrometry (GIRMS).

Analysis of Dose water enrichment

The isotopic enrichment of stock ^{18}O normalized water and deuterated water need to be ascertained in terms of atomic percent excess (APE) of the label claim. For this purpose, the stock water must be diluted with double distilled water (DDI), since the isotopic enrichment of the stock dose water falls beyond the range of values the mass spectrometer can measure with sensitivity. The actual APE can then be back calculated from the measured isotopic enrichment of the diluted dose water and DDI (see Appendix 1).

Analysis of urine samples by gas-isotope-ratio mass spectrometry

Sample Preparation for ^{18}O Analysis Urine samples were allowed to thaw completely at room temperature. The sample (0.2 ml) was deposited in a pyrex glass tube and frozen in an isopropyl alcohol-dry ice slush bath. Air in the tube was removed by attaching the tube to a vacuum line. The urine aliquot was allowed to thaw under vacuum and the dissolved gases escaped and pumped out. Once the urine was re-frozen, the vacuum line was flushed with a standard carbon dioxide gas and a fixed amount of carbon dioxide was introduced into the tube. Together with the urine aliquot, this carbon dioxide was then kept frozen in a liquid nitrogen bath until the tube was sealed with a gas-oxygen torch. The sealed tube was submerged in a water bath at 25 degrees Celsius for a minimum of 4 days to allow the urine to equilibrate with carbon dioxide.

After equilibration, the urine aliquot was frozen in an isopropyl alcohol bath. The tube was cracked open under vacuum to release carbon dioxide, which was transferred into a collection tube using liquid nitrogen. The collection tube was sealed with a gas-oxygen torch.

Sample Preparation for Deuterium Analysis Again, urine samples were allowed to thaw completely and to attain room temperature. Four microlitre aliquots were pipetted into 6 mm diameter short pyrex tubes which, using ultratorrs, were attached to open ended pyrex tubes. (Ultratorrs are connective devices fitted with rubber rings to provide air-tight connection between two pieces of glassware.) These urine aliquots were frozen in a isopropyl alcohol-dry ice slush bath. Air in the pyrex tubes was removed via the vacuum line. The open ended pyrex tubes were sealed at one end using a gas-oxygen torch. The

sealed end of the tubes were then submerged in the slush bath and the water in frozen urine in the short pyrex tubes was allowed to thaw and drain down to the sealed end and freeze. After removing the empty short pyrex tubes, 125 mg of pre-weighed zinc pellets were added to the water. These zinc pellets were frozen in the water when air in the tubes was pumped out via the vacuum line. The water and zinc were then sealed in the vacuum tubes using a gas-oxygen torch. By heating the sealed tubes at 450 degrees Celsius for 30 minutes, metallic zinc reacted with water to generate zinc oxide and to release hydrogen gas.

The sealed collection tubes were attached to the inlet ports on the mass spectrometer (VG Optima; Fison). Cracking the tubes released carbon dioxide or hydrogen gas into the mass spectrometer to be analyzed. ^{18}O abundance and deuterium abundance of the samples relative to that of the laboratory standard (distilled tap water) which was in turn compared with standard mean ocean water (SMOW) and standard light Antarctic precipitation (SLAP). The $^{18}O/^{16}O$ and $^{2}H/^{1}H$ of SMOW are defined as zero; where as the $^{18}O/^{16}O$ and $^{2}H/^{1}H$ of SLAP are -55.5 and -428 , respectively (Gonfiantini, 1984). There was a trend for CVs to increase as isotopic enrichment decreased. CVs for ^{18}O abundance analysis was 4.5% for the laboratory working standard. CVs for deuterium enrichment analysis was 4.8% for SMOW and 2.3% for SLAP. Using triplicates, CVs for samples were calculated. CVs for ^{18}O abundance analysis were 7.2% and 0.3% for the baseline samples and high enrichment samples, respectively. CVs for deuterium enrichment analysis were 1.4% and 0.5% for the baseline samples and high enrichment samples, respectively. The results were given in δ per millilitre units, i.e. isotopic enrichment

relative to the laboratory working standard. The δ_{sample} and $\delta_{baseline}$ values were normalized against SMOW and SLAP and then converted to atomic percent excess (APE):

$$APE = 100 \times R/(R+1) \quad [1]$$

where R = ratio of excess isotope

$$= R_{standard} \times (\delta_{sample} - \delta_{baseline})/1000 \quad [2]$$

and $R_{standard}$ is the ratio of heavy to light isotope of standard mean ocean water (SMOW), $R_{standard} = 0.00205$ for ^{18}O and $R_{standard} = 0.00015576$ for deuterium

Calculation of total body water (TBW) using isotopic dilution space of ^{18}O

TBW was calculated based on ^{18}O dilution space and a correction factor of 1.01 (Davies and Wells, 1994) using the following equation:

$$TBW\ (litres) = (TBW_{initial} + TBW_{final})/2 \quad [3]$$

$$TBW_{initial}\ (litres) = [dose\ (g) \times APE_{dose\ water} / molecular\ weight_{dose\ water}] \times (18.02/APE_{plateau\ sample}) \times 0.001/1.01 \quad [4]$$

$$TBW_{final}\ (litres) = Body\ weight_{final}\ (kg) \times [TBW_{initial}\ (litres)/Body\ weight_{initial}\ (kg)] \quad [5]$$

where dose is the number of grams of ^{18}O in the oral dose;
$APE_{dose\ water}$ is the ^{18}O atomic percent enrichment of the dose water;
$molecular\ weight_{dose\ water}$ is the molecular weight of the dose water, since molecular weight of $H_2O = 18.016$ and $H_2{}^{18}O = 20.158$, therefore, the molecular weight of 9.2% APE dose water $= (0.092 \times 20.158) + (1-0.092) \times 18.016 = 18.213$
$APE_{plateau\ sample}$ is the atomic percent enrichment of the sample at isotopic plateau, which occurs at 5 hours post dose and was calculated by back extrapolation (Davies et al., 1994; Winthrop, personal communication; Salazar et al., 1994):

$$\delta_{day1\ sample} = \delta_{plateau} \times e^{-kt} \quad [6]$$

where $\delta_{day\ 1\ sample}$ is the corrected δ of the Day 1 sample;

$\delta_{plateau}$ is the corrected δ of the 5 hours post-dose sample (plateau sample);
t is the time elapsed between the plateau sample and the Day 1 sample in days;
k is the elimination constant of ^{18}O; k was calculated using APE of the day 1 and day 7 samples (Schoeller et al., 1986):

$$k = (\ln APE_{day7} - \ln APE_{day1}) / \Delta t \quad [7]$$

where Δt is the time difference between the two sample in days

$APE_{plateau}$ was determined using equations [1] and [2]

TBW volume was corrected for new water intake by subtracting free water in study formula consumed between dosing and urine sampling (Brunton et al., 1996).

Calculation of carbon dioxide production rate (rCO_2) (Jones et al., 1987)

Carbon dioxide production rate (rCO_2) was calculated using the equation published by Jones et al. (1987):

$$rCO_2 = 0.445\ N\ (1.01k_o - 1.04k_h) \times 1000 \quad [8]$$

where rCO_2 is in litres day^{-1}
N is mean dilution space for ^{18}O, in litres
k_o & k_h are the elimination constants for ^{18}O and deuterium respectively

Calculation of Total Energy Expenditure (TEE)

Total Energy Expenditure (TEE) was determined from rCO_2 and the dietary respiratory quotient using the equation of DeWeir (1949):

$$TEE\ (kJ\ day^{-1}) = 4.63\ rCO_2 + 16.49\ rCO_2 / RQ \quad [9]$$

where RQ is the dietary respiratory quotient (Jones et al., 1987) which was calculated from the composition of the diet using the equation of Southgate & Durnin (1970):

$$RQ = (p \times 0.81) + (f \times 0.71) + (c \times 1.00) + (a \times 0.67) \quad [10]$$

where p, f, c and a are the fraction of total metabolizable energy contributed by protein, fat, carbohydrates and alcohol respectively. The fraction of energy contributed by each of the nutrients was determined by multiplying the Southgate and Durnin factors of 4, 9, 3.75 and 7 kcal g^{-1} with the protein, fat, carbohydrate and alcohol contents of the diet.

3.2.7 Statistical Analysis

Measurements at term corrected age and at 3 months corrected age were compared using a paired t-test (MYSTAT version 2.1, Wylie College Software; Evanston, IL). CVs for isotopic analysis was calculated using triplicates of each of a randomly selected baseline sample and a high enrichment sample. The agreement between whole body soft tissue composition measured by DXA and that determined by oxygen-18 dilution was assessed using the method by Bland and Altman (1986). Pearson's r was calculated for the relationships between i) total energy expenditure and weight and ii) total energy expenditure and fat free mass (SAS, SAS Institute Inc., Cary, NC).

3.3 Results

3.3.1 Subjects

Eleven subjects (7 males and 4 females) who fit the inclusion criteria were recruited from the neonatal intensive care units of St. Joseph's Hospital in Hamilton and Kitchener-Waterloo Hospital. The clinical characteristics of the subjects are described in table 3.3.1.1.

3.3.2 Dietary Intake

Formula intake

Mean daily formula consumption adjusted for body weight is shown in figure 3.3.1. The subjects consumed significantly more formula at term corrected age than at 3 months corrected age ($p<0.0005$). The reduction in intake was significant in both male ($p<0.0005$) and female subjects ($p<0.05$).

Energy and Protein intake

From the results of bomb calorimetry and micro-Kjeldahl analysis, energy and concentration of the study formula were determined to be 2.86 ± 0.06 kJ per gram (662.3 ± 0.5 kcal per litre) and protein concentration was 16.6 ± 0.5 g per litre respectively. Based on the formula intake information above, both energy and protein intake of the subjects (Figure 3.3.2) were significantly higher at term corrected age than at three months corrected age ($p<0.0005$).

Table 3.3.2.1 Clinical characteristics of subjects

	BOYS (n=7)	GIRLS (n=4)
Gestational Age (wk)	29.5 ± 3.0[a]	28.8 ± 2.9
Birthweight (g)	1192 ± 322	1321 ± 375
PMA at entry to study (wk)	36.9 ± 0.9	35.9 ± 0.7

[a] mean ± SD; PMA post-menstrual age

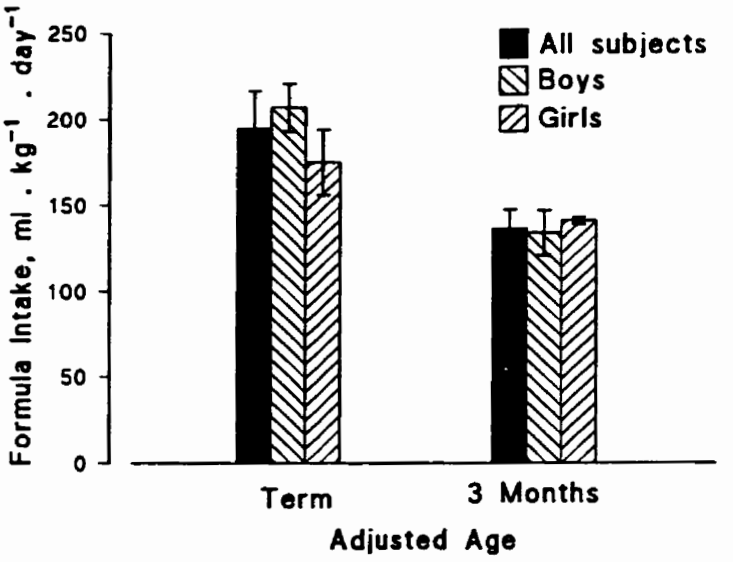


Figure 3.3.1 Study formula consumption of the subjects determined by 5-day weighed intake record at term and 3 months corrected age. Formula intake decreased from term to 3 months corrected age ($p<0.0005$ for all subjects; $p<0.0005$ for the male subjects and $P<0.05$ for the female subjects).

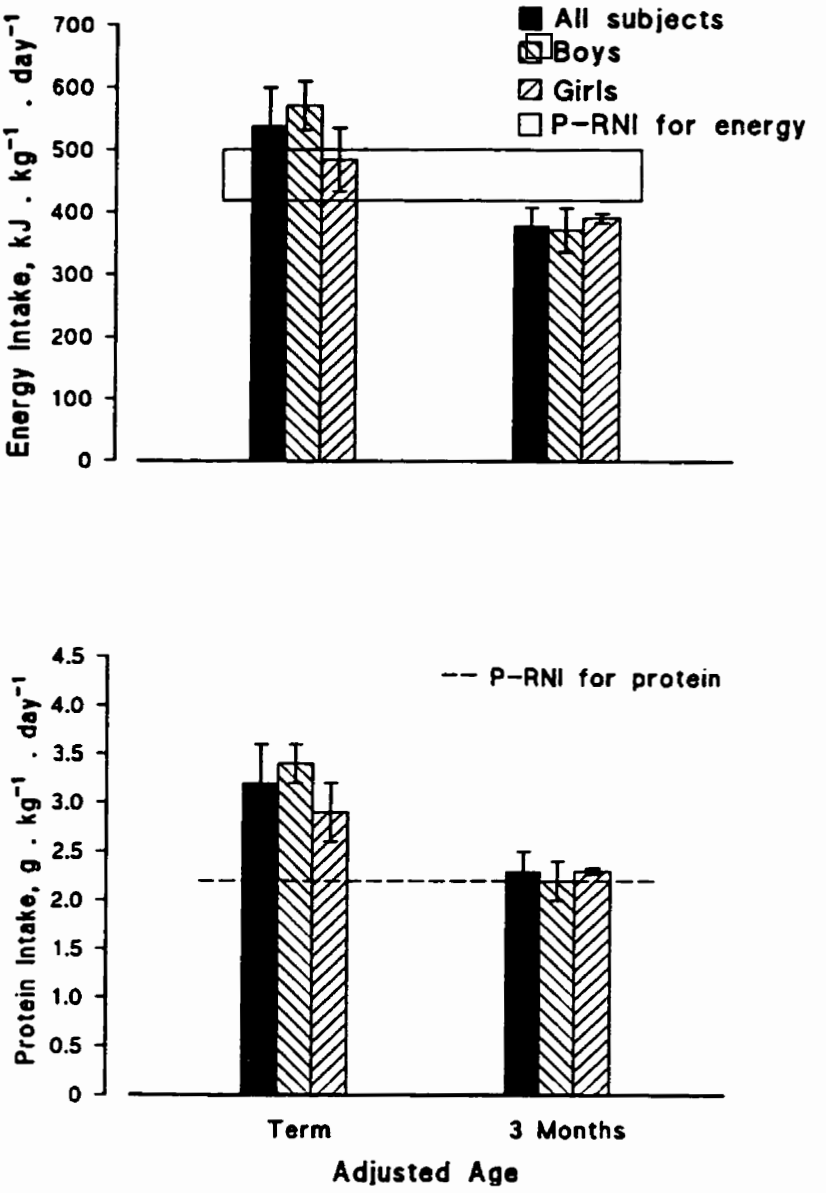


Figure 3.3.2 Gross energy and protein intake of the subjects calculated from 5-day weighed intake record. The decreased in energy and protein intake from term to 3 months corrected age is statistically significant ($p<0.0005$ for all subjects; $p<0.0005$ for the male subjects and $p<0.05$ for the females). P-RNI: recommended nutrient intake for premature infants during the post-discharge period (Canadian Pediatric Society, 1995).

3.3.3 Growth

Weight

The mean weight of the male subjects was below the 5th centile of the size-at-birth (SAB) standards at entry to the study (Figure 3.3.3). At the term corrected visit, their mean weight was at the 25th centile of the NCHS reference curves and increased towards the 50th centile by the 3 months corrected visit. At entry to the study, the mean weight of the female subjects was between the 5th and the 25th centiles of the SAB reference standards. Their mean weight was between the 50th and the 75th centiles at the term corrected visit and remained so till the 3 months corrected visit.

The male subjects gained 30$\pm$7 g per day between the term corrected visit and the 3 months corrected visit, and the female subjects gained 26$\pm$5 g per day during this time period. The rate of weight gain ($g.kg^{-1}.day^{-1}$) between entry to the study and the term corrected visit was greater than that between the term corrected visit and the 3 months corrected visit ($p<0.0005$ for all subjects; $p<0.005$ for the male subjects and $p<0.01$ for the females).

Length

At entry to the study, the mean length of both male and female subjects (Figure 3.3.4) fell below the 5th centile of the SAB standards. At the term corrected visit, the mean length of the male subjects was just above the 5th centile. Their mean length increased slightly to just below the 25th centile by the 3 months corrected visit. The mean length of the female subjects, however, rose to between the 25th and the 50th centiles at the term corrected visit and remained so at the 3 months corrected visit.

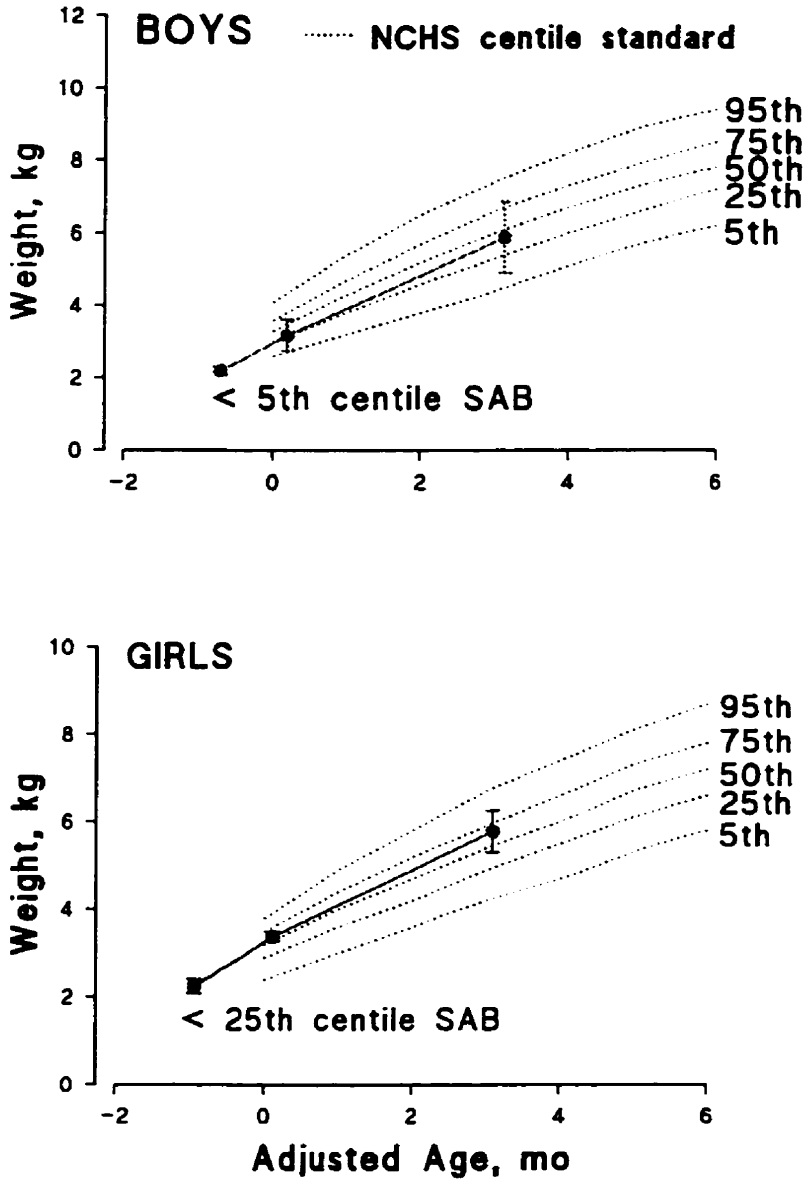


Figure 3.3.3 The mean weight of the subjects at entry to the study, term corrected age and three months corrected age. The reference growth curves are the National Centre for Health Statistics (NCHS) centile standards (Hamill et al., 1979). SAB: Size-at-birth standards for Hamilton, ON (Blinder et al., 1984)

65Weight-for-age Z-Scores and length-for-age Z-Scores

The NCHS weight-for-age Z-scores for all subjects (Figure 3.3.5A) scattered between +1 and -2 at the term corrected visit. The mean weight-for-age Z-score for all subjects was -0.36 with a standard deviation of 0.77. By three months corrected age, 6 of the 11 subjects had positive weight-for-age Z-scores and the mean was 0.023 ± 0.88. The length-for-age Z-scores (Figure 3.3.5B) for 10 of 11 subjects fell between 0 and -3 at the term corrected visit and this trend continued to the three months corrected visit. The mean length-for-age Z-score was -1.22 ± 0.86 at term corrected visit and showed a significant improvement to -0.75 ± 0.77 by the 3 months corrected visit ($p<0.05$).

However, when the weight-for-age Z-scores were compared with the length-for-age Z-scores at term corrected age, the weight-for-age Z-scores were significantly higher ($p<0.01$). The length-for-age Z-scores improved significantly by 3 months corrected age but remained lower than the weight-for-age Z-scores ($p<0.05$).

Head Circumference

At entry to the study, the mean head circumference of both male and female subjects (Figure 3.3.6) were below the 50th centile of the SAB standards. At the term corrected visit, the mean head circumference of the male subjects was at the 75th centile of the NCHS reference curves and by 3 months corrected age, their mean head circumference was between the 50th and 75th centiles. The mean head circumference of the female subjects tracked between the 75th and the 95th centiles from the term corrected visit to the three months corrected visit.

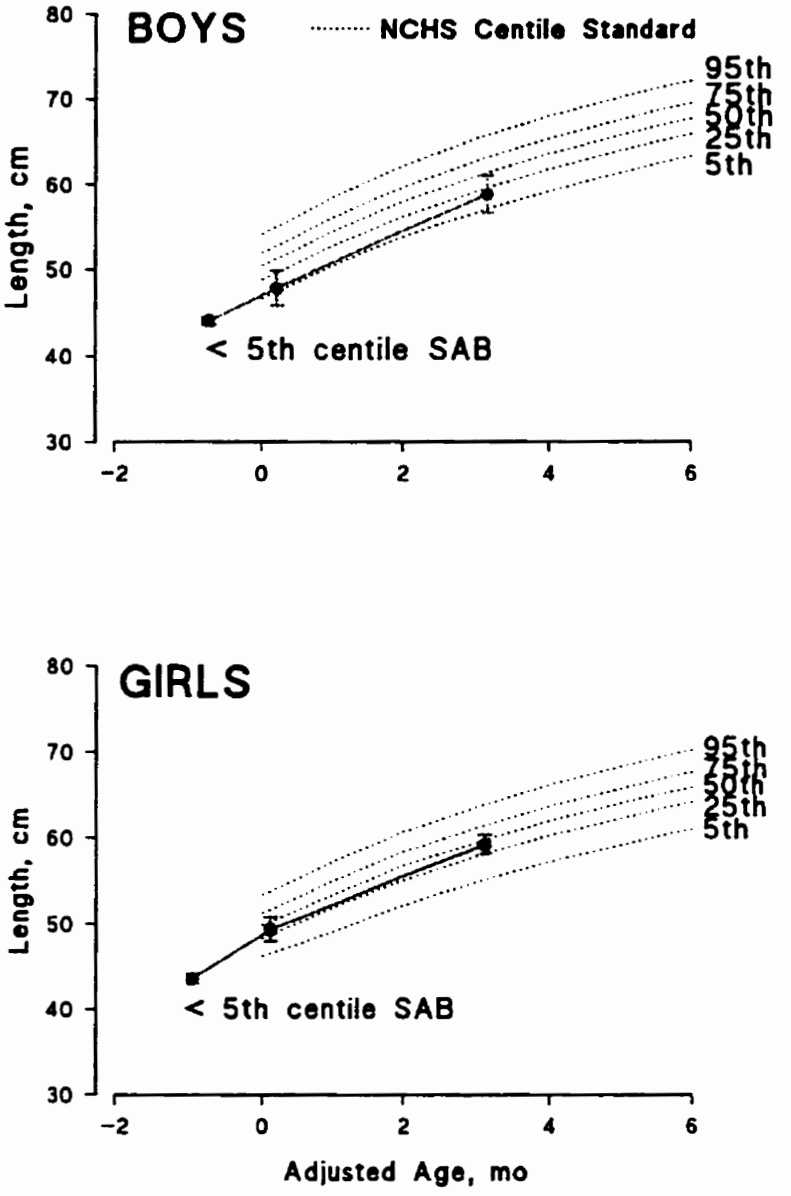


Figure 3.3.4 The mean length of the subjects at entry to the study, term corrected age and three months corrected age. The reference growth curves are the National Centre for Health Statistics (NCHS) centile standards (Hamill et al.,1979). SAB: Size-at-birth standards for Hamilton, ON (Blinder et al., 1984)

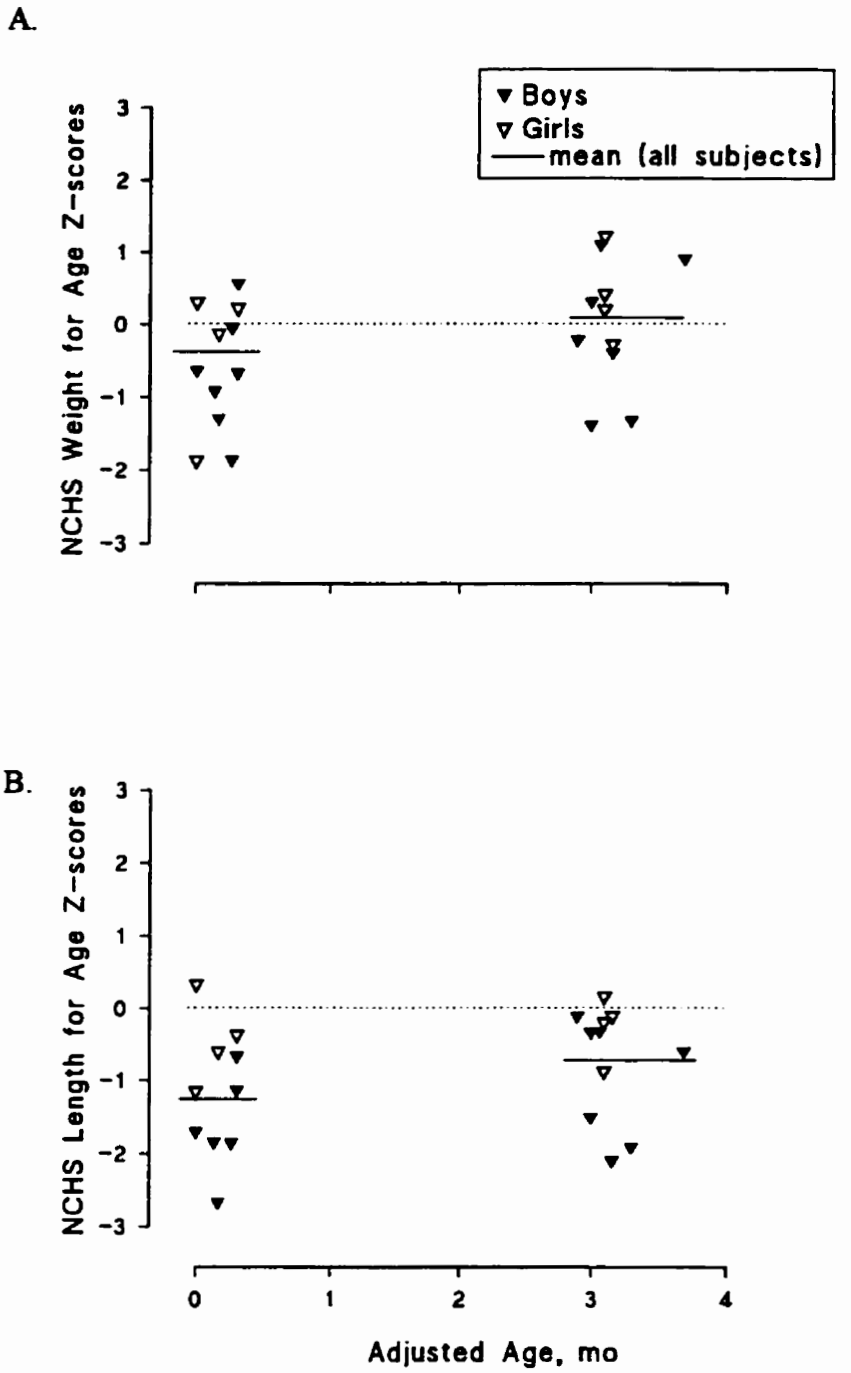


Figure 3.3.5 A. The National Centre for Health Statistics (NCHS) weight-for-age Z-scores of the subjects. The mean weight-for-age Z-scores of all subjects were -0.36 and 0.023 at term and 3 months corrected age, respectively. B. The NCHS length-for-age Z-scores of the subjects. The mean length-for-age Z-scores were -1.22 and -0.75 at term and 3 months corrected age, respectively, and the difference was significant ($p<0.05$).

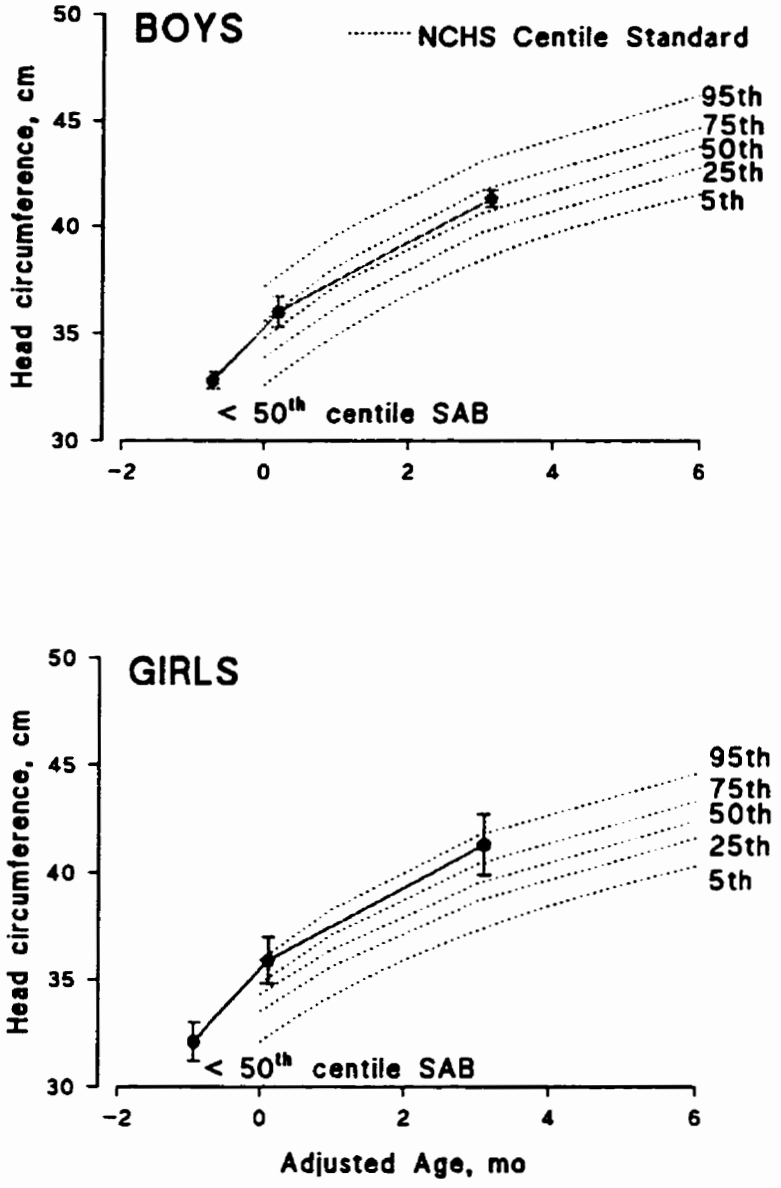


Figure 3.3.6 The mean head circumference of the subjects at entry to the study, term corrected age and three months corrected age. The reference growth curves are the National Centre for Health Statistics (NCHS) centile standards (1970). SAB: Size-at-birth standards for Hamilton, ON (Blinder et al., 1984)

3.3.4 Body Composition

Dual-energy x-ray absorptiometry scans (Figure 3.3.7) revealed that at term corrected age, 79% of the body weight the male subjects was fat free mass and 21% was fat. By 3 months corrected age, these subjects had 69% fat free mass and 31% total body fat. The female subjects had 82% fat free mass at term corrected age and 72% fat free mass at 3 months corrected age. Their total body fat contributed to 18% of their body weight at term corrected age and 28% of their body weight at 3 months corrected age. Percentage fat free mass decreased in 9 out of 10 subjects between the two follow-up visits. The mean percentage fat free mass of all ten subjects decreased from $80.3 \pm 6.4\%$ at term corrected age to $70.4 \pm 6.8\%$ at 3 months corrected age ($p<0.01$).

When the percentage fat free mass of the subjects were compared with that of term born, formula fed infants (Randall Simpson et al., 1995) (Figure 3.3.8), the male subjects scattered between the mean plus and minus 3 standard deviations of term born male infants. The percentage fat free mass of the female subjects fell within the mean plus or minus 2 standard deviations of term born female infants (Figure 3.3.8).

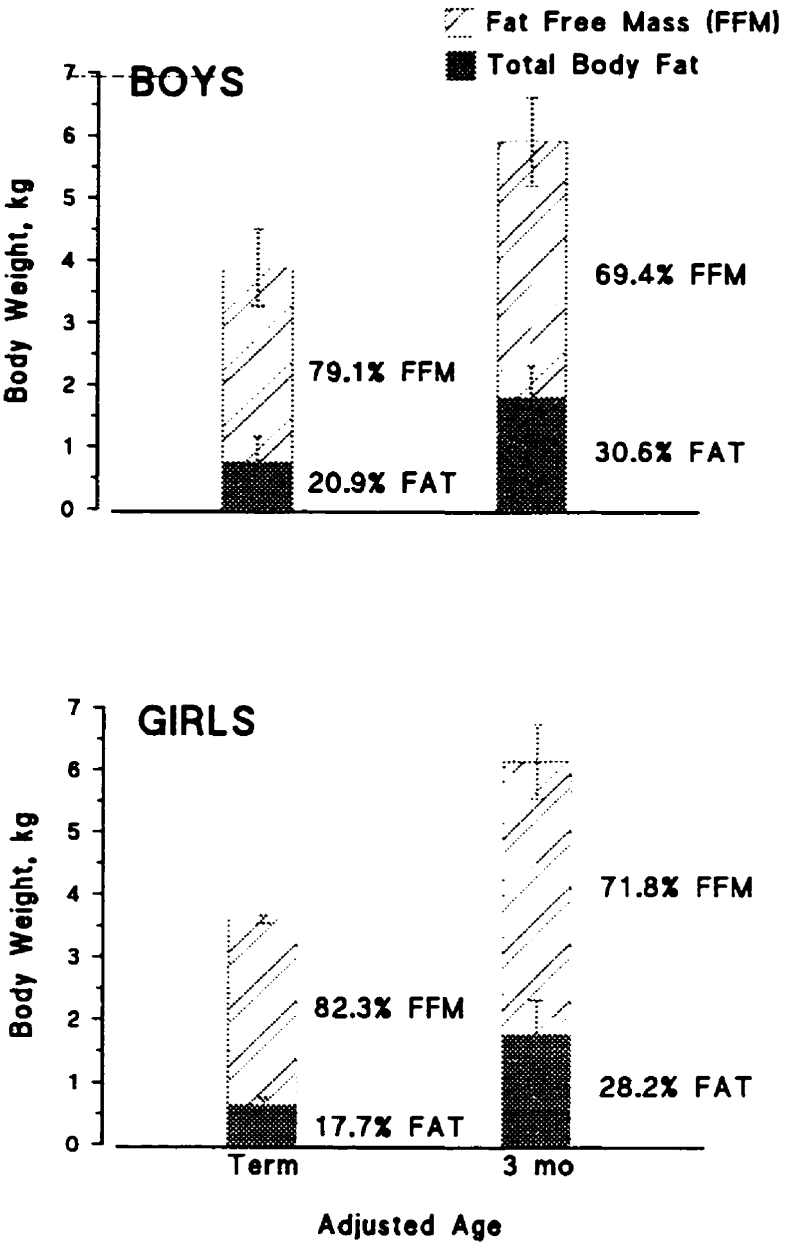


Figure 3.3.7 Body composition of 6 male subjects and 4 female subjects at term corrected age and at 3 months corrected age, determined by dual-energy x-ray absorptiometry.

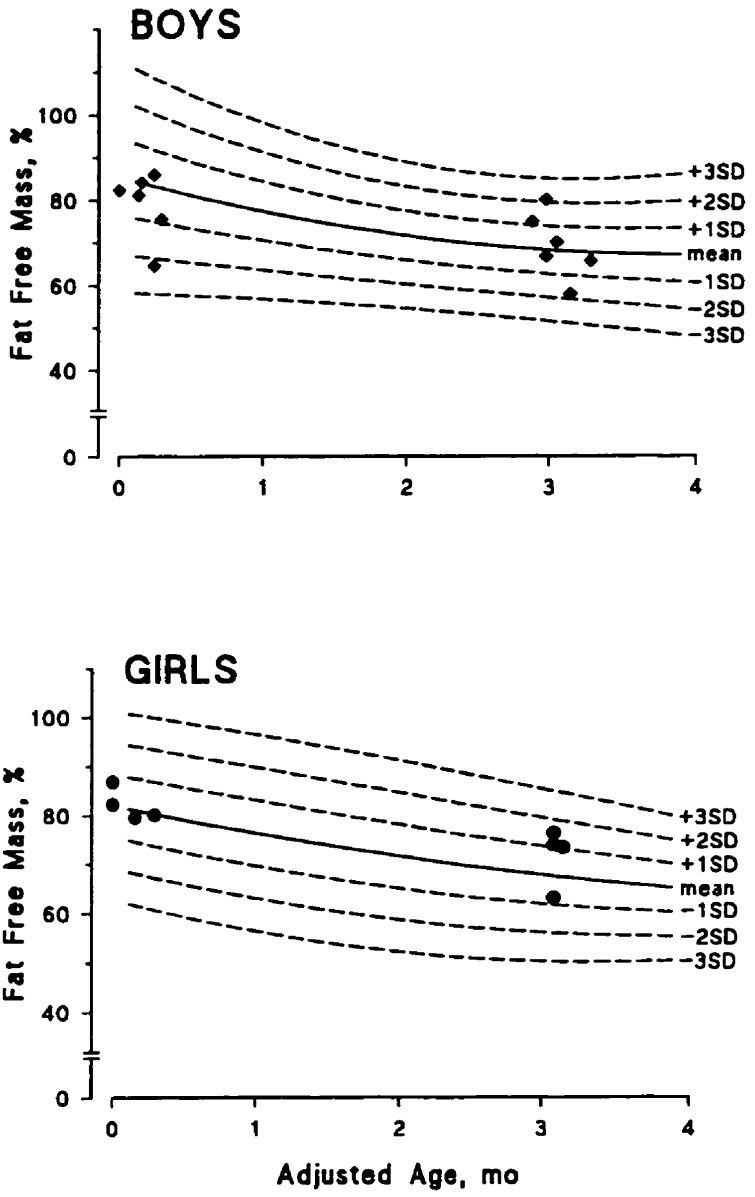


Figure 3.3.8 Percentage of fat free mass of 6 male subjects and 4 female subjects as determined by dual-energy x-ray absorptiometry (DXA). The reference curves are generated by smoothing mean percentage of fat free mass of formula fed, term born infants determined by DXA at birth, 2 and 4 months of age (Randall Simpson et al., 1995).

Body composition determined by oxygen-18 isotopic dilution

Percentage of total body water at term and 3 months corrected age is depicted in figure 3.3.9. There was a significant decrease in the percentage of total body water from term corrected age (64.3% $\pm$ 5.4%) to 3 months corrected age (56.5% $\pm$ 6.3%) ($p<0.05$).

Body composition determined by oxygen-18 isotopic dilution versus DXA

Body composition determined by ^{18}O dilution and by DXA are shown in table 3.3.4.1. Results of paired t-test showed that the percentage fat free mass and percentage total body fat determined by the two methods at term and 3 months corrected age did not differ significantly. When the difference in percentage fat free mass determined by ^{18}O dilution and by DXA was plotted against the mean of the two methods (Bland and Altman, 1986) (figure 3.3.10), 40% of the measures differed from the mean of the two methods by greater than 5%.

Table 3.3.4.1. Body composition determined by oxygen-18 dilution and by DXA. CA: corrected age; *Ref.*[+]: body composition of reference children (Fomon et al., 1982)

Term CA	*Oxygen-18 dilution*		*DXA*		*Ref.*[+]
Subject #	%FFM	%fat	%FFM	%fat	%FFM
1	69.9	30.1	75.6	24.4	
2	67.5	32.5	80.2	19.8	
3	70.5	29.5	81.2	18.8	
4	74.9	25.1	84.3	15.7	
5	79.6	20.4	82.4	17.6	
6	82.8	17.2	79.7	20.3	
7	79	21	82.3	17.7	
8	86.4	13.6	86	14	
9	83.6	16.4	64.8	35.2	
10	90.9	9.1	86.9	13.1	
mean	78.5	21.5	80.3	19.7	86
(SD)	(7.7)	(7.7)	(6.4)	(6.4)	

3 mo CA	*Oxygen-18 dilution*		*DXA*		*Ref.*[+]
Subject #	%FFM	%fat	%FFM	%fat	%FFM
1	66.5	33.5	70.2	29.8	
2	56.9	43.1	74	26	
3	67.5	32.5	75.1	24.9	
4	70	30	58	42	
5	88.5	11.5	65.9	34.1	
6	71.7	28.3	73.5	26.5	
7	81.2	18.8	76.5	23.5	
8	73.5	26.5	80.2	19.8	
9	70.3	29.7	66.9	33.1	
10	59.1	40.9	63.2	36.8	
mean	70.5	29.5	70.4	29.6	76
(SD)	(9.3)	(9.3)	(6.8)	(6.8)	

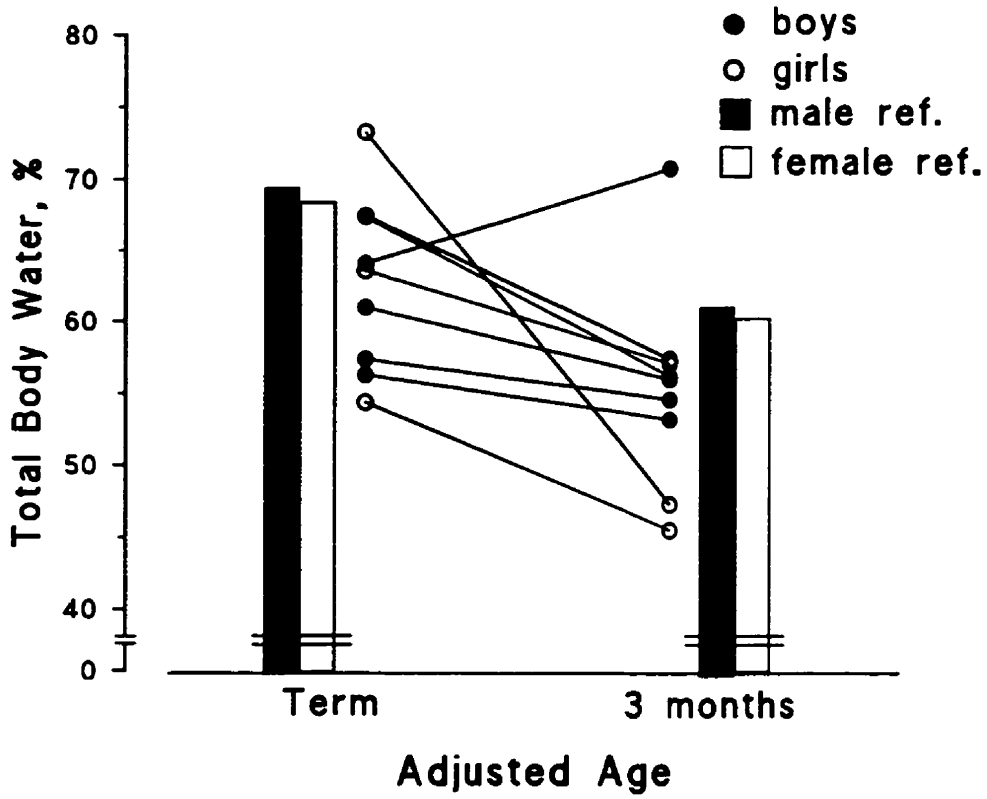
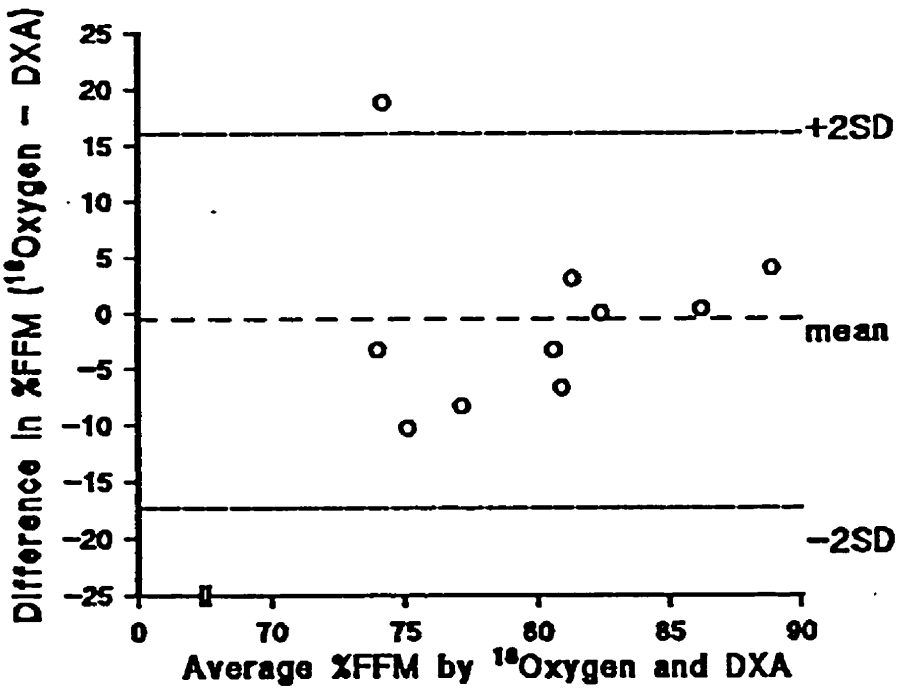


Figure 3.3.9 Percentage total body water at term and 3 months corrected age determined by oxygen-18 dilution. Percentage of total body water decreases from term corrected age (mean = 64.3%) to 3 months corrected age (mean = 56.5%) ($p<0.05$).

A. Term corrected age



B.. 3 months corrected age

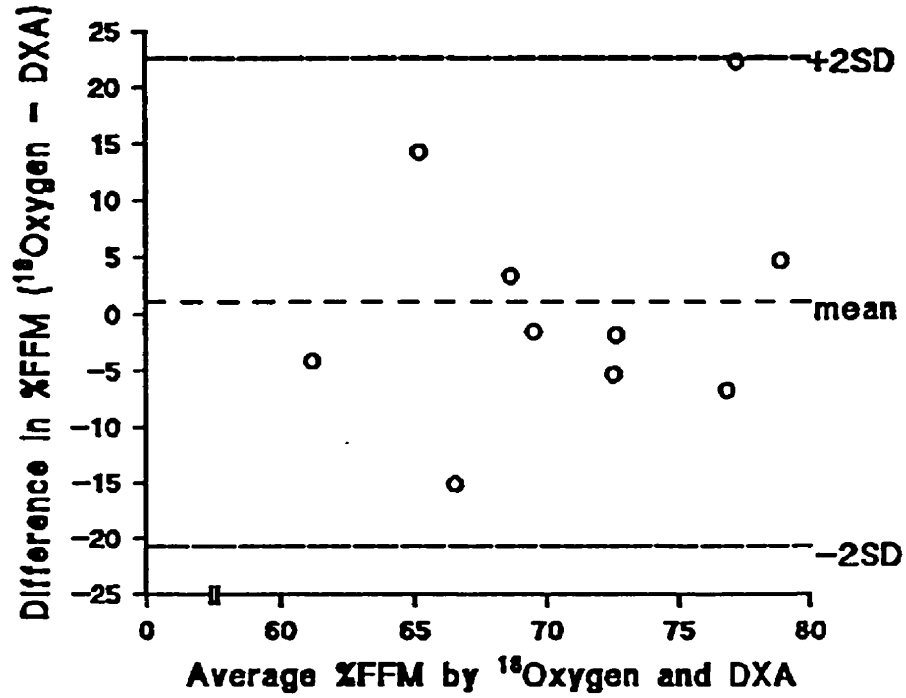


Figure 3.3.10 Test of agreement (Bland and Altman, 1986) in % fat free mass (FFM) measurement between ^{18}oxygen dilution and DXA. The mean difference between methods was 0.6% at term corrected age and 1.1% at 3 months corrected age.

3.3.5. Energy Expenditure

Energy expenditure ($kJ.kg^{-1}.day^{-1}$) of 5 of the 7 male and all 4 female subjects at term and three months corrected age are depicted in figure 3.2.11. Longitudinal energy expenditure data were not available due to an error resulting in a negative value for rCO2 in one infant; and for the other infant, one measurement was rejected as an outlier.

The energy expenditure of the 9 remaining subjects at 3 months corrected age (224 $\pm$ 47 $kJ.kg^{-1}.day^{-1}$) did not change from the energy expenditure at term corrected age (233 $\pm$ 93 $kJ.kg^{-1}.day^{-1}$) significantly. Paired-t test showed that the percentage of gross energy intake which was expended increased significantly from term (mean = 42.8$\pm$16.2%) to 3 months corrected age (mean = 59.1$\pm$13.5%) ($p<0.05$) (see Appendix 2).

Total energy expenditure of the subjects correlates with body weight ($R^2=0.53$; $p<0.005$) (figure 3.3.12). Body composition determined by DXA at the term and 3 months corrected visits showed that there was a significant association ($R^2=0.38$; $p<0.01$) between total energy expenditure ($kJ.day^{-1}$) and fat free mass (figure 3.3.13).

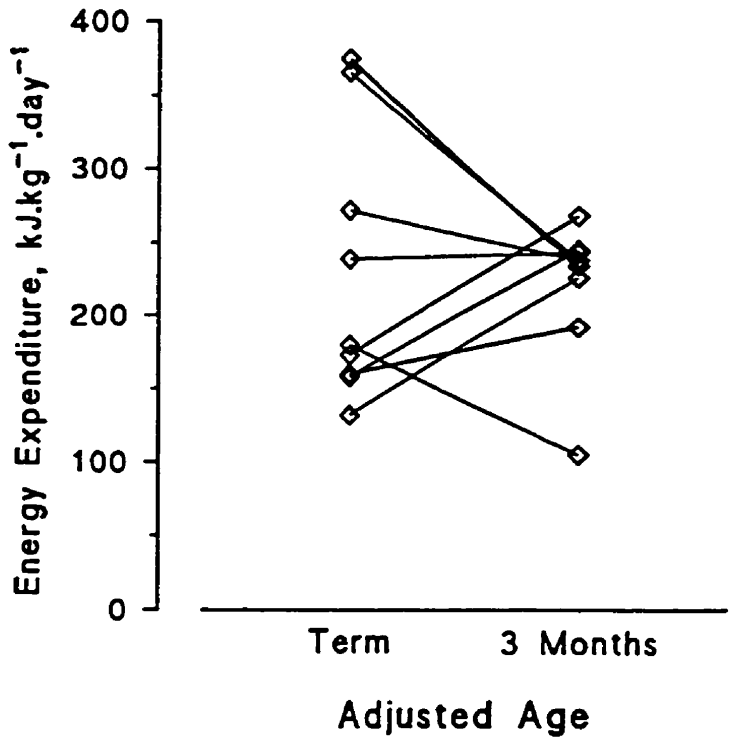
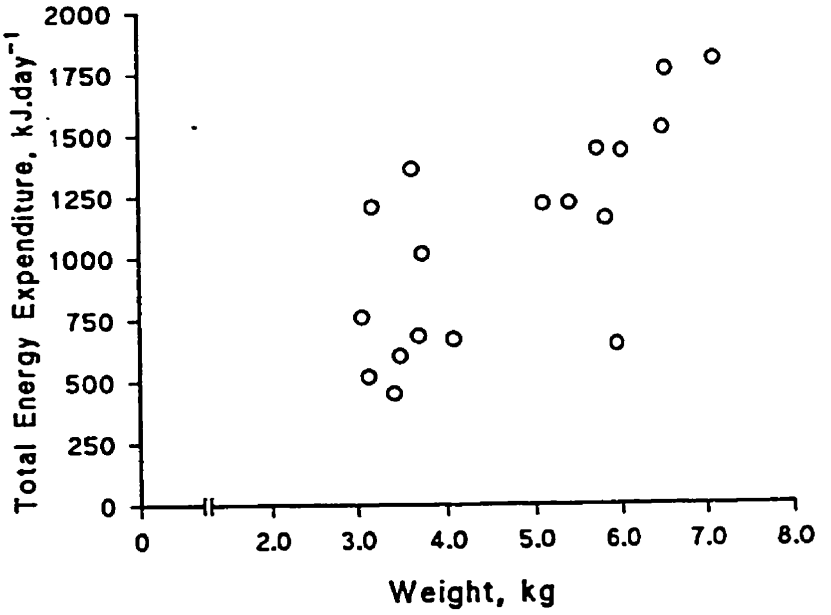


Figure 3.3.11. Energy expenditure at term and three months corrected age determined by doubly labelled water method. Energy expenditure of the subjects did not change significantly from term corrected age (mean = 233 $\pm$ 93kJ.kg^{-1}.day^{-1}) to 3 months corrected age (mean = 224 $\pm$ 47kJ.kg^{-1}.day^{-1}).

A.



B.

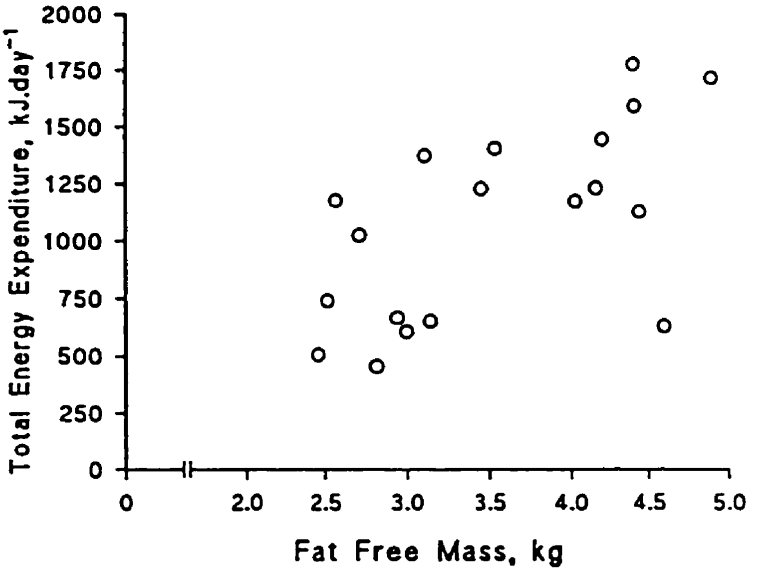


Figure 3.3.12. A. There was a significant correlation between the total energy expenditure and body weight of the subjects ($R^2 = 0.53$; $p<0.005$). B. The correlation between total energy expenditure and fat free mass of the subjects was significant ($R^2 = 0.38$; $p<0.01$).

Section 3.3 Discussion

Energy expenditure

This study is the first to measure total energy expenditure of healthy, formula-fed preterm infants as free-living subjects. The only reported values of energy expenditure in preterm infants were from infants measured at younger ages, usually less than 38 weeks PMA (Jensen et al., 1992; Westerterp et al., 1991). The infants in the validation study by Jones et al. (1987) had a mean gestational age of 38 $\pm$ 2 weeks but these infants were not representative of formula-fed, healthy infants because some were receiving parenteral nutrition after abdominal surgery. The reference data published by Davies et al. (1989) included energy expenditure of term infants measured at 1.5 months and approximately 50% of the infants in the sample were breast-fed.

The data on energy expenditure in this present study are generalizable to healthy preterm infants fed standard formula designed for term-born infants. Although this study is not a randomized controlled trial, the rigor of the various procedures involved in the doubly labeled water method, the objective assessment of dietary intake, and the well-defined subjects characteristics all give strength to the validity of the data.

Contrary to hypothesis 1 stated in section 3.1.2, energy expenditure in preterm infants in this present study showed no change from term to three months corrected age. The hypothesis was based on the perceived potential of the subjects to achieve catch-up growth by maintaining rapid growth beyond term corrected age. In healthy term-born infants who did not experience catch-up growth, Davies et al. (1989) reported a trend for energy expenditure to increase from 1.5 months to 6 months of age. Their rapid growth, increased food intake, as well as increased physical activity towards 6 months of age are

likely to play a role in this trend of increased energy expenditure. The reasons for the different observations in the preterm subjects in this present study may be related to the increase in the total amount of formula consumed (not adjusted for body weight), which can result in an increase in energy spent as the thermic effect of food. Also, the lower rate of growth between the two follow up visits compared with that between entry to the study and term corrected age suggest a reduction of energy spent in depositing new tissue. The trend towards a higher percentage of total body fat from term to 3 months corrected age indicates an increased proportion of energy retained is stored as fat. The combined effects of these observations may help to explain the absence of a significant change in expenditure measurements at term and 3 months corrected age.

Energy expenditure of these healthy preterm infants at 3 months corrected age also tends to be lower than that of BPD infants (Brunton et al., 1996). In the past, researchers would have attributed this difference to the increased work of breathing in the BPD infants (De Gamarra, 1992). The etiology of BPD may mean inflammation of lung tissues thus elevating energy expenditure. Also, as these sick infants recover and grow rapidly, an increased amount of the energy will be spent in tissue synthesis (Brunton et al., 1996). This present study and that of Davies et al. (1996) found a positive relationship between energy expenditure and fat free mass. In BPD infants, energy expenditure was found to have poor correlation with body weight (Brunton et al., 1996) but the relationship between energy expenditure and fat free mass was not discussed. If the severity of lung disease is the primary factor that causes elevated energy expenditure in infants with BPD, total energy expenditure is unlikely to correlate with fat free mass.

The subjects in this study were fed standard formula to reflect the effects of current nutritional care practice on energy metabolism and growth patterns of preterm infants. Given the strength of these data on energy expenditure, they are useful for future research in a number of ways. These data can serve as a basis for comparison in studies of energy metabolism of sick preterm infants on a similar diet. The mean and standard deviation values can be used to determine the sample size of future intervention studies where the energy intake of the preterm subjects may be manipulated. Interestingly, although there were only half the number of subjects in this study (n=9) compared to that of Brunton et al. (1996) (n=19), the standard deviations in energy expenditure at 3 months corrected age of this group of subjects (47 kJ per kg per day) was dramatically smaller than that of BPD infants at the same age (129 kJ per kg per day) (Brunton et al., 1996). This difference is likely due to the etiology of BPD, i.e., the varying degree of recovery from lung disease results in considerable variations in energy expenditure within infants with BPD. Although this difference is unlikely to be caused by methodological differences alone, these should be considered as well. This study employed a different approach from Brunton et al. (1996) to calculate mean isotopic dilution space, N, which was used in the calculation of rCO_2. In this study, the mean of the dilution space of oxygen-18 at the beginning of the metabolic period, and that at the end of the metabolic period, was used to determine N and in turn rCO_2; whereas Brunton et al. (1996) did not determine the isotopic dilution space at the end of the metabolic period, but calculate N as the mean of the initial dilution space of the two tracers, oxygen-18 and deuterium.

Body composition: the relationship with growth, energy intake and energy expenditure

The measured longitudinal changes in body composition were not found to be significant most likely due to the small sample size and not the lack of precision of DXA. Based on the CV of 0.6% for fat free mass determined by the piglet experiments of Brunton et al. (1997), DXA should be able to detect a true change in fat free mass if the measured change in fat free mass is greater than 1.5% between visits. Therefore, the measured 10% decrease in percentage fat free mass is unlikely to be just an artifact. These longitudinal body composition data provide insight on the mean and variability of body composition of healthy, formula fed preterm infants, and like the energy expenditure data, can aid in sample size calculations of future studies of the quality of growth of preterm infants using DXA technology.

In order to assess the quality of growth of formula fed preterm infants, these longitudinal body composition data were compared with those of healthy, formula fed term infants. The results of DXA measurements in a group of formula fed, term-born infants collected by our laboratory between 1992 and 1994 were chosen for comparison because any previous published reference data were obtained using methods other than DXA (Fomon, 1967; Fomon et al., 1982). Fomon et al. (1982) measured body composition of infants and children using total body potassium-40 and deuterium dilution, which are both indirect measures of soft tissue composition. Reference body composition data derived from cadaver analysis of stillbirths (Fomon, 1967) may not serve as a meaningful comparison because of the changes in feeding practice during the past three decades and differences in methodology. Sauve and Geggie (1991) reported significantly

lower tricep skinfolds in preterm infants from 4 to 24 months corrected age than in term infants. On the contrary, the soft tissue composition of the 10 preterm participants of this thesis research was found to be comparable to their term born counterparts at term and three months corrected age. Unfortunately, Sauve and Geggie (1991) did not measure body composition prior to 4 months corrected age to allow for comparison with our findings. Also, it is questionable how the investigators control for their bias since it is difficult to remain blinded to the infants' prematurity, especially when the preterms are significantly smaller as reported.

While it is a posititve factor that the subjects of this study had a soft tissue composition which was comparable to term infants at term and three months corrected age, the subjects demonstrated disproportionate growth. Like other formula fed preterm infants studied by our laboratory (Weiler et al., 1997), these subjects were also short for their weight at term corrected age. Thus it appears that under current nutritional care practice, disproportionate growth affects not only preterm infants with severe BPD who receive dexamethasone treatment (Weiler et al., 1997), but also healthy preterm infants. This finding is also supported by a recent randomized controlled trial (Wheeler et al., 1996) in which healthy preterm infants fed standard term formula had a weight that was at minus one standard deviation from the mean of the growth curves of Babson et al. (1976) at term corrected age, whereas their length was at minus two standard deviations from the mean, i.e. these infants also appeared to be short for their weight.

Also consistent with the previous findings in our laboratory is the preservation of head circumference of the subjects. Even the male subjects, whose weight at entry to the

study indicated growth faltering in their neonatal course, had a mean head circumference which falls between the 25th and the 50th centiles of the SAB standards at entry to study. It has been suggested (Pilon and Fox, 1992) that in the presence of inadequate nutrient intake to meet nutrient needs, energy will be conserved to sustain vital physiological processes. In the case of low nutrient reserves and limited intake such as during the neonatal course of a preterm infant, brain growth may take precedence over weight gain and linear growth. Others (Lubchenco et al., 1966; Fujimoto et al., 1991) suggest that extrauterine head growth may differ from intrauterine head growth in the absence of pressure exerted on the fetus's head by the amniotic fluid. Therefore intrauterine growth curves such as the SAB standards may not be the appropriate tool for assessing postnatal head growth in preterm infants. The ideal tool should address the degree of prematurity, postnatal age as well as neurological out come of preterm infants. In some of the cases in this thesis research, striking head circumference gains between visits were noted. This is important because a dramatic increase in head circumference is a symptom of hydrocephalus. However, our clinical staff have not been alerted of any incidence of hydrocephalus in infants in this sample who had follow-up visits with the Growth and Development Clinic at MUMC.

We obtained detailed dietary intake data at two time points during the study, it was possible to explore potential dietary factors that might influence both the size of the infants and their body composition (Bell., 1994). The subjects were fed a standard formula from discharge to reflect the effects of current nutrition practice on the growth of preterm infants after hospital discharge. The observed pattern of reduction in forumula intake has

been reported previously in formula fed preterm infants after hospital discharge (Wauben et al., unpublished data). Using weighed intake records, Lucas et al. (1992) also demonstrated a steady trend of reduction in formula intake from term to 12 weeks corrected age and the trend continued until the end of the study period. Reduction in formula consumption was expected after 4 months corrected age as solid foods were introduced but no information on total energy intake was given. Sauve and Geggie (1991) reported a trend of preterm infants (of similar gestational age and birthweight to infants in this thesis research) to increase their energy intake from approximately 475 kJ to over 550 kJ per kg per day between 4 and 12 months corrected age. The validity of these figures was questionable because the investigators suspected caregivers of over-reporting the infants' intake. Unfortunately, the infants' dietary intake prior to 4 months corrected age was not reported. Based on the weighed intake records of Lucas et al. (1992) and this present research, it is apparent that average energy intake decreases rather than increasing or remaining constant during the early months of the post-discharge period.

In the 11 participating infants, the observed decrease in intake was not a result of the introduction of solid food. Only one subject was fed infant cereal and another consumed fruit juice (which was discontinued two weeks prior to dosing of stable isotopes) at 3 months corrected age. The study formula remained as the sole food source of all the rest of the infants thus preserving the uniformity of the composition of the diet throughout the study. Therefore, the reduction in formula consumption caused the significant decrease in protein intake. It is unclear whether measured intake is mediated by

food-intake regulation processes by the infants, or perhaps a change in the behaviour of caregivers, which causes this reduction in intake.

The observed reduction in formula consumption seems to contradict the perceived need for the infants to achieve catch-up growth. However, the weight of all subjects were above the 5th centile of the NCHS growth standards by 3 months corrected age. This improvement in weight was most striking in the male subjects. Although they were appropriate for gestational age at birth like the female subjects, the weight of the male infants at entry to the study indicated that they suffered some growth failure during their stay in the N.I.C.U. Interestingly, their improvement in weight by term corrected age corresponded to protein and energy intake above the upper boundaries of the P-RNI values. Whereas the female infants who did not suffer growth faltering tended to energy intakes within the P-RNI range. This suggests that preterm infants who have suffered growth failure may have nutrient needs which exceed the P-RNI values just prior to reaching term corrected age. However, caution must be used when interpreting data from such a small sample size. In fact, Lucas et al. (1992) found no significant correlation between formula consumption and size at discharge in sample of 31 preterm infants whose weight was between the 10th and 3rd centiles of the Gairdner and Pearson (1971) charts. In the present sample, the female infants caught up in weight by term corrected age but the boys seemed to be approaching the 50th centile of the NCHS curves at 3 months corrected age. Although

intake is unaffected by size at discharge (Lucas et al., 1992), it is not known whether size at discharge affects the time needed to catch up after discharge.

This energy and protein intake at or above the P-RNIs at term corrected age and the subsequent reduction in dietary intake of the subjects corresponded with a weight gain (in grams per day) similar that of term born infants. These data on weight gain of term infants, reported by Nelson et al. (1989), were generated based on longitudinal measurements of over 700 formula fed, term infants whose weight was similar to that of the subjects at 1 and 12 weeks of age. Although the energy and protein intake of these term infants was unclear, it was likely that they met the recommended nutrient intake (RNI) for energy (500 to 420 kJ per kg per day) and protein, as indicated by their growth. The RNIs during the 0 to 3 months period are not different from the P-RNI values for energy and protein during the post-discharge period for preterm infants. The Nutrition Committee of the CPS (1995) states that by reaching the P-RNIs for energy and protein, growth rates equivalent to or greater than those of term infants can be attained. From the growth pattern observed in the 11 subjects, this statement by the CPS remains to be verified.

In this present sample, the significant improvement in length-for-age Z-score of the subjects by 3 months corrected age did not completely compensate for disproportionate growth. Recent literature (Wheeler et al., 1996) suggests that post-discharge nutritional intervention has a positive effect on linear growth. Preterm infants who continued to receive preterm formula after hospital discharge (at approximately 35 weeks PMA) for 8 weeks, had greater mean length than those who were switched to a standard term formula. This difference was still significant even when differences at entry were taken into account statistically. Since the two formulas were iso-energetic but the preterm formula had a

higher protein concentration (18.5 g per litre instead of 15 g per litre as well as a higher whey to casein ratio.), it appeared that increased protein to energy ratio of the feeds possibly contributed to improved length gain. Since the preterm formula group had better mean linear growth, their weight to length ratio appeared less disproportionate at term corrected age than for those fed standard term formula. In this present sample of healthy preterm infants, even though the length for age Z-scores improved significantly from term to 3 months corrected, the length for age Z-scores of the subjects remained significantly lower than the weight for age Z-scores for term infants at 3 months corrected age. It appeared that the intake of the subjects may not be adequate to promote and sustain linear catch-up growth despite acceptable weight gain. Thus in order to increase protein intake without further increasing energy intake, the protein to energy ratio of the feeding must be increased. Whether it is protein or energy which determines food intake remains controversial.

From these data, it appears that the feeding of standard formula supports weight gain to reach between the 25th and the 75th centiles, and this weight gain is accompanied by a body composition similar to term infants, even as the fraction of energy intake expended increased in 7 of the 9 subjects. In these subjects who are short for their weight, promoting length gain and maintaining a desirable body composition will require careful manipulation of diet composition. It is known that at similar levels of protein intake, increasing energy intake increases fat deposition, which may not contribute very much to weight gain (Putet et al., 1987; Putet, 1993). Therefore, increasing energy intake alone does not necessarily result in greater weight gain (Bell, 1994). At similar levels of

metabolizable energy intake, increasing protein intake increases lean mass gain. Lean mass has a higher water content and results in a greater weight gain (Putet et al., 1987). Also, total energy expenditure may increase due to higher energy cost of protein accretion compared with fat accretion (Catzeflis et al., 1985) and lean tissues are more metabolically active than adipose tissue (Linder, 1991). Consequently, net energy stored from dietary intake may decrease due to the higher metabolic cost of protein accretion compared with fat (Catzeflis et al., 1985). Together, metabolizable energy intake and protein-energy ratio affect energy balance and influence growth (Bell, 1994). This supports the need to improve the protein to energy ratio of the post-discharge diet of preterm infants since excesses in energy intake alone increase fat deposition. Lucas et al. (1992) found no difference in the skinfold measurements between preterm infants fed standard formula and those fed a protein and energy enriched discharge formula. The preservation of fat to lean ratio may be attributed to the slight increase of protein to energy ratio in the discharge formula in the study by Lucas et al. (1992).

If the protein to energy ratio of the diet is raised to help linear growth, and the infants maintain their formula consumption as in the study by Lucas et al. (1992), their protein intake (in g per kg per day) will be increased. Theoretically, an increase in protein intake can lead to elevated energy expenditure thus affecting energy balance in preterm infants. Although there is evidence (Wheeler et al., 1996) showing the positive effect of protein on linear growth, more research is required to determine the effects of a high protein to energy ratio on metabolic response, energy balance as well as body composition.

Body composition: ^{18}O dilution versus DXA

Theoretically, whole body fat free mass and fat mass can be calculated from TBW data. However, body composition measurements using different methods may not agree. Previous body composition measurements performed in our laboratory employed DXA technology and this study provided the opportunity to compare measurements by ^{18}O dilution with DXA in formula fed, healthy preterm infants at and beyond term corrected age. Although DXA has been validated for use in preterm infants (Brunton et al., 1993 and Picaud et al., 1995), both ^{18}O dilution and DXA are still relatively new methods of assessing body composition. Although paired-t test showed that the mean percentage fat free mass did not differ significantly between methods, this test does not reveal information about the magnitude or direction of the differences in individual pairs of measurements. Correlation analysis is not appropriate in this study because the narrow range of values does not generate good correlation regardless of the true relationship between the two methods. Even a correlation coefficient of 1 does not guarantee agreement (Bland and Altman, 1986) because data points which lie along any straight line parallel to the line of equality will yield perfect correlation . Using the test for agreement by Bland and Altman (1986), body composition determined by ^{18}O dilution did not agree with the measurements by DXA at term and 3 months corrected age in this group of 10 healthy preterm infants. This finding supports findings of a similar test of agreement between the two methods in preterm infants with BPD (Brunton et al., 1996). Previously, we suspected the disagreement found in body composition measurements associated with BPD was possibly the result of a difference between infants with BPD and the healthy

infants participating in this research. However, the disagreement between methods in healthy preterm infants indicates that the etiology of BPD is unlikely to cause disagreement between isotopic dilution measurements and DXA, but rather, the two methods are simply not interchangeable. Bland and Altman (1986) state that when precision of one method is poor, the agreement between two methods is bound to be poor. This could explain our findings in view of the 13% error reported when oxygen-18 was validated for oral dosing in piglets (Bayley and Whyte, 1985).

Methodological issues encountered while conducting the study

This study examined a number of methodological issues (see section 2.1.2) relevant to the use of the doubly labeled water method in free-living premature infants. During the course of the study, a number of pertinent observations were made. Some of these issues are outlined below and should be considered in future studies employing the doubly labeled water method.

Although 0.2 ml of dose water samples were retained for analysis, the energy expenditure data were calculated based on a dose water sample drawn directly from the vacutainer and carefully diluted with DDI. During the method development stage of the study, an attempt was made to analyze the 0.2 ml samples from the two test infants. Dilution of these samples were conducted based on the proposed calculations of the M.Sc. candidate. The resulting enrichment appeared erroneous (Brady, 1996). Alternatively, the dose water dilution protocol employed Brunton et al. (1996) (see appendix 1) was used and water samples were drawn directly from the vacutainer. This approach, however, does not measure the enrichment at the time of dosing. While urine samples are stored at -70

degrees Celsius to prevent evapouration and changes in isotopic enrichment, it is not known whether the enrichment of stock oxygen-18 normalized water and deuterated water changes during storage in the refrigerator (>0 degrees Celsius). For future studies, 0.2 ml of the dose water retained at the time of two separate dosing attempts should be analyzed to determine the stability of enrichment over time. Error in our first attempt lies in the dilution procedure itself, rather than in the calculations, and careful documentation as well as weighing of the syringe (which stores the 0.2 ml sample) should correct the problem.

In this study, sampling error can be minimized but not totally eliminated. The caregivers were telephoned to be reminded to collect urine samples but there was no guarantee that they would use the right vials for the corresponding samples. Interestingly, both infants with problematic results have a twin. It is conceivable that this complicates the collection procedure. For future attempts, it may be worthwhile to provide caregivers help with urine collection at the appointed time, especially when complications are predicted.

The impact of isotopic equilibration time on the calculation of TBW was discussed under method development in section 2.1.2. Based on the isotopic equilibration time observed in two healthy preterm infants, the findings justified using the back extrapolation approach to determine plateau enrichment, which is likely to be more accurate than using a 5 hour sample. In this study, the "5-hour" sample was collected at any time between 2.5 hours and 9 hours post dose. However, whether equilibration in a 34 week PMA infant is same as that in a term corrected or 3 months corrected is unclear. Serial urine sample

collection in the home may provide the answer but demands excellent compliance from caregivers, plus many other practical considerations. This remains an issue to be explored.

Jensen et al. (1992) found only negligible change in the percentage of TBW during the metabolic period. Adopting this finding in the set up of the protocol of this study allowed for the calculation of final TBW volume using the initial percentage of TBW. This approach to determining final TBW volume should be used for future studies as well because it can reduce isotope needs and cost of analysis considerably.

Conclusions

This study was the first attempt to measure total energy expenditure in formula fed, healthy preterm infants at term corrected age and beyond. Although the mean energy expenditure were similar from term to 3 months corrected age, there was considerable variability in energy expenditure at each time point, and the energy expended accounted for an increase in the percentage of their gross energy intake in 7 of the 9 subjects. Body composition of these infants was comparable to that of their term born counterparts and there was an increase in the percentage total body fat and a decrease in fat free mass between term and 3 months corrected age in 9 of the 10 subjects, as hypothesized. However, these preterm infants remained short for their weight at 3 months corrected age. Their rate of weight gain prior to term corrected age was greater than that between term and 3 months corrected age, possibly due to the tendency for increasing fat gain towards 3 months corrected age.

In these infants who were fed standard formula, except for their negative mean length-for-age Z-scores, catch-up growth resulted in weight, head circumference and body

composition comparable to term born infants and occurred at energy and protein intakes which tend to exceed and were more variable than the P-RNI values. Although the P-RNIs published by CPS in 1995 are the first set of recommendations which includes specific recommended nutrient intake values for the post-discharge period, the appropriateness of these values need to be assessed because they have been established based only on a small body of research and consensus. The outcome measures of post-discharge nutrition intervention which have been examined include bone mineralization (Chan, 1992; Bishop et al., 1993), protein status (Chan et al., 1994) vitamin status (Carlson et al., 1994) and growth (Lucas et al., 1992; Chan et al., 1994; Wheeler et al., 1996) and the results are promising. Energy metabolism after hospital discharge was previously unexplored until this study was conducted, thus fulfilling the objective of determining total energy expenditure. The objective of measuring body composition was achieved using two methods: DXA and ^{18}O dilution. Although previous findings from our laboratory lead to the hypothesis that the two methods agree within the population of healthy, preterm infants, the findings of this thesis research suggest the opposite.

Growth data from our laboratory and pertinent literature show that preterm infants indeed do not complete catch-up growth during hospital stay, and under current nutritional care practice, they remain short for their weight beyond term corrected age. Therefore, more research in energy metabolism in relation to growth and body composition is needed to help improve the P-RNIs and to better nourish preterm infants in their early life.

Section 3.5 Future Directions of Research

The data on energy expenditure of free-living, healthy preterm infants fed standard formula provided the mean and standard deviation values needed to calculate an appropriate sample size for future studies in energy metabolism in this population. Further studies using a sufficiently large sample size are needed to confirm the findings of this study.

A number of methodological issues pertaining to the use of the doubly labeled water method in free-living, formula-fed preterm infants remains to be explored. Serial urine collection at home performed supervised by the investigators will be ideal for ascertaining isotopic equilibration time at and beyond term corrected age. This will require careful planning and cooperation of the caregivers. Another concern is the potential changes in stock oxygen-18 normalized water and deuterated water during refrigeration. If the enrichment of these stock solutions remains stable, analysis of dose water retained from each dosing attempt will not be necessary, thus reducing the analytical costs of using the doubly labeled water method.

The results of this study suggest that nutrition intervention intended to correct disproportionate growth must do so without drastically changing the infants' soft tissues composition. A randomized controlled trial using a protein enriched formula as intervention is needed to ascertain if an increased protein to energy ratio can indeed improve length gain in preterm infants experiencing disproportionate growth. The impact of an increase in protein to energy ratio on body composition, energy intake and energy

expenditure must be studied in order to help these infants to achieve and maintain a soft tissue composition similar to that of their term counterparts.

Together, careful documentation of food intake, anthropometric data and body composition measurements throughout the first year of life will provide insight on the relation between nutrition and growth during this critical period of early catch-up growth. The understanding of this relationship is crucial to the improvement of feeding recommendations for free-living preterm infants.

REFERENCES

Association of Official Analytical Chemists. Official methods and analysis. 10th ed. Washington, D.C.; Association of Official Analytical Chemists 1965; 15-16.

Babson, S.G. and Benda, G.I. (1976) Growth graphs for clinical assessment of infants of varying gestational age. J.Pediatr. 89:814-20

Bell, E.F. (1994) Diet and body composition of preterm infants. Acta. Paediatr. Suppl. 405:25-8

Bishop, N.J., King, F.J. and Lucas, A. (1993) Increased bone mineral content of preterm infants fed with a nutrient enriched formula after discharge from hospital. Arch. Dis. Child. 68:573-8

Bland, M.J. and Altman, D.G. (1986) Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1:307-10

Blinder, I.N., McClemont, S., Anderson, G.D. and Sinclair, J.C. (1984) Size-at birth standards for an urban Canadian population. Can.Med.Assoc.J. 130:133-40

Brady, S. (1996) The Doubly Labeled Water Technique: Equilibration Determination and Total Body Water Calculation in the Premature Infant. Undergraduate thesis in fulfillment of Biochemistry 4B06, McMaster University.

Braillon, P.M., Salle, B.L., Brunet, J., Glorieux, F.H., Delmas, P.D. and Meunier, P.J. (1992) Dual energy x-ray absorptiometry measurement of bone mineral content in newborns: Validation of the technique. Pediatric Research 32(1):77-80

Brooke, O.G. (1980) Energy balance and metabolic rate in preterm infants fed with standard and high-energy formulas. Br.J.Nutr. 44:13-23

Brooke, O.G. (1986) Energy Needs in infancy. In Energy and Protein Needs during infancy, ed. Fomon, S.J. and Heird, W.C. Academic Press, Inc. Orlando, FL.

Brooke, O.G. and Kinsey, J.M. High energy feeding in small for gestation infants. Archives of Disease in Childhood. 60:42-6, 1985.

Bruck, K. et al. (1962) Neutral temperature range and range of thermal comfort in premature infants. Biol.Neonate. 4:32-51

Brunton, J.A., Atkinson, S.A., Saigal, S., Withrop, A.L. and Brady, S. (1996) The measurement of energy expenditure in infants using doubly labelled water. Ph.D. Thesis, McMaster University.

Brunton, J.A., Bayley, H.S. and Atkinson, S.A. (1993) Validation and application of dual-energy x-ray absorptiometry to measure bone mass and body composition in small infants. Am.J.Clin.Nutr. 58:839-45

Brunton, J.A., Weiler, H. and Atkinson, S.A. (1997) 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. Pediatr. Res. 41:590-6

Butte, N.F. (1988) Energy Requirements During Infancy. In Nutrition During Infancy, ed. Tsang, R.C. and Nichols, B.L., Hanley & Belfus, Inc., Philadelphia, PA

Butte, N.F. et al. (1996) Report of the working group on energy requirement of infants, children and adolescents. European J.Clin.Nutr. 50 suppl.:s188-9

Carlson, S.E., Peeples, J.M., Werkman, S.H. and Koo, W.W.K. (1995) Plasma retinol and retinol cinding protein concentration in premature infants fed preterm formula past hospital discharge. European J.Clin.Nutr. 49:134-6

Catzeflis, C. et al. (1985) Whole body protein synthesis and energy expenditure in very low birthweight infants. Pediatr.Res. 19:679-87

Chan, G.M. (1992) Performance of dual-energy x-ray absorptiometry in evaluating bone, lean body mass, and fat in pediatric subjects. J.Bone.Min.Res. 7(4):369-74

Chan, G. (1993) Growth and bone mineral status of discharged very low birth weight infants fed different formulas or human milk. J. Pediatr 123:439-43

Chan, G.M., Borschel, M.W. and Jacobs, J.R. (1994) Effects of human milk or formula feeding on the growth, behaviour, and protein status of preterm infants discharged from the newborn intensive care unit. Am.J.Clin.Nutr. 60:710-6

Coward, W.A. (1988) The doubly-labelled-water method: principles and practice. Proc. Nutr. Soc. 47:209-18

Davies, P.S.W, Cameron, J.E. and Lucas, A. (1989) Reference data for total energy expenditure in early infancy. In Activity, Energy Expenditure and Energy Requirements of Infants and Children, ed. Schurch, B and Scrimshaw, N.S. International Dietary Energy Consultancy Group, Cambridge, MA.

Davies, P.S.W., Clough, H., Bishop, N.J., Lucas, A., Cole, J.J. and Cole, T.J. (1996) Total energy expenditure in small for gestational age infants. Arch.Dis.Child. 74:F208-10

Davies, P.S.W. and Wells, J.C.K. (1994) Calculation of total body in infancy. European Journal of Clinical Nutrition 48:490-5

De Weir, J.B. (1949) New methods for calculating metabolic rate with special reference to protein metabolism. J.Physiol. 109:1-9

De Gamarra, M.E., Schutz, Y., Catzeflis, C., Freymond, D., Cauderay, M., Calame, A., Micheli, J.L. and Jequier, E. (1987) Composition of weight gain during the neonatal period and longitudinal growth follow-up in premature babies. Biol.Neonate 52 suppl:181-7
Fomon, J. (1967) Body composition of the male reference infant during the first year of life. Pediatrics 40: S63-S70

Fomon, S.J., Filmer, L.J.Jr., Thomeas, L.N., Anderson, T.A. and Nelson, S.E. Influence of formula concentration on caloric intake and growth of normal infants. Acta. Pediatr. Scand. 64:172-81, 1975.

Fomon, S.J., Haschke, F., Ziegler, E.E. and Nelson, S.E. (1982) Body composition of reference children from birth to age 10 years. Am.J.Clin.Nutr. 35:1169-75

Freymond, D., Schutz, Y., DeCombaz, J., Micheli, J.-L. and Jequier, E. (1986) Energy balance, physical activity and thermogenic effect of feeding in premature infants. Pediatr.Res. 20:638-45

Fujimoto, S., Ishikawa, T., Togari, H., Kanayama, M., Okajima, K. and Inukal, K. (1991) Postnatal head growth in very premature infants with good outcome. Acta.Pediatr.Jpn. 33:83-6

Gairdner, D. and Pearson, J. (1971) A growth chart for premature and other infants. Arch.Dis.Child. 46:783-7

Giofiantini, R. (1984) Report on advisory group meeting on stable isotope reference samples for geochemical and hydrological investigations. Vienna, Austria: International Atomic Energy Agency.

Goran, M.I. (1994) Application of the doubly labeled water technique for studying total energy expenditure in young children: a review. Pediatric Exercise Science 6:11-30

Gotfredsen, A., Jensen, J., Borg, J. and Christiansen, C. (1986) Measurement of lean mass and total body fat using dual photon absorptiometry. Metabolism 35:88-93

Hamill, P.V.V., Drizd, T.A., Johnson, C.L., Reed, R.B., Roche, A.F. and Moore, W.M. (1979) Physical growth: National Centre for Health Statistics percentiles. Am.J.Clin.Nutr. 32:607-29

Jones, P.J.H., Winthrop, A.L., Schoeller, D.A., Swyer, P.R., Smith, J., Filler, R.M. and Heim, T. (1987) Validation of doubly labeled water for assessing energy expenditure in infants. Pediatr. Res. 21(3):242-6

Jensen, M.D. (1992) Research techniques for body composition assessment. J. Am.Diet.Assoc. 92:454-60

Jensen, C.L., Butte, N.F., Wong, W.W. and Moon, J.K. (1992) Determining energy expenditure in preterm infants: comparison of $H_2{}^{18}O$ and indirect calorimetry. Am. J. Physiology 236:R685-692

Lebenthal, E. and Leung, Y.K. (1988) Feeding the Premature and Compromised Infant: Gastrointestinal Considerations. Pediatric Clinics of North America 35:215-37
Lifson, N. and McClintock, R. (1966) Theory of use of the turnover rates of body water for measuring energy and material balance. J.Theo.Biol. 12:46-74

Linder, M.C. (1991) Nutritional Biochemistry and Metabolism With Clinical Applications. 2nd ed. Elsevier Science Publishing Company Inc., New York, NY.

Lucas, A., King, F. and Bishop, N.B. (1992) Postdischarge formula consumption in infants born preterm. Arch. Dis. Child. 67:691-2

Luchenco, L.O., Hansman, C. and Boyd, E et al. (1966) Intrauterine growth in length and head circumference as estimated from live births at gestational ages from 26 to 42 weeks. Pediatrics 37:403-8

Lukaski, H.C. (1987) Methods for the assessment of human body composition: traditional and new. Am. J. Clin. Nutr. 46:53-56

Mazess, R.B., Peppler, W.W. and Gibbons, M. (1984) Total body composition by dual-photon absorptiometry. Am.J.Clin.Nutr. 40:834-9

Micheli, J.L. and Schutz, Y. (1987) Protein metabolism and postnatal growth in very low birthweight infants. Biol.Neonate 52 suppl:25-40

Miller, D.S. and Payne, P.R. (1965) A ballistic bomb calorimeter. Br.J.Nutr. 13:501

Nelson, S.E., Rogers, R.R., Ziegler, E.E. and Fomon, S.J. (1989) Gain in weight and length during early infancy. Early Hum.Dev. 19:223-9

Nutrition Committee, CPS (1995). Nutrient needs and feeding of premature infants. Can.Med.Assoc.J.152:1765-85

Picaud, J.-C., Rigo, J., Nyamugabo, K., Milet, J. and Senterre, J. (1995) Evaluation of dual-energy X-ray absorptiometry for body-composition assessment in piglets and term human neonates. Am. J. Clin. Nutr. 63:157-63

Pilon & Fox, ed. (1992) Fetal and Neonatal Physiology. W.B.Saunders Company Inc., Philadelphia, PA.

Putet, G. et al. (1987) Energy balance and composition of body weight. Biol.Neonate 52:17-24

Putet, G. (1993) Energy. In Nutritional Needs Of The Preterm Infant, ed. Tsang, R.C. et al., Cadceus Medial Publishers, Inc., Pawling, NY.

Putet, G., Senterre, J., Rigo, J. and Salle, B. (1984) Nutrient balance, energy utilization, and composition of weight gain in very low birthweight infants fed pooled human milk or a preterm formula. J.Pediatr. 105:79-85

Reichman, B., Chessex, P., Verellen, G., Putet, G., Smith, J.M., Heim, T. and Swyer, P.R. (1983) Dietary composition and macronutrient storage in preterm infants. Pediatrics 72:322-328

Roberts, S.B. (1989) Use of the doubly labeled water method for measurement of energy expenditure, total body water, water intake, and metabolizable energy intake in humans and small animals. Can. J. Physiol. Pharmacol. 67:1190-8

Roberts, S.B., Coward, W.A., Schlingenseipen, K.-H., Nohria, V. and Lucas, A. (1986) Comparison of the doubly labeled water method with indirect calorimetry and a nutrient-balance study for simultaneous determination of energy expenditure, water intake, and metabolizable energy intake in preterm infants. Am. J. Clin. Nutr. 44:315-22

Roberts, S.B. and Lucas, A. (1987) Energetic efficiency and nutrient accretion in preterm infants fed extremes of dietary intake. Human Nutr.Clin.Nutr. 41C:105-13

Roberts, S.B. and Young, V.R. (1988) Energy costs of fat and protein deposition in the human infant. Am.J.Clin.Nutr. 48:951-5

Salazar, G., Infante, C. and Vio, F. (1994) Deuterium equilibration time in infant's body water. European.J.Clin.Nutr. 46:475-81

Schoeller, D.A. (1988) Measurement of energy expenditure in free-living humans by using doubly labeled water. J. Nutr. 118:1278-89

Schoeller, D.A., Dietz, W., van Santen, E. and Klein, P.D. (1982) Validatio of saliva sampling for total body water determination by $H_2{}^{18}O$ dilution. Am.J.Clin.Nutr. 35:591-4

Schoeller, D.A., Ravussin, E., Shutz, Y., Acheson, K.J., Baertschi, P. and Jequier, E. (1986) Energy expenditure by doubly labeled water: validation in humans and proposed calculation. Am.J.Physiol. 250:R823-830

Schoeller, D.A. and van Santen, E. (1982) Measurement of energy expenditure in humans by doubly labeled water method. J.Appl.Physiol. 53:955-9

Schoeller, D.A.,Van Santen, E., Peterson, D.W., Dietz, W., Jaspan, J. and Klein, P.D. (1980) Total body water measurement in humans with ^{18}O and ^{2}H labeled water. Am.J.Clin.Nutr. 33:2686-93

Schoeller, D.A. and Taylor, P.B. (1987) Precision of the doubly labelled water method using the two-point calculation. Human Nutrition: Clinical Nutrition 41C:215-23

Schulze, K.F., Stefanski, M., Masterson, J., Spinnaazola, R., Ramakrishnan, R., Dell, R.B. and Heird, W.C. (1987) Energy expenditure, energy balance, and composition of weight gain in low birth weight infants fed diets of different protein and energy content. J. Pediatr. 110(5):753-9

The Scientific Review Committee. (1990) Nutrition Recommendations. Health and Welfare Canada, Ottawa, Canada.

Southgate, D.A.T. and Barrett, I.M. (1966) The intake and excretion of calorific constituents of milk by babies. Br.J.Nutr. 20:363-72

Southgate, D.A.T. and Durnin, J.V.G.A. (1970) Caloric conversion factors: An experimental re-assessment of the factors used in the calculation of the energy value of human diets. Br.J.Nutr. 24:517-35

Suave, R.S. and Geggie, J.H. (1991) Growth and dietary status of preterm and term infants during the first two years of life. Can. J. Public Health 82:95-100

Swyer, P.R. (1987) New perspectives in neonatal nutrition. Biol.Neonate 52 suppl.:4-16
Roberts,S.B. et al. (1988) Energy expenditure and intake in infants born to lean and overweight mothers. N.Engl.J.Med. 318:461-6

Venkataraman, P.S. and Ahluwalia, B.W. (1992) Total bone mineral content and body composition by x-ray densiometry in newborns. Pediatrics 90(5):767-70

Weiler, H.A., Paes, B., Shah, J.K. and Atkinson, S.A. (1997) Longitudinal assessment of growth and bone mineral accretion in prematurely born infants treated for chronic lung disease with dexamethasone. Early Human Development 47:271-86

Welle, S. (1990) Two-point vs. multipoint sample collection for the analysis of energy expenditure by use of the doubly labeled water method. Am. J.Clin.Nutr. 52:1134-8

Westerterp, K.R. et al. (1991) Comparison of short term indirect calorimetry and doubly labeled water method for the assessment of energy expenditure in preterm infants. Biol.Neonate 60:75-82

Wheeler, R.E. and Hall, R.T. (1996) Feeding of premature infant formula after hospital discharge of infants weighing less than 1800 grams at birth. J. Perinatology 16:111-6

Whyte, R.K., Bayley, H.S. and Schwarcz, H.P. (1985) The measurement of whole body water by $H_2{}^{18}O$ dilution in newborn pigs. Am.J.Clin.Nutr. 41:801-9

Whyte R.K., Haslam, R., Vlainic, C., Shannon, S., Samulski, K., Campbell, D., Bayley, H.S. and Sinclair, J.C. (1983) Energy balance and nitrogen balance in growing low birth weight infants fed human milk or formula. Pediatr. Res. 17:891-898

Wilson, D.C. and McClure,G. (1994) Energy requirements in sick preterm babies. Acta.Pediatr.Suppl. 405:60-4

Wolfe, R.R. (1992) Radioactive and Stable Isotope Tracers in Biomedicine. Wiley-Liss Inc., New York, NY.

Wong, W.W., Butte, N.F., Garza, C. and Klein, P.D. (1990) Comparison of energy expenditure estimated in healthy infants using the doubly labelled water and energy balance methods. Eur. J. Clin. Nutr. 44:175-84

Wong, W.W. & Schoeller, D.A. (1990) Mass spectrometric analysis. In the doubly labelled water method for measuring energy expenditure, ed. Prentice, A.M. IAEA, Vienna, Austria.

Ziegler, E.E., Biga, R.L. and Fomon, S.J. (1981) Nutritional Requirements of the Premature Infant in Textbook of Pediatric Nutrition. ed. Suskind, R.M. Raven Press, NY.

APPENDIX 1 Calculation of oxygen-18 enrichment of stock oxygen-18 normalized water

0.1 g aliquots of oxygen-18 stock solutions were diluted with 50 g double distilled water (DDI). Duplicates of the diluted stock solutions and DDI were analyzed using GIRMS. The enrichment of the stock solution were back calculated from the result of GIRMS.

Step 1: Determining the amount of oxygen-18 in 50 g of DDI

Mean corrected $\delta_{DDI} = -8.236$

$$\delta_{DDI} = 1000 \times [(R_{DDI} / R_{std}) - 1]$$

$$R_{DDI} = [(\delta_{DDI} / 1000) + 1] \times R_{std}$$
$$= [(-8.236/1000)+1] \times (0.002005)$$
$$= 2.00334 \times 10^{-3}$$

$$APE_{DDI} = 100\% \times R_{DDI} / (R_{DDI} + 1)$$
$$= 0.1999372\%$$

i.e., in 50 g of DDI, 0.1999372% was oxygen-18. Amount of oxygen-19 in DDI

$$= 50 \text{ g} \times 0.1999372\ \%$$
$$= 0.0999686 \text{ g}$$

Step 2: Determining the amount of oxygen-18 in diluted stock solutions

For Lot # SY-1138, mean corrected $\delta_{SY\,1138} = 94.604$
For Lot # SY-4767, mean corrected $\delta_{SY4767} = 96.990$

For Lot # SY-1138:

$$R_{SY\,1138} = [(\delta_{SY\,1138} / 1000) + 1] \times R_{std}$$
$$= [(94.604/1000)+1] \times (0.002005)$$
$$= 2.19468 \times 10^{-3}$$

$$APE_{SY\,1138} = 0.2189873\ \%$$

i.e., in 50 g of diluted SY-1138, 0.2189873 % was oxygen-18. Amount of oxygen-19 in diluted SY-1138

$$= 50 \text{ g} \times 0.2189873 \%$$
$$= 0.109734$$

Amount of oxygen-18 in diluted solution contributed by 0.1030 g of stock solution SY-1138

$$= 0.109734 - 0.0999686 \text{ g}$$
$$= 9.76545 \times 10^{-3}$$

Therefore the APE of SY-1138

$$= 100\% \times 9.76545 \times 10^{-3} \text{ g } {}^{18}O / 0.1030 \text{ g stock solution}$$
$$= 9.5 \%$$

For Lot # SY-4767:

$$R_{SY\,4767} = [(\delta_{SY\,4767}/1000) + 1] \times R_{std}$$
$$= [(96.990/1000)+1] \times (0.002005)$$
$$= 2.19946 \times 10^{-3}$$

$$APE_{SY\,4767} = 0.2194633 \%$$

i.e., in 50 g of diluted SY-4767, % was oxygen-18. Amount of oxygen-19 in diluted SY-4767

$$= 50 \text{ g} \times 0.2194633 \%$$
$$= 0.1097316$$

Amount of oxygen-18 in diluted solution contributed by 0.1060 g of stock solution SY-4767

$$= 0.1097316 - 0.0999686 \text{ g}$$
$$= 9.76304 \times 10^{-3}$$

Therefore the APE of SY-4767

$$= 100\% \times 9.76304 \times 10^{-3} \text{ g } {}^{18}O / 0.1060 \text{ g stock solution}$$
$$= 9.2 \%$$

APPENDIX 2 Percentage of gross energy intake expended

The percentage of gross energy intake expended increased in 7 of the 9 subjects.

	Percentage of gross energy intake expended	
Subject #	Term CA	3 mo CA
1	26.8	62
2	39.6	27.5
3	28.1	57.2
4	40.3	65.1
5	66.3	71
6	31.4	59
7	33	67.7
8	49.1	73.4
9	70.9	60
mean	42.8	59.1
SD	16.2	13.5

APPENDIX 3

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PARENT INFORMED CONSENT FORM AND EXPLANATION SHEET

Follow-up Nutrition (FUN) in Formula Fed Premature Babies

During early life in hospital, premature babies receive specially designed formulas or fortifiers for breast milk which promote bone and body growth. At time of discharge from hospital or term corrected age whichever is earlier, they are then fed standard formulas designed for normal term babies. Premature babies often take months to years to achieve growth similar to term babies. There is some belief that feeding premature babies special formulas after hospital discharge will help these babies to grow better. We are therefore investigating if feeding premature babies after discharge from hospital until 12 months with a formula containing extra protein, minerals and vitamins will result in better growth and development in the first year of life compared to standard term formula.

We ask your permission to enrol your baby in this study as your baby is formula fed and has reached term corrected age/discharge from hospital. A total of 60 similar babies will participate in this study. Your baby will have an equal chance of receiving either one of the two formulas. Neither we nor you will be aware of which formula your baby will receive. This information will be stored in a secret code on a computer, available to your doctor should there be a need to know for medical reasons.

In this study, we will measure your baby's formula and food intake, energy expenditure, blood level of nutrients, growth (weight, length, head circumference) and quality of growth in the form of bone mineralization, body composition and behavioural development. The attached sheet contains an EXPLANATION OF TESTS.

Some of the tests will be repeated at five follow-up visits at term, 3, 6, 9 and 12 months corrected age in the Growth and Development Clinic of Children's Hospital of Chedoke-McMaster. We will collect a one quarter (1/4) teaspoonful sample of blood by heel prick once in hospital and at term 3, 6, 9 and 12 months follow-up visits. Parking costs to attend these visits will be paid by the research team.

There are no risks associated with any of the procedures apart from minor pain and discomfort associated with blood sampling and the small dose of radiation involved from measurements of bone and body composition, the TOTAL AMOUNT of which does not

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exceed that of one standard baby chest x-ray. Your baby will have the benefit of careful monitoring of nutrition and growth. The formula will be provided free of charge until 12 months corrected age. The knowledge obtained from this study will allow us to make feeding recommendations for premature babies in the first year of life. All the information gathered on your baby for this study will be entirely confidential.

Dr. S. Atkinson (905 521-2100, x 5644) and Dr. B.A. Paes (905 521-6039) are the investigators. Please call them should you have any questions about the study. Your decision whether or not to have your baby participate in this study will not in any way adversely affect your baby's routine care.

CONSENT

I have read and understood the consent form and Explanation sheet entitled, *"Follow-up Nutrition in Formula Fed Premature Babies"* which has been explained to me by _____. I also understand that I am free to withdraw my baby from this study at any time, even after signing this consent form, and this will in no way affect my baby's present or future medical care.

_____	_____
Parent/Guardian	Witness
_____	_____
Signature	Signature
_____	_____
Date	Date

EXPLANATION of TESTS

Follow-up Nutrition in Formula Fed Premature Babies

1) **Nutrient Intake:**

To evaluate your baby's diet we will ask you to record your baby's intake of formula and solids for 5 days before each follow-up visit.

2) **Energy Expenditure**

This will be done twice; once at discharge from hospital and then again at 3 months corrected age. To do this, we will add a few millilitres of a specially prepared water to your baby's formula twice only. This water is labelled with heavy hydrogen and oxygen atoms which normally occur in nature, are NOT radioactive and have no harmful effects. Your baby will have urine samples collected at specific times after receiving the special water over a 5 day period.

3) **Blood Level of Nutrients**

A quarter teaspoonful of blood will be obtained by pricking the heal. A blood sample will be collected five times during the study, once in hospital and then again at 3, 6, 9 and 12 months of age.

4) **Growth**

Your baby will have weight, length and head circumference measured at discharge from hospital and at all follow up visits.

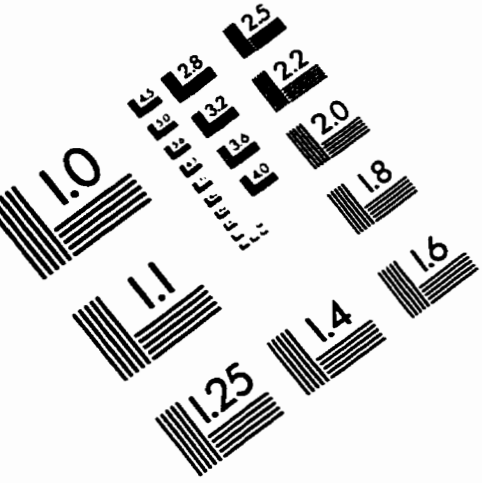
5) **Quality of Growth.**

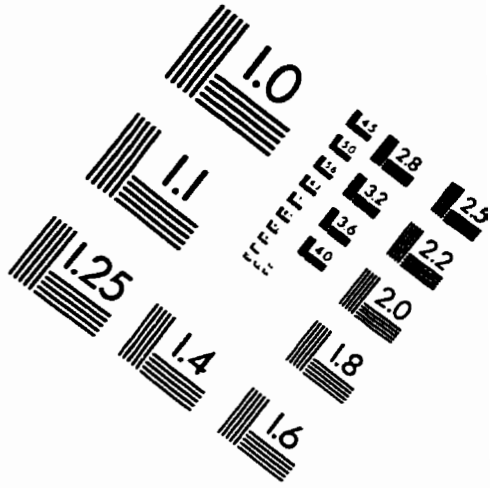
In order to find out about the quality of growth of your baby a simple, non-invasive scanner while your baby lies sleeping for usually less than 15 minutes, will be used to measure bone mineral content and the amount of lean and body fat tissue. This scan involves a small radiation exposure for which the total for ALL measurements over the whole year is les than a routine baby chest x-ray.

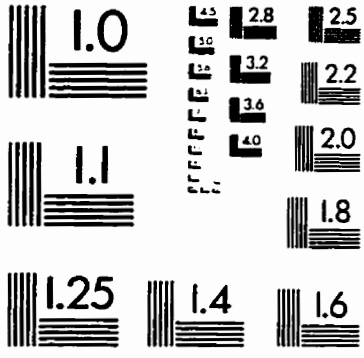
6) **Neurological and Developmental Outcomes**

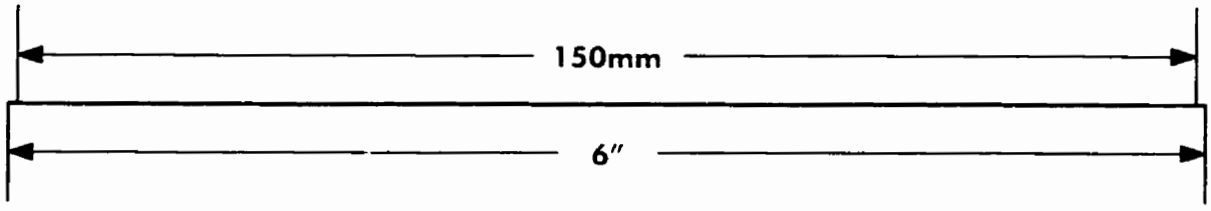
At the Growth and Development clinic visits, all premature babies (weighing les than 1500 g at birth) are routinely assessed for their neurological and developmental progress. Babies weighing more than 1500 g at birth will be assessed by the research team. Your baby will have a detailed assessment of development by administering standardized tests - Bayley Scales of Infant Development and DeGangi test of motor and neurological function at 6 and 12 months of age. At these times we will ask you to complete a questionnaire on your baby's behaviour. (Maternal Ratings of Infant Temperament using Questionnaire of Carey and McDevitt).

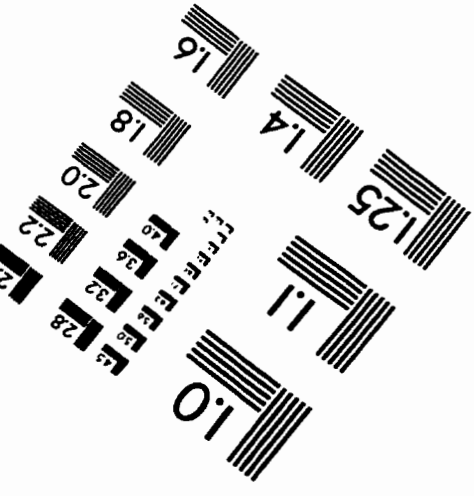
IMAGE EVALUATION
TEST TARGET (QA-3)

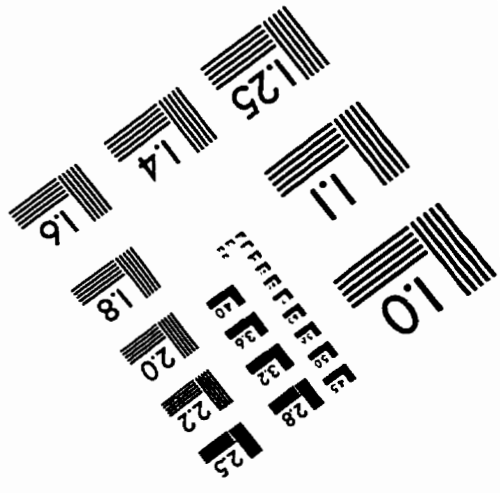












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