STUDIES OF NUTRITION AND GROWTH IN INFANTS WITH CHRONIC CARDIOPULMONARY DISEASE

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For Val, Natasha and Jessica
and for my parents

Abstract

Hypotheses

(1) Nutritional status is impaired in symptomatic congenital heart disease (CHD) in infancy, and this is related to an inadequate positive energy balance. (2) Undernutrition precedes the development of bronchopulmonary dysplasia (BPD) in preterm infants, and there is a subsequent persistent deficit in energy balance, bone mineral content (BMC) and growth.

Objectives

To study (1) the effect of CHD on growth and energy balance in infancy (2) macronutrient intake prior to the development of BPD (3) the effect of BPD on energy balance and BMC and the effect of dexamethasone used to treat BPD on BMC.

Background

Poor growth is seen commonly in chronic disease of the heart and lungs (CCPD) and is important because: (a) the disease and its treatment may compromise nutrition (b) good nutrition may influence the outcome of the condition and adult health. Body growth in infants is dependent upon a sufficiently positive balance of protein and energy, and certain micronutrients are important for aspects of specific organ development. There are few previous studies looking at specific aspects of nutrition in young infants with CCPD.

Methods

CHD Energy balance measurements were carried out on 21 infants with CHD, post-term age [median (range)] 49 days (-9 to 246) and in 9 controls, post-term age 35 days (-14 to 86). Energy intake (EI) and losses (EL) were measured by bomb calorimetry (18 CHD, 5 control), resting energy expenditure (REE) by indirect calorimetry over several hours (14

CHD, 9 control), and anthropometry performed. Metabolizable energy intake (MEI) was calculated as EI-EL, and energy available for deposition (EAD) as MEI-REE.

BPD 195 consecutive infants of <32 weeks gestation had weekly anthropometry and records of achieved nutritional intake. 54 of these had dual energy X-ray absorptiometry of the forearm for bone mineral content (BMC). Case control studies were done on nested cohorts within this group: (1) macronutrient intake and growth in 20 babies with BPD and 20 gestation and birthweight matched controls, (2) BMC in 10 babies with BPD and 10 gestation and birthweight matched controls, (3) BMC in 15 BPD babies treated with dexamethasone and 15 untreated BPD controls. In a separate convenience sample of 4 infants with BPD and 4 preterm controls EI and EL were determined by bomb calorimetry.

Results

CHD There was a fall with age in z-score for body size: for weight 1SD in 21 days, length 1SD in 43 days and head circumference 1SD in 37 days. Weight gain over 5 days [median (quartiles)] was less in cardiac infants [11.0g/d (2.5-16.7)] than in controls [39.0g/d (20.0-47.5)], P=0.0034. There was no statistically significant difference in EI, EL, or MEI between the groups. Sleeping oxygen uptake [SV_{O2}, median (range)] was similar in CHD infants [9.93ml/kg/min (7.7-13.88)] and controls [9.23ml/kg/min (7.5-11.66)], with the highest values in 3 of 4 infants with persistent cardiac failure and pulmonary hypertension. Respiratory quotients were similar. SV_{O2}/kg correlated inversely with log [summed skinfold thicknesses] (r^2=0.671; P=0.0001) and with body mass index. There was a positive correlation in CHD infants between weight gain and MEI %RDA (r^2 =0.28, P=0.024), and energy available for deposition (r^2 =0.43, P=0.05).

BPD BPD infants had lower early macronutrient intake than controls [median (quartiles)]: for first week EI, BPD = 262 kJ/kg/d (210-282), control = 347 kJ/kg/d (293-

372), P=0.003. BPD infants had a lower proportion of EI as enteral feeds than controls in the first 2 weeks [median (quartiles)]: BPD = 68% (31.3-79.2), control = 91% (78.0-93.0), P=0.0025. The rate of weight gain [slope (95% CI)] was less in babies with BPD [151.5 g/week (135.5-167.6)] than in controls [192.2 g/week (178.5-205.9)], P<0.05. There was no demonstrable difference in z-score values for weight, length, head circumference or body mass index at discharge. In the small sample studied, there was no difference in EI, EL, or MEI. For MEI, BPD = 592 kJ/kg/d (425-741), control = 565 kJ/kg/d (527-737). For all babies measured (n=54), BMC at birth [median (quartiles)] was 1.79mg/mm (1.57-2.03), with a fall in the first 5 weeks by 0.23mg/mm (0.09-0.41), followed by a rise, with a value at 10 weeks of 1.99mg/mm (1.69-2.16). There was no detectable difference between the BPD and control groups. Dexamethasone had no effect on the postnatal trend in BMC, but was associated with slower forearm growth: length increase in 5 weeks [median (quartiles)] for steroid group =7.54mm (0.6-7.6), for controls =11.6 (5.4-24.5), P=0.019.

Conclusions

CHD There appears to be a progressive postnatal deterioration of nutritional status in hospitalized infants with CHD. This is associated with a tendency to lower energy intake, and in addition raised REE in some infants. Availability of energy appears to be a limiting factor for growth in this group.

BPD There is a shortfall of nutrient intake in the first two weeks, particularly via the enteral route, in preterm infants who later develop bronchopulmonary dysplasia. The subsequent rate of weight gain is slower for several weeks in these babies. There appears to be no abnormality of energy intake or losses. There is a large deficit in BMC in preterm infants at term, with no additional effect of BPD. Systemic steroid treatment slows linear growth, without any apparent effect on bone mineralization.

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Contents

Chapter 1 Introduction

1.1 NUTRITION AND GROWTH IN CHRONIC DISEASE

Many chronic diseases are associated with physiological changes which result in impaired nutritional status. In some of these diseases, undernutrition may adversely affect the progression of the condition. In chronic cardiopulmonary disease (CCPD), gross protein-energy insufficiency might impair respiratory muscle strength and function, and a shortfall of certain micronutrients might adversely affect myocardial and pulmonary function (Anker and Sharma 2002, Schols 2002). There are additional potential costs of undernutrition in early life. Poor nutrition in the first few months of life may compromise brain growth and maturation (Dobbing 1981). Poor body growth is a common feature of chronic disease in childhood, and its degree often relates to the severity of the underlying condition. Small size may affect the older child's psychological adjustment, and delay the onset of puberty (Ehlers 1978, Linde et al 1967). In addition, there is accumulating evidence that early nutrition influences the development of adult disease (Lucas 1990). This is of growing relevance, since with improvements in care the life expectancy of sufferers from chronic disease is increasing.

There has been much controversy about the mechanisms causing impaired nutrition in various chronic conditions. Is inadequacy of nutrient intake or retention the primary cause, or are metabolic alterations to blame? Into the latter category fall endocrine changes resulting in increased basal metabolism, and increased work of the heart and breathing. If there are metabolic changes, are these related to a basic cellular abnormality present at birth, or are they the result of correctable anatomical or physiological abnormalities related to the underlying condition? The syndrome of cachexia in chronic disease, which has been recognised for some time, is now seen to be

the result of a complex combination of endocrine, inflammatory and other influences in addition to poor intake (Conraads et al 2002).

There are many theoretical reasons why undernutrition may exacerbate some disease processes, and there is reason to believe that in some conditions improving nutritional intake can reduce disease severity (for example, Borowitz 1996).

Key to the study of nutrition in chronic disease is the assessment of nutritional status. Nutritional status can be studied in one of three main ways: (a) measurement of body composition (by anthropometry as well as by more sophisticated techniques) –this provides a static assessment of nutritional adequacy which can be compared to measurements in healthy children, although the mechanisms of undernutrition cannot be elucidated in this way (b) assessment of nutrient balance –measuring the intake and output of energy and protein, for example (c) measurement of a bodily function which is dependent upon the adequacy of one or more nutrients.

Two groups of chronic conditions will be considered here (a) congenital heart disease (b) bronchopulmonary dysplasia, or chronic lung disease in preterm infants.

1.2 NUTRITION AND GROWTH IN CONGENITAL HEART DISEASE

1.2.1 Growth in congenital heart disease

It has been recognized for some time that children with congenital heart disease (CHD) are frequently small for their age. Mehrizi and Drash (1962) demonstrated the extent of the problem in a large study, which showed that nearly one third of such children of all ages were below the third centile for weight and height. Evidence of undernutrition is present in a high proportion of children hospitalized with congenital heart disease (Cameron et al 1995).

Corrective surgery is the ultimate aim, and may be the best ultimate solution to poor growth in CHD. Advances in surgical technique have made complicated surgery possible in younger infants. However, surgery may have to be delayed, and may be more hazardous in undernourished or ill children (Haydock and Hill 1986).

The evidence from the literature suggests that a large number of factors, prenatal and postnatal, may contribute to growth retardation in CHD.

(i) Prenatal growth

The birthweight of infants with CHD is not, as a whole, significantly different from that of normal infants (Mehrizi and Drash 1962, Feldt et al 1967, Suoninen 1971). Levy et al (1978), however, found an increased incidence of light-for-dates babies in "severe" CHD.

Suoninen (1971) found a higher incidence of prematurity in CHD, mainly in infants with patent ductus arteriosus. In this condition, it is obviously possible that prematurity was, in some cases, contributing to the continuing patency of the ductus.

A subgroup of infants with CHD is born small for gestational age with malformation syndromes, including those resulting from genetic abnormalities and from intrauterine infection (Jackson 1968). In these, growth impairment is likely to be due to a primary defect in morphogenesis affecting many tissues of the body, and is less likely to benefit from therapeutic measures (Shelton 1980).

A recent large epedemiological study suggested that there were differences in body proportions at birth in certain types of congenital heart disease unassociated with extracardiac abnormalities, and that the pattern varied with the type of cardiac malformation (Rosenthal 1996).

(ii) Postnatal growth

Many studies have documented the postnatal growth of children with CHD. They vary enormously in the size of the population studied, criteria for selection of cases, the grouping of children in disease categories, and the definition of growth impairment.

Some of the earliest published studies, by Adams et al (1951 and 1954) showed large variations in the growth of children with CHD, the most consistently small being those with left to right shunts. They also demonstrated some postoperative improvement in the growth of children who had a patent ductus ligated, soon after this operation was first introduced.

A summary of some of the findings of other studies is as follows:

Weight is usually affected more than height (Linde et al 1967, Miller et al 1969, Suoninen 1971). Boys are affected more severely than girls in most lesions (Linde et al 1967, Suoninen 1971). There is often acceleration in growth, particularly in weight, after surgery (Umansky and Hauck 1962, Feldt et al 1969, Suoninen 1971), although this is not always the case (Maxwell 1966). Body size is usually closer to normal at adolescence, whether there has been surgical intervention or not (Linde et al 1967).

Clinical parameters of growth other than weight and height have not been well documented in these children.

(iii) Causes of poor growth

Many hypotheses have been put forward to explain postnatal growth retardation in CHD. Approaches to investigation have been equally diverse, and clear conclusions cannot be drawn from the literature.

The main hypotheses fall into the following categories, which are obviously not mutually exclusive: (1) familial or hereditary factors (2) haemodynamic abnormalities

(3) cellular abnormality (4) endocrine changes (5) recurrent infection (6) undernutrition (7) hypermetabolism.

(1) Familial or hereditary factors The siblings of undersize children with CHD show a normal distribution of weight and height (Maxwell et al 1966, Linde et al 1967). Thus familial small size is probably not important. Jackson (1968) suggested that recognizable chromosomal anomalies were associated with 3 to 5% of cases of CHD; also that recessive or polygenic inheritance may be important. Small size may, of course, be part of such a genetic syndrome.

(2) Haemodynamic abnormalities These may be expected to cause poor growth for several reasons: (a) poor peripheral systemic blood flow in children with heart failure, and tissue hypoxia and acidosis resulting in poor peripheral utilization of nutrients (b) impaired food intake due to respiratory embarrassment and anorexia (c) malabsorption due to venous congestion and/or hypoxia of the bowel (d) increased work of the heart and breathing, leaving less energy for growth.

Few clear-cut correlations have been found between the presence or severity of haemodynamic abnormality and the degree of growth retardation. The most seriously retarded group in most studies is that with cyanotic conditions (Mehrizi and Drash 1962, Linde et al 1967, Suoninen 1971). However, no relationship seems to exist between the degree of arterial hypoxaemia and the degree of growth retardation (Feldt et al 1969, Linde et al 1967, Suoninen 1971). Of the acyanotic group, children with VSD causing heart failure in infancy were severely affected (Feldt et al 1969). Miller et al (1969) found no good correlation between any haemodynamic values and growth failure in VSD, although there was some association between pulmonary hypertension and poor weight gain.

(3) Cellular factors Study of muscle specimens in children with CHD has shown a reduction in cell number in relation to age, and probably in relation to height (Cheek 1968). In post-mortem studies (Naeye 1967), those who died in the perinatal period had cellular changes suggestive of mild intrauterine growth retardation; those who died aged 1 month to 8 years showed changes suggestive of chronic undernutrition; those who died later had abnormalities related to chronic hypoxia. Adipose tissue cellularity is reduced in children with cyanotic CHD (Baum and Stern 1977), suggesting that hypoxaemia affects adipocyte multiplication in infancy.

(4) Endocrine changes Hait et al (1972) demonstrated abnormal oral glucose tolerance with high blood sugar values and low insulin response to hyperglycaemia in infants with congestive cardiac failure. They suggested that this may be due to high circulating catecholamine levels suppressing insulin, or secondary to protein-calorie malnutrition, which is known to cause abnormal carbohydrate metabolism, with impaired insulin response to glucose (Becker 1983).

Another study (Gacs et al 1971) suggested low blood sugar levels in cyanotic children, possibly related to decreased release from glycogen in the liver. The same authors showed normal fasting levels of adrenal corticosteroids and growth hormone response to insulin stress in cyanotic children.

(5) Infections It has been suggested that frequent infections, especially of the respiratory tract, contribute to growth retardation in CHD. Suoninen (1971) found a positive correlation in coarctation of the aorta only. Maxwell et al (1966) did not find any link.

(6) Undernutrition and (7) Hypermetabolism Undernutrition may be one common pathway by which many of the abnormalities described above result in growth

retardation. As stated previously, post-mortem changes reminiscent of chronic undernutrition are found in many instances.

There are several reasons, in theory, why growth retardation in CHD may be associated with undernutrition. In general terms, normal postnatal growth requires an adequate supply of structural elements for tissue synthesis (e.g. proteins), of energy (the energy cost of growth), and certain micronutrients required as cofactors for normal metabolism.

An inadequate supply of any nutrient may be due to (a) poor intake (poor feeding, vomiting) (b) poor intestinal absorption (c) poor peripheral utilization (due to haemodynamic disturbances) or (d) increased utilization resulting in increased demand.

Little work has been done on nitrogen balance in CHD. Iber et al (1967) found somewhat increased stool albumin losses, but a markedly positive nitrogen balance in the face of poor growth in a small number of infants. They suggested increased insensible loss of nitrogen, possibly as free nitrogen gas in expired air. This finding may merely reflect the tendency inherent in balance studies to underestimate nutrient losses and thus overestimate nutrient retention (Hegsted 1976).

There may be inadequate energy substrate to supply the energy cost of growth, which is a combination of the energy stored in the components of new tissue and the energy used in the anabolic processes of tissue synthesis (Widdowson 1961). It is one component of the energy balance of an individual, which is the main subject of the present study.

1.2.2 The Principles of Energy Balance

The concept of "energy balance" is a specific application of the Law of Conservation of Energy. There is every reason to believe that it is as applicable to a

biological system as to a physical one. Thus, there is no net energy gain or loss from a closed system, but there may be conversion of energy from one form to another.

An energy balance equation can be written for an individual as:

Energy ingested (gross or thermochemical energy) = **Total energy expenditure (TEE)** (metabolism incl. heat loss) **+ Energy losses (in stools, urine and vomitus)** **+ Energy stored in new tissue**

Gross or thermochemical energy is the energy released by complete oxidation of a food substance, and is usually measured using a bomb calorimeter.

(i) Total energy expenditure

In a thermoneutral environment (when energy does not need to be expended for thermoregulation),

Total energy expenditure (TEE) = **Resting energy expenditure (REE)** (including energy used for tissue synthesis) **+ Diet-induced thermogenesis + Energy used in activity**

In adults, "resting energy expenditure" becomes "basal energy expenditure" after a period of fasting (usually 12 hours), when dietary thermogenesis is no longer significant. This cannot ethically be measured in young children.

(ii) Energy cost of growth and metabolizable energy intake

The energy cost of growth comprises the chemical energy stored in new tissue together with the portion of total energy expenditure used to fuel growth.

Energy cost of growth = Energy stored in new tissue + Energy used for tissue synthesis

The energy cost of growth has to come from that part of ingested energy available to the body allowing for losses in vomitus, stool, and urine, that is metabolizable energy.

Metabolizable energy = Ingested energy – losses in vomitus, stool and urine
(MEI) (EI) (EL)

The energy available for deposition will, in theory, be reduced if (a) ingested energy is reduced (b) energy losses are increased in stools, or by vomiting or in the urine or (c) energy expenditure is increased.

(iii) Special considerations in childhood

Evidence, mainly from cross-sectional data, suggests that energy expenditure expressed per kg body weight increases during the first 2 years of life (Schofield 1985). During this time the contribution of physical activity increases. Overall, values then fall towards adulthood, an important cause of this change being a fall in the relative contribution to body weight by metabolically active organs (particularly brain, liver, kidneys and heart which together form 19.5% of body weight in the newborn and 4.9% in the adult, Holliday 1971). In infancy, rapid growth and the different quality and frequency of feeding probably also contribute to higher energy expenditure.

1.2.3 Methods available for investigating energy balance

The experimental study of energy balance involves the accurate determination of ingested energy, and of losses in stool, urine, and vomitus, and the measurement of energy expenditure. The energy available for deposition has to be derived from these.

(i) Energy Intake and Losses

Measurements of intake and losses is, in many ways, easier to perform in young infants, since their diet consists of milk alone, and their limited mobility enables the easier collection of excretory products and measurement of metabolic rate.

Metabolic cradles, which restrain infants and thus make the complete collection and separation of excretory products possible, are non-physiological and cannot, for obvious reasons, be used for prolonged periods.

Several careful studies have been done in neonates, including those of low birth weight, with little disturbance to their normal activities (Brooke et al 1979, Reichman et al 1982). Little work has been done in older infants or in children with CHD.

Over several days, milk intake is measured, and accurate timed collections made of vomitus and excreta. The energy content of food and excreta is derived from either (a) chemical analysis of their carbohydrate, fat and protein content, and subsequent calculation of energy values using energy conversion factors (e.g. Atwater factors) derived from measurements of metabolizable energy, or (b) measurement of their heat of combustion in a bomb calorimeter, giving a value for gross or thermochemical energy (Miller and Payne 1959). The thermochemical energy value of a food is higher than the calculated metabolizable energy, which takes into account incomplete absorption and incomplete oxidation of protein.

In nutrient balance studies there is a bias towards overestimation of intake because of failure to take account of all spillages and regurgitations, and towards underestimation of losses because (a) collection of stools, urine and vomitus may be incomplete (b) losses in shed skin and in sweat are usually ignored. This leads to an overall bias towards overestimation of metabolizable energy intake (Hegsted 1976).

(ii) Energy expenditure

Energy expenditure (EE) has, traditionally, been measured by direct or indirect calorimetric methods.

(1) Direct and indirect calorimetry Direct calorimetry involves the measurement of the heat produced as a by-product of metabolism, in a chamber made of materials of

accurately known specific heats. The equipment needed is costly and complicated to use, and this type of method is little used in clinical research.

Indirect calorimetry involves the measurement of some variable, other than heat production, directly related to energy expenditure. Examples include heart rate (Spady et al 1976), evaporative water loss (Puyau 1969, Kennaird 1976), and respiratory gas exchange. Stable isotope techniques developed more recently, although indirectly measuring gas exchange, are usually considered separately.

(2) Respiratory gas exchange This method of measuring energy expenditure was predicted by Lavoisier, who recognized that an animal's oxygen consumption (V_{O2}) and carbon dioxide output (V_{CO2}) depended upon activity, food and temperature. It is based upon the assumption that the metabolic processes contributing to energy expenditure use oxygen and produce carbon dioxide in a completely aerobic and predictable way.

In theory, gas exchange can be measured very accurately by analysis of inspired and expired gases. Energy expenditure is then calculated as follows (Davson and Eggleton 1968):

(a) Measure oxygen consumption, carbon dioxide output and urinary nitrogen excretion (as a measure of protein metabolism).

(b) Calculate the respiratory quotient (RQ, carbon dioxide output/oxygen uptake), which is dependent upon the mixture of substrates utilized. The use of carbohydrate alone (most commonly glucose, derived from other carbohydrates or by gluconeogenesis from fats or amino acids) results in an RQ of 1.00; fat metabolism gives an RQ of near 0.7; and protein around 0.8.

(c) Derive non-protein V_{O2}, V_{CO2} and non-protein RQ from total V_{O2}, V_{CO2}, RQ and urinary nitrogen, taking into account the fact that protein is incompletely oxidised

(published tables) - this provides an indication of the proportion of fat and carbohydrate metabolised.

(d) Calculate energy expenditure from tables of energy value per litre oxygen at different levels of non-protein RQ (Zuntz and Schumberg, mod. by Lusk, mod. by McClendon, in Harper et al 1979). This value ranges from 19-21 kJ per litre of oxygen consumed in the range of RQ 0.7-1.0.

Several studies have measured oxygen uptake alone and used an assumed RQ. This would lead to a maximum error of about ±3% within the physiological range of RQ.

If urinary nitrogen excretion is not measured, the maximum associated error is 3.2% (see Appendix).

In practice, a large variety of techniques has been developed for the measurement of respiratory gas exchange. There has been little standardization of techniques, even within the paediatric age group, and with some techniques little validation.

(3) Open and closed circuit methods There are two main types of method for measuring gas exchange directly: (a) open circuit, (b) closed circuit. In a closed circuit system, the subject is contained in a chamber which forms part of a closed system through which air recirculates. Carbon dioxide is absorbed out and measured; oxygen uptake is measured either as the decrease in the volume of the system, or the amount of oxygen which has to be added to keep the volume of the system constant. Such a system needs to be strictly airtight, and gas concentrations and temperatures precisely controlled; volume changes need to be measured accurately. This makes the method complicated and expensive. Access to the subject is not possible, and studies can therefore only be performed over short periods.

An open circuit method was first described by Pettenkofer and Voit (1862). This type of method is much more versatile, and always involves the subject breathing in a

moving, continuously replenished stream of air. With the subject inside a chamber, Pettenkofer described the carbon dioxide output of an animal as:

CO_2 output = airflow x CO_2 concentration difference in entry and exit gases.

To be entirely accurate, oxygen and carbon dioxide concentrations in, and flow rates of incoming and outgoing air streams need to be measured. Then,

CO_2 output = (Flow out x CO_2 conc. of air out) - (Flow in x CO_2 conc. of air in)

In practice this can be simplified by assuming that there is no difference between the two flow rates (for errors involved see Appendix):

CO_2 output = Flow out x insp.-exp. conc. difference of CO_2.

Identical equations, substituting **O_2** for **CO_2**, can be used for oxygen uptake.

The earliest open circuit measurements of gas exchange involved the accurate collection and later chemical analysis of expired gases. This made the equipment unwieldy and complicated to use. The availability, more recently, of instruments capable of automatic, continuous gas analysis, has made open circuit systems more flexible.

Open circuit systems have in common a pump to draw air through the circuit, a flow meter, and an oxygen analyser (with or without a carbon dioxide analyser). They differ mainly in the way in which they interface with the subject. This varies from a funnel or facemask held over the child's face, to a headbox, or a whole body chamber in which the child is enclosed.

Masks and headboxes provide a small dead-space. This means that large flow rates need to be used to prevent re-breathing of carbon dioxide, and sensitive gas analysers are needed to detect changes in concentration of the very diluted gases. Also, although theoretically, breath-by-breath changes in gas exchange can be recorded with little damping, this is not necessary for most purposes, and a more averaged recording showing major changes is more useful. Workers who have used such a method have

usually added some mechanical or electronic damping device, for this reason. Masks and headboxes restrain infants, and may by their presence alter the child's breathing pattern. They are therefore only suitable for short-term measurements of sleeping gas exchange, and for use with sedated children, for example during cardiac catheterization. Whole body chambers provide some freedom of movement for the infant, and because of the dead space, some damping of changes in expired gas concentration.

(4) The influence of thermal environment The thermal environment (a concept which includes ambient temperature, its gradient with skin temperature, air humidity, clothing and radiant and convective stresses) has a significant influence on energy expenditure in infancy. The neutral thermal environment is one in which metabolic rate and evaporative heat loss are at a minimum. Cold stress increases metabolic rate by invoking non-shivering thermogenesis (mainly in brown fat) and shivering (Bruck and Wunnenberg 1966). It also causes vasoconstriction, which results in skin cooling in the acral areas. Overheating may affect metabolic rate by eliciting behavioural responses (Harpin et al 1983). It has been suggested that the optimal thermal environment may be different in babies with congenital heart disease (Kennaird 1976).

(5) Resting and Total Energy Expenditure Energy expenditure is usually measured with a view to calculating the energy requirements of an individual or group. In order to do this REE alone cannot be used and TEE needs to be estimated. Thus, either a correction has to be made for activity and thermogenesis, or TEE itself needs to be measured. In some conditions associated with an increased REE, there appears to be a balancing reduction in energy expended in activity (Macallan et al 1995), thus leading to incorrect extrapolation from REE to energy requirements.

Many indirect calorimetric studies have used measurements of gas exchange of short duration during sleep, to derive resting metabolic rate. Stothers and Warner (1978)

found that in the neonate, oxygen consumption varied in different sleep states. In nutritional studies, measurements of longer duration with the subject in different states of wakefulness enable total energy expenditure to be derived more precisely. Several such prolonged studies have been performed in low-birthweight neonates, (e.g. Chessex et al 1981, Gudinchet et al 1982, Abdulrazzak et al 1983), but few in larger infants or in CHD. Methods used to calculate gas exchange and derive energy expenditure values from these involve certain assumptions and vary enormously in complexity in the literature.

Since the early 1990s stable isotope methodology, which allows measurement of TEE in free-living subjects, has taken increasing prominence in energy balance research.

(6) The doubly labelled water method In the most widely used stable isotope method, doubly labelled water (water enriched with the naturally occurring stable isotopes deuterium or ^{2}H, and ^{18}O) is ingested by the subject. CO_2 production is then derived from the difference in rate of disappearance of the two isotopes from the body water pool (^{2}H being incorporated into water, and ^{18}O into water and CO_2). This technique can be used in free-living subjects without interfering with normal activity and environment, and is used to derive total energy expenditure, assuming the respiratory quotient.

A body of work from the Dunn Nutrition Unit in Cambridge, UK, has added considerably to the literature on energy expenditure in infancy and has challenged traditional thinking on energy balance. These studies used the doubly labelled water technique to estimate total energy expenditure and body composition, in addition to traditional indirect calorimetry and anthropometry.

This work has suggested significantly lower energy requirements for infants and children than had been recommended in the past (Davies et al. 1995, Davies et al. 1997). Furthermore, in healthy subjects there appeared to be no relationship between fatness in

childhood and (a) total energy expenditure or behaviour in infancy (Wells, Cole et al. 1996; Wells, Stanley, et al. 1996; Davies et al 1991) or (b) milk volume or energy intake in infancy (Wells, Stanley et al. 1998).

Using stable isotope techniques to estimate total energy expenditure and fat free mass simultaneously, Davies et al showed that fat free mass is the best corrector for body size for the purposes of expressing energy expenditure in young infants. Even after allowing for this, small for gestation infants appeared to have higher energy expenditures for a given fat free mass (about 20% more) than appropriately grown infants (Davies et al. 1996). Body weight$^{0.5}$ appeared to provide a reasonable estimate of metabolic body size in healthy infants (Wells & Davies 1995).

There were differences in sleeping metabolic rate measured by indirect calorimetry between breast and formula fed babies, which appeared explicable by differences in FFM, but in addition a gender difference which appeared independent of FFM. With age and growth there is a change in the metabolic activity per gram of fat free mass, because of an increase in the contribution of muscle (metabolically relatively inactive) compared to other organs (relatively active). In addition, there is a reverse in the relative contributions of physical activity (increasing) and growth (decreasing) to energy expenditure with age in the first year (Wells & Davies 1998).

Assumptions are made during stable isotope studies about isotope dilution spaces. These change with age in healthy children (Wells, Ritz, et al. 1998), mainly as a result of a reduction in percentage of body water, and are likely to be affected by various disease states. Thus equations used to derive energy expenditure using assumptions based on healthy children will need to be modified when studying children with cardiac failure in whom there are basic changes in body fluid compartments which may alter further with drug therapy (Mitchell et al. 1994).

1.2.4 Energy balance in chronic disease

Insights into the interaction between chronic disease and nutritional status can be gained from the body of recent literature on the nutritional consequences of cystic fibrosis and human immunodeficiency virus disease. This literature also illustrates the difficulties of performing and interpreting studies of energy metabolism in chronic disease.

Cystic fibrosis is associated with a progressive deterioration in growth and nutritional status throughout childhood. Malnutrition is an adverse prognostic factor, and avoiding it may improve outcome (Levy et al 1985). A negative energy balance is central to malnutrition in cystic fibrosis, as in many chronic diseases, although there is debate about the role of different components of energy balance (Reilly et al 1997). The inflammatory process may play a role in the pathogenesis of malnutrition, particularly through the influence of cytokines, which are associated with anorexia and hypermetabolism (Bell et al 2000).

Decreased EI, increased EE, impaired digestion and absorption, and excessive losses might all contribute to a negative energy balance in cystic fibrosis (Reilly et al 1997). Short periods of very low intake, particularly with intercurrent infection, as well as sustained subnormal intake probably play a large part in malnutrition in this condition. Fat digestion is suboptimal, even with good pancreatic supplementation. Undigested carbohydrates, normally reabsorbed after fermentation by colonic bacteria, appear unchanged in the stool. Many studies have shown increased REE, but this does not necessarily equate to increased TEE. This may be due to a "compensatory" decrease in activity. The contribution of a genetic component to hypermetabolism is unclear despite a large number of studies on this subject. There is a close inverse relationship between REE and lung function; REE is not raised in those with good lung function and

nutritional status. The link between lung function and REE may be related to (1) the cost of tissue synthesis related to protein turnover (2) the energy cost of breathing (3) beta-agonist treatment increasing REE (4) mild chronic inflammation or infection – this may be mediated by cytokines (anti-inflammatory drugs have been shown to promote weight gain). Hypermetabolism during acute pulmonary exacerbations may cause a cumulatively negative energy balance. The increased REE appears to be balanced by a reduction in physical activity, except in moderate to severe lung disease, when the compensation may not be complete. Interestingly, the relationship between TEE and weight loss in HIV infected subjects is the opposite of that expected -wasted patients have a low TEE (Macallan et al 1995). One possible explanation is that the energy cost of particular activities may be altered as part of the disease process.

1.2.5 Energy balance in congenital heart disease

(i) General issues

In heart disease, there are several theoretical reasons why energy balance may be affected (1) intake may be reduced because of anorexia, reduced respiratory reserve, and a feeling of fullness because of slow gastric emptying (2) losses may be increased because of (i) malabsorption because of hypoxia or venous congestion of the bowel or (ii) increased vomiting (3) expenditure may be increased. Pittman and Cohen (1964) stated that a diseased adult heart may use up to 30% of the body's oxygen uptake for its metabolism, as opposed to 10% in a normal heart. It should be remembered that in the infant the partitioning of energy expenditure is very different to that in the adult, the organs contributing a greater proportion of energy expenditure, thus making any increase potentially more significant. It has been suggested that individuals with heart failure have an increased sympathetic drive (Chidsey and Braunwald 1965, Lees et al 1965). The

sympathetic nervous system stimulates brown fat metabolism which, some believe, plays a key role in controlling metabolic rate (Rothwell and Stock 1981).

(ii) The literature

The published evidence about energy balance in CHD is conflicting, but is summarized below.

In infants, a subnormal ingested energy in relation to weight has been demonstrated (Kreiger 1970, Huse et al 1975). Another study showed adequate energy and nutrient intake in older children (Strangeways et al 1978).

Forced feeding (Kreiger 1970) or continuous or overnight tube feeding (Vanderhoof et al 1982, Schwarz et al 1990) improved anthropometric measures.

Cavell (1981a and b) performed studies of gastric emptying using a tracer in the feed and measuring its disappearance from gastric residuals. They showed that gastric emptying was slower in infants with heart disease, and suggested that this might cause a feeling of fullness, leading to reduced food intake. Adding a glucose polymer to increase energy density, despite further decreasing gastric emptying, increased the throughput of calories into the small intestine (measured as disappearance from the stomach). However, energy retention was not measured, and in fact one infant developed diarrhoea presumably because of an osmotic effect.

Intestinal absorption has not been well studied in CHD. Sondheimer and Hamilton (1978) found excessive intestinal protein loss in some children, though not to the extent seen in conditions associated with malabsorption such as coeliac disease. No other consistent digestive tract abnormality was found, in particular there were no abnormalities on intestinal villous biopsy. Vaisman et al (1994) found no overall increase in stool fat or energy losses in infants with congenital heart disease treated with diuretics, but found an association between losses and total body water in these babies.

Energy expenditure in CHD has been assessed mainly by measuring respiratory gas exchange. This has usually been done over short periods with the child asleep or sedated. As well as helping in the study of energy balance, it is useful to the cardiologist for the calculation of cardiac output and systemic and pulmonary blood flow, from variables measured at cardiac catheterization.

Some workers have found increased resting metabolic rate in relation to weight in CHD (Lees et al 1965, Stocker et al 1972, Kraus and Auld 1975). Others found it was normal (Cheek 1968, Huse et al 1975). The infants with higher metabolic rates were often the most undernourished in these studies. However, expressing EE per unit body weight rather than per unit fat free mass (a more recent practice) will tend to give higher levels in thin babies because of their greater proportion of metabolically active lean tissue. In one study (Kennaird 1976), acyanotic conditions were associated with an abnormally high metabolic rate, and cyanotic conditions with a subnormal rate. In the latter, metabolic rate was increased when surgery removed the cyanosis. The author suggested that the thermoneutral range of temperature resulting in minimal oxygen consumption was different from normal in cyanotic babies (higher) and acyanotic babies in heart failure (lower).

A study using the doubly labelled water method showed a significant increase in total daily energy expenditure with lower than expected intake in infants with CHD (Barton et al 1994). In another study children between the ages of 4 and 33 months were studied before and after cardiac surgery (Mitchell et al 1994). In nearly 30% of cases there was a significant elevation of energy expenditure, which fell to within the normal range immediately postoperatively.

(iii) Difficulties with interpretation of the evidence

The literature on energy balance in childhood and in CHD needs cautious interpretation for the following reasons:

(a) The populations studied are of widely different age ranges and cardiological diagnoses. It is important to take the age of subjects into consideration, independently of body size, because the apportionment of ingested energy to the different components of energy balance changes markedly with age. This is particularly true of the proportion of ingested energy used for growth; it decreases from about ⅓ of ingested energy up to the age of 2 months, to less than 5% after 5 months (based on normative growth data and assuming a constant energy cost of growth).

(b) Until recently, there has been a paucity of normative data on energy balance, and it has been difficult to obtain control subjects for such studies. The normal data available relates mainly to neonates and young infants. Energy balance has been studied in detail in neonates of low birthweight (e.g. Brooke et al 1979, Reichman et al 1982), but not so much in older infants. Fomon (1971) measured the voluntary intake of full term newborn infants fed a cow's milk formula. Recommended dietary allowance (RDA) values have been based, in the past, on such studies of observed intake (DHSS 1979). More recently, energy requirements in infants have been calculated from measurements of energy expenditure using the doubly labelled water technique added to an estimate of stored energy (with knowledge of the rate of weight gain and the body composition of reference infants of different ages) (Davies, Ewing et al 1989, Prentice et al 1988). These results suggest that older methods had overestimated requirements by 42-46kJ/kg/day in infants up to 6 months.

(c) The methods used vary widely, especially in the measurement of metabolic rate. These will be discussed later.

(d) In most studies, only selected aspects of energy balance have been measured, and over relatively short periods of time.

(e) There is disagreement about the best way to present data. A special difficulty is the issue of relating energy balance to measures of body size.

In nutritional studies, comparison needs to be made between individuals of different sizes and states of nutrition. Thus energy balance values should be expressed in terms of some aspect of body size enabling direct comparison. Weight has been used in most clinical studies (e.g. kJ/kg body weight). The criticism of this is that the body composition of a growth-retarded child is very different from that of a normally grown child. Specifically, a growth-retarded individual has a larger proportion of lean tissue, which is metabolically more active than adipose tissue, and this may result in an artificially high value for metabolic rate when related to weight.

Lean body weight would be a logical choice, but is technically difficult to measure. Nutritionists have tried, mathematically, to derive measures relating to this. Kleiber (1961) thought that the measure "weight$^{3/4}$" enabled comparison of energy values between individuals of widely varying sizes (for example, a mouse and an elephant). It is felt that this may be less applicable to individuals within a narrower range of size (e.g. different human individuals) (Davies, Cole et al 1989). More recently fat free mass has been derived using techniques such as stable isotope measurement of total body water, total body electrical conductivity (TOBEC, Hashimoto et al 2002), and dual energy X-ray absorptiometry (DEXA, Schmelzle and Fusch 2002).

One theory suggests that the number of cells in an organism, rather than its weight, is the determinant of its metabolic rate (Cheek 1968, Shelton 1980). Thus an underweight child with a normal cell number for age would have a "high" metabolic rate in relation to weight in comparison to a normally nourished child of the same age.

Some workers feel that nutritional requirements and metabolism relate more closely to surface area (Butler and Richie 1960).

1.2.6 Hypothesis and study objectives

The hypothesis was that infants with CHD show poor body growth because of an abnormality in the partitioning of energy balance, hence leaving less energy available for deposition.

The main objective was to measure growth and the components of energy balance in infants with CHD and controls. In order to carry this out, two intermediate objectives had to be achieved:

(a) To adapt and validate available methods of investigating energy balance for studying the partition of daily energy balance in infants.

(b) To measure respiratory gas exchange over prolonged periods in infants using indirect calorimetric methodology, and hence derive resting energy expenditure.

The approach was to carry out a descriptive study of hospitalized infants with congenital heart disease and to compare them with controls. No formal estimation of sample size was performed.

1.3 NUTRITION AND GROWTH IN BRONCHOPULMONARY DYSPLASIA

1.3.1 Definition and pathogenesis of bronchopulmonary dysplasia

Bronchopulmonary dysplasia (BPD) or chronic lung disease in preterm infants has been a significant problem because of the improved survival of the most preterm infants related to better cardio-respiratory intensive care. It is a cause of prolonged hospitalization, and these infants have a higher mortality and long-term morbidity (Eber and Zach 2001). In the years since the current work was carried out, the pattern of neonatal chronic lung disease has altered, probably because of improvements in obstetric

care and early neonatal respiratory care, so that it is now predominantly a condition seen in babies born below 28 weeks gestation or 1kg birthweight (Bancalari et al 2003).

It is very likely to be multifactorial in causation, and numerous factors have been implicated. The quality of early nutrition in preterm infants may have a bearing on many of these factors and may thus affect respiratory outcome.

(a) Structural and biochemical lung immaturity. Low gestational age and birthweight are the strongest risk factors for BPD. This may reflect the effects of ventilation on anatomically underdeveloped lungs with poorer antioxidant defences, but may also reflect the greater likelihood of smaller babies encountering the other risk factors.

(b) Mechanical trauma from ventilation. Most babies with chronic lung disease have required artificial ventilation for early lung disease. Although there is little evidence from prospective controlled trials that a particular aspect of artificial ventilation is critical, it is generally accepted that positive pressure is contributory (Taghizadeh and Reynolds 1976, Attar and Donn 2002).

(c) Oxygen toxicity The occurrence of BPD has been related to the duration of exposure to high concentrations of inspired oxygen (Edwards et al 1977). Exposure of experimental animals to 100% oxygen results in lung changes similar to BPD (Winter and Smith 1972). Antioxidant enzyme systems are probably immature and other antioxidant factors may be deficient in preterm infants, particularly those that develop BPD (Frank and Sosenko 1987).

(d) Infection. Respiratory colonization with organisms that are normally of low virulence is typical of preterm babies who are ventilated. These organisms may set up low-grade inflammation contributing to lung damage. Ureaplasma and mycoplasma have been implicated in the causation of preterm delivery and neonatal lung disease, although

the evidence for an aetiological role is conflicting (van Waarde et al 1997). The concurrence of systemic infection and a persistent ductus arteriosus significantly increase the risk of BPD, and this may be because infection results in the release of mediators which delay the closure of the ductus (Gonzales et al 1996).

(e) Inflammation This is part of the pathological process in the respiratory disease of preterm infants. There is cellular and humoral evidence of active inflammation in neonates with chronic lung disease (Pierce and Bancalari 1995).

(f) Increased interstitial fluid in the lungs. High fluid intakes and the presence of a patent ductus arteriosus have been associated with an increased risk of developing BPD, possibly because of their common effect on increasing interstitial fluid in the lungs (Tammela 1995).

(g) Recurrent milk aspiration. The predisposition of preterm infants with respiratory difficulty to gastro-oesophageal reflux, and the detection of fat-laden macrophages in the bronchoalveolar fluid suggests that this might be important in at least some infants (Radford et al 1995).

(h) Deficiency of micronutrients Deficiency of vitamin A may lead to poor epithelial healing. Pathological changes in the lungs are very similar to those of BPD (Zachman 1985). Inositol, a phospholipid which is thought to enhance the synthesis of surfactant in the lungs, may be functionally deficient in preterm infants (Howlett and Ohlsson 2000).

Past studies of chronic lung disease are confounded, even in just quoting incidence, by differences in definition. The original definition of bronchopulmonary dysplasia was: "The continued requirement for added oxygen at 28 days postnatal age in a baby who initially required ventilation for lung disease, and who has radiological changes compatible with BPD". Each of the components of this definition has been

modified by different workers. This definition may no longer be very useful for clinical and research purposes, particularly since the most premature infants can be expected to require respiratory support for longer in relation to lung immaturity and poor respiratory drive. A modified definition taking account of the corrected age of the infant may be more appropriate (Shennan et al 1988), although this approach may exclude babies with a significant clinical problem, and has been questioned again recently (Bancalari et al 2003).

1.3.2 Undernutrition in the causation of BPD

A deficiency of macro- and micronutrients may play an important role in the pathogenesis of bronchopulmonary dysplasia, although it is only recently that an attempt has been made to study this (Frank and Sosenko 1988). Undernutrition may predispose the baby to the harmful effects of many of the other insults implicated in causation. The rationale of suggesting a pathophysiological link between BPD and undernutrition can be understood by considering the situation commonly confronted by a very low birth weight infant. The baby of 1kg has a non-protein caloric reserve of only about 110kcal/kg (520 kJ/kg), which is barely sufficient for a day's total energy requirements. Respiratory disease is likely to increase energy demands by up to 25% (Weinstein and Oh 1981), and the pain and stress related to intensive care pose a further catabolic threat (Rogers 1992). Undernutrition might potentiate oxidative injury, and impair protective responses against infection (Farthing and Keusch 1985). Inadequate rib mineralization and intercostal muscle strength may lead to inefficiency of chest wall movement (Glasgow and Thomas 1977), and thus prolong the need for potentially damaging ventilatory support.

It is interesting that preterm infants who are also small for gestational age, although having a reduced likelihood of early neonatal lung disease, are more likely to develop

chronic lung disease. This may partly be the result of restriction of lung growth *in utero*, and also because of a deficiency of lung-protecting nutrients (Lal et al 2003).

(i) Macronutrients

Deficiency of macronutrients (protein and energy) may lead to an inability to grow and repair immature, damaged lung tissues. In experimental newborn animals, moderate undernutrition results in severely restricted lung growth, and markedly increases the mortality related to hyperoxia (Frank and Groseclose 1982). There is some evidence that gross undernutrition precedes the development of BPD in very low birth weight babies (Wilson et al 1991). However, the same author did not show a reduction in the incidence of BPD in this population with a package of measures resulting in an increase in nutritional intake (Wilson et al 1997).

(ii) Micronutrients

Micronutrients important for the protection of the lung from damage and for lung growth and repair may also be deficient.

(a) Anti-oxidant defences. Vitamin E is an important natural antioxidant, and is difficult to supply in quantities approaching those accreted transplacentally in the 3rd trimester of pregnancy, particularly if enteral nutrition has to be delayed (Gutcher et al 1984). The ratio of vitamin to polyunsaturated fatty acids (PUFAs) in the diet seems crucial in protection against oxygen toxicity (Witting 1980). Vitamin E is probably provided in sufficient amounts in infants fed milk. However, parenteral nutrition solutions may have low levels of biologically active vitamin E in the presence of large quantities of PUFAs (Gutcher et al 1984). Supplementation with pharmacological doses of vitamin E has not been shown to improve respiratory outcome (Watts et al 1991), and may predispose to necrotising enterocolitis and sepsis (Johnson et al 1985).

Copper, zinc, selenium and manganese are essential components of the antioxidant enzymes superoxide dismutase and glutathione peroxidase, which are present in low levels in preterm infants (Walravens 1980, Forman et al 1983). Serious deficiency of these trace elements is unlikely to occur unless infants are fed parenterally for a prolonged period.

The sulphur-containing amino-acids cysteine and methionine also have important anti-oxidant properties, probably because they bind oxygen free radicals and their deficiency has been shown to increase mortality resulting from hyperoxia in rats (Forman et al 1983).

(b) Vitamin A. Vitamin A is involved in the regulation of growth and differentiation of many tissues, and maintains the integrity of epithelial cells. It may also have a role in immune function. Vitamin A stores are low in preterm infants (Shenai et al 1981), and stay especially low in infants who develop BPD (Shenai et al 1985). It is difficult to deliver sufficient vitamin A postnatally, especially by the parenteral route, because of light degradation and adherence to the tubing of giving sets during administration of parenteral nutrition (Green et al 1987). Intervention studies have shown conflicting results, and this may be because of differences in patient populations, and in the route and dosage of supplementation. In a recent meta-analysis, the conclusion was that vitamin A supplementation (given enterally or intramuscularly) resulted in a modest reduction in the combined outcome of death or oxygen requirement at one month of age in babies of birthweight $\leq$1500g, and in a reduction in need for supplemental oxygen at 36 weeks postconceptional age in babies of birthweight <1000g (Darlow and Graham 2002).

(c) Inositol A single large randomized double blind study (Hallman et al 1992) supplementing preterm infants receiving parenteral nutrition in the first five days of life

showed an increased survival without requirement for supplemental oxygen at 28 days. A meta-analysis suggests an additional reduction in grade 3 or 4 intraventricular haemorrhage and in significant retinopathy of prematurity (Howlett and Ohlssen 2000).

1.3.3 Feeding practice and the causation of BPD

Whilst adequacy of nutrition has been recognised as of theoretical importance for babies with chronic lung disease, various aspects of enteral and parenteral feeding have been under suspicion in exacerbating respiratory disease, the resulting caution creating a further threat to the nutritional status of these infants. Concerns about the possible deleterious effects of enteral feeding on respiratory disease as well as the risk of necrotising enterocolitis have led to the practice of delaying milk feeding. Recurrent silent aspiration of intragastric milk feeds has been proposed as an aetiological factor in chronic lung disease, although gastro-oesophageal reflux appears to be no more (and perhaps even less) likely in ventilated infants (Newell et al 1989). Evidence linking the speed of introduction of enteral feeds with necrotizing enterocolitis is, at best, equivocal. Some studies suggest that the likelihood of necrotizing enterocolitis may even be reduced by early enteral feeding (LaGamma 1985). The better enteral tolerance of expressed breast milk and the protection it provides against necrotising enterocolitis (Lucas and Cole 1990) have further reduced any rationale to delay enteral feeding for a prolonged period.

Complications related to the use of older formulations and the previously less controlled administration of parenteral nutrition solutions have led to great caution in their use. The pulmonary complications of parenteral lipid emulsions (Hammerman and Aramburo 1988) are probably avoided if dosage is regulated, and blood monitored for lipaemia (Spear et al 1986). Parenteral lipid preparations provide an important source of energy, without some of the side effects of intravenous dextrose. Dextrose when given

alone has been shown to increase metabolic rate and carbon dioxide production because it is converted to lipid, a process which is energy-requiring and has a high respiratory quotient (VanAerde et al 1986). Intravenous lipid is also an important source of essential polyunsaturated fatty acids, which may themselves have an antioxidant role (Sosenko et al 1988). On the other hand, total parenteral nutrition cannot easily provide the preterm infant's requirements of vitamin A (photodegradation, Gillis et al 1983) and vitamin E, calcium and phosphate (limitations of solubility: Shine and Farwell 1984) and some of the trace elements.

The conflicting concerns in sick infants about, on the one hand adequacy of nutrition, and on the other the possible side effects of feeding have resulted in a lack of consensus about this important aspect of neonatal intensive care, and there have been widely varying policies among units (Churella et al, 1985).

1.3.4 Growth in BPD

Good growth is an important aim in itself, but also reflects adequacy of nutrition in chronic disease. Several studies have looked at long-term growth in infants with chronic lung disease. Markestad and Fitzhardinge (1981) looked at growth in 20 babies with BPD, without comparison with a control group. They found that all infants were at or below the 3rd centile for weight and length at term. Over the next two years, although some catch-up growth occurred, expected size had not been achieved in anything but head circumference. Acceleration in linear growth usually coincided with a marked improvement in clinical respiratory status. Meisels et al (1986) studied 17 babies with BPD with preterm controls matched for birthweight and gestation, and found twice as many BPD babies as controls to be less than the 10th centile for weight and length in the 2nd year (although this was not statistically significant). Davidson et al (1990) compared 30 infants with BPD with 41 preterm controls, although it was found difficult to match

for birthweight and gestation. They found lower weight and height centiles in female BPD infants throughout the first 2 years of life, although in the group as a whole, the difference was only significant at the final assessment at 21 months. Vohr et al (1982), in a controlled study did not find any difference in growth during the first 3 years in BPD and control infants.

Growth retardation in these babies may occur partly because they are the most preterm, and the sickest babies, under the greatest nutritional stress during their acute illness. Even when stable, these babies are as a whole restricted in their fluid (and hence nutrient) intake because of worries about worsening interstitial lung fluid, or precipitating cardiac failure. They may not feed well orally because of dyspnoea and other coexistent complications of prematurity.

Dexamethasone has been used extensively for the treatment of lung disease in neonates. It has been found to help wean such infants off the ventilator (Avery et al 1985), and there is a suggestion that it might be helpful early in the course of lung disease in preterm infants. Dexamethasone treatment is associated with an acute slowing of growth (Skinner et al 1997).

There has been a suggestion that chronic or recurrent low grade hypoxaemia may contribute to growth failure in BPD (Moyer-Mileur et al 1996).

It is not clear what part is played by "emotional" stunting because of abnormal environment and the recurrent stresses these babies endure for long periods.

Recent work suggests the importance of inflammatory mediators in modulating nutritional status in adult chronic obstructive pulmonary disease and cystic fibrosis (Bell et al 2000). There is certainly evidence that bronchopulmonary dysplasia is associated with a persistent inflammatory response (Groneck and Speer 1995), and this may contribute to cachectic changes in BPD, although this has not been studied.

1.3.5 Energy balance in BPD

Energy is an important component of nutrition in preterm infants. Because a large proportion of energy intake is used for growth (up to 50%, Reichman et al 1982), energy is likely to be a limiting factor for growth.

There is some evidence that bronchopulmonary dysplasia is associated with hypermetabolism as assessed by indirect calorimetric measurements of respiratory gas exchange (Yeh et al 1989, Weinstein and Oh 1981, Kurzner et al 1988a). This may partly be the result of the increased work of breathing (Kurzner et al 1988b), and possibly cardiac work especially if there is coexistent pulmonary hypertension, and may result in a reduction in the amount of energy available for deposition. There has been some criticism about the methodology and interpretation of the studies of metabolic rate (Kalhan and Denne 1990). Measurement of oxygen uptake by indirect calorimetry relies on measuring small differences in oxygen concentration between inspired and mixed-expired gases. In babies receiving supplemental oxygen, small and undetected variations in inspired oxygen concentration will have a large effect on measured oxygen uptake. None of these studies included any validation of the methodology specifically for oxygen-dependent subjects.

Energy intake was found to be reduced in infants with BPD early in their life (mean 60 days postnatal age, Yeh et al 1989), but not later (6 months corrected age, Kurzner et al 1987). Both these studies showed no difference in energy absorption from the diet. The study of Yeh et al (1989), in addition, indicated a low energy cost of growth in BPD, suggesting an altered composition of tissue gain.

Dietary caloric supplementation with glucose polymers (Yunis and Oh 1989), and theophyllines used to improve pulmonary function (Gerhart et al 1979) may increase energy expenditure for the same reason as intravenous glucose. Steroids used to wean

babies with BPD off ventilatory support cause catabolism, with a likely increase in muscle protein breakdown (Brownlee et al 1992).

1.3.6 Bone mineralization in bronchopulmonary dysplasia

There is very rapid accretion of bone mineral during the last trimester of pregnancy. Poor bone mineralization (metabolic bone disease or osteopenia of prematurity) is seen commonly in very low birth weight infants (Ryan 1998). It is thought to be mainly due to a postnatal shortfall of mineral intake, and there have been reports of achievement of intrauterine rates of mineralization with supplemented feeds (Chan et al 1988). The shortage of mineral supply may be made worse by the therapeutic use of diuretics and steroids used to produce short-term improvement in pulmonary function in infants with chronic lung disease (Shrivastava et al 2000). This may contribute to the retarded linear growth seen in preterm infants, although if their smaller body size is taken into account bone mineral content has caught up by the age of 1 year (Congdon et al 1990). In theory, poor mineralization of the ribs might play a part, during the neonatal period, in perpetuating chronic respiratory disease by causing atelectasis due to inefficient splinting of the chest wall (Glasgow and Thomas 1977).

Greer and McCormick (1987) compared VLBW infants with and without BPD matched carefully for gestation and birthweight, and did not find that bone mineralization was affected by the presence of BPD. They did not specifically look at the effect of drug therapy. Adrenal corticosteroids may, in addition to their effect on bone mineral, retard bone growth by suppressing collagen synthesis, and by slowing the function of the bone growth plate, perhaps by reducing pituitary growth hormone secretion or the production of local growth factors (Preece 1976).

1.3.7 Hypothesis and study objectives

The hypothesis was that evidence of impaired nutritional status precedes (and might contribute to) the development of chronic lung disease, this deficit persisting once BPD is established, and being associated with poor body growth.

The objectives were to:

(a) Measure growth and macronutrient intake in the first few weeks of life in babies who go on to get BPD and in controls

(b) Measure energy balance in infants with established BPD and controls

(c) Measure forearm bone mineral content longitudinally from birth in babies with BPD and controls.

The approach was to carry out descriptive studies of nutrition in infants with BPD and compare them to control preterm infants without BPD. No formal estimation of sample size was performed for these studies.

Chapter 2 Methods

2.1 CONGENITAL HEART DISEASE

For Results, see 3.1
For Discussion, see 4.1

These studies were carried out over the 2 years 1981-1983. An initial period was spent in development and validation of methodology. Studies of energy balance and growth were then carried out on inpatients recruited from the cardiology and general paediatric wards in the Royal Liverpool Children's Hospital. This hospital provided a tertiary referral service for paediatric cardiology and cardiothoracic surgery for Merseyside and North Wales.

Energy balance studies were carried out on infants with and without CHD. For ease of description, the studies are considered in 3 parts:

(1) Measurement of Energy Intake and Losses (in stool, urine, and vomitus),

(2) Measurement of Energy Expenditure or Metabolic Rate,

(3) Measurement of Growth.

2.1.1 Ethical approval and Consent

The study was approved by the Liverpool Paediatric Sector Ethical Committee.

Parents or guardians were fully informed about the study, and written consent was obtained.

2.1.2 Study design

For this explanatory study, a convenience sample of hospitalized infants with CHD and healthy infants of a similar age range was enrolled and the groups compared from the point of view of nutritional status.

2.1.3 Selection of cases

Stable hospitalized infants with CHD but without any major associated abnormality were eligible for the study. The requirements of the energy balance study

were such that infants needed to have limited independent mobility and still be dependent on napkins (for the measurement of intake and losses), and able to be nursed in a metabolic chamber for several hours (energy expenditure). They were thus usually below the age of 6 months post-term age. Controls were healthy infants on the general paediatric wards, admitted or kept in hospital following an acute illness for predominantly non-medical reasons.

2.1.4 Energy Intake and Losses

Infants were nursed in a hospital cot on a ward in the Royal Liverpool Children's Hospital. They were studied over a period of 3-5 days when their clinical condition was judged to be stable. Their feeding regimen and treatment were not changed, and they were not subjected to major procedures such as cardiac catheterization during the study period. They were examined daily, and the following recorded: (a) sleeping heart rate, (b) sleeping respiratory rate, (c) presence or absence of central cyanosis, (d) liver size, (e) peripheral oedema. Blood samples were taken at least on alternate days for (i) haematocrit, (ii) urea and electrolytes, (iii) capillary blood gases.

(i) Energy Intake

Infants were included in this part of the study only if their diet consisted solely of commercial formula milk. Most infants were fed pre-packed commercial formula milk. Two infants were fed formula milk made from powder in the hospital milk kitchen. In the latter cases, a whole day's supply was reconstituted, and the required number of bottles filled from this supply and sealed in the milk kitchen. The feeding regimen for each infant was decided upon by the medical and nursing staff responsible for the care of that infant, well before the start of the study, as that most appropriate to his/her clinical condition. Individually numbered milk bottles were weighed before and after feeds (Mettler top pan balance).

Samples of each type of pre-packed formula, and samples of each batch of locally prepared milk were analysed for energy content. 100g aliquots (measured on a Mettler top-pan balance) were frozen in round-bottom flasks. The weight of dry residue was measured and its heat of combustion determined by bomb calorimetry on at least 10 occasions. The mean was taken from readings showing a standard error of less than 3%, and hence the energy content of the milk calculated (Miller and Payne 1959).

(ii) Energy Losses

(1) Collections Infants wore closely fitting disposable napkins (Boots, disposable) with porous napkin-liners (Boots, 5-star), the whole being held onto the infant with a plastic napkin-tie. This system provided some separation of stools (retained by the napkin-liner) from urine (soaked into the napkin).

Infants were nursed on large absorbent bibs (Molynlycke, Sweden) to collect milk spillages and vomitus.

Napkins and bibs were changed as soon as they were seen to be soiled, and stored in individual sealed "freezer bags" in a freezer at -20^{0}C.

Extra napkin-liners and bibs were used, as appropriate, to clean the infant's skin when changing.

Topical applications to the napkin area were avoided during the study.

50mg of Carmine powder (sterilized by the local pharmaceutical quality control lab. to avoid contamination, Lang et al 1967) was mixed with the first feed of the balance period, and the first feed after its completion (see Fig. 2.1). This marker has a gut transit time similar to food, and appears unchanged in the stool (Rose 1964). Stool collections were started with the first appearance of the first marker, and stopped with the first appearance of the second marker.

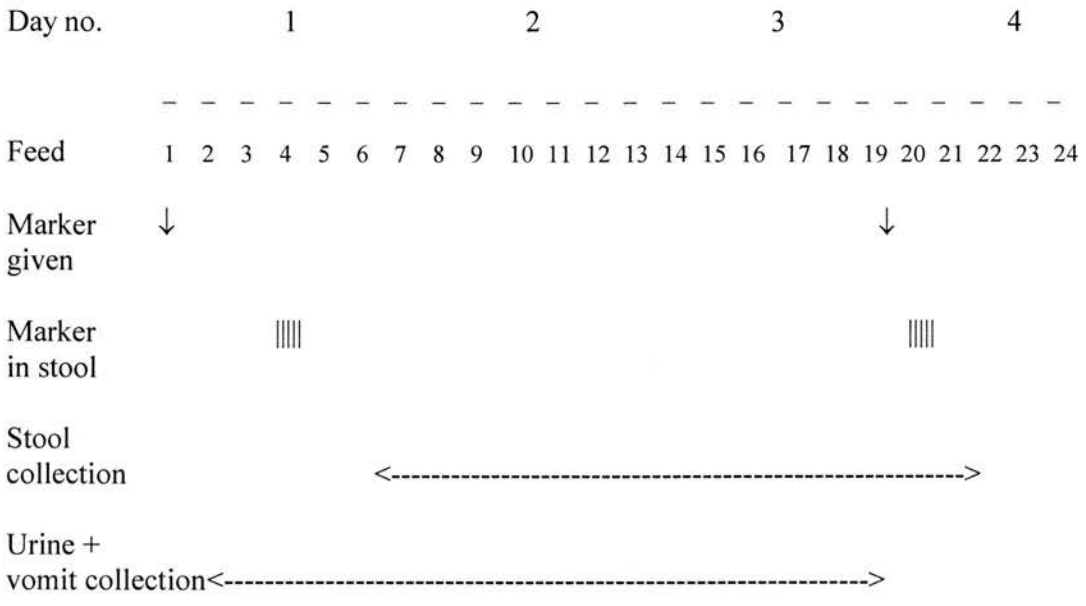

Fig. 2.1 Diagrammatic Representation of 3 day study of energy intake and losses.

Nurses instructed in the procedures involved were responsible for most of the day-to-day care of infants, involving feeding and changing. They also recorded the bottle numbers used at each feed, and the length of time taken over feeding. They stored bottles, containing any unfinished milk, as well as used napkins, bibs and liners, individually wrapped, in a freezer used specifically for this purpose and kept by the infant's cot. The nurses were supervised daily, and practice-runs exposed possible technical problems, and improved techniques. Napkins were checked to fit closely to the infant; any loose folds were gathered together with surgical tape. Infants were nursed and fed always with bibs strategically placed to catch vomits. Initially, major accidental losses were common but with time, these became less frequent. Any such major losses were reported, and resulted in that study being abandoned or restarted.

(2) Processing (a) Urine and vomitus For ease of handling, urine and vomitus were dealt with together (see p49-50).

Napkins and bibs were weighed individually and labelled before use. A day's collection of used napkins and bibs was thawed from the freezer and re-weighed

individually. Thus the weight of urine and vomitus collected each day was calculated. After weighing, napkins and bibs were thoroughly shredded by hand, in a plastic basin, with 1 to 3 litres of tap water. The resulting pulp was filtered in portions through a 500ml Buchner funnel using Whatman filter paper into a 5l flask connected to the laboratory wall suction unit. Measured volumes of water were used to wash each funnel-full, and filtered for a measured time, after which residual water was forced out using a large weight on the pulp.

Four 250ml aliquots of the thoroughly mixed filtrate (measured with a graduated measuring cylinder) were frozen at -20^{0}C for 48 hours in Pyrex-glass round-bottom flasks of known weight. The frozen samples were freeze-dried (Edwards Modulyo freeze-drier, Edwards High Vacuum, UK) to obtain a small residue whose weight was determined by weighing the re-warmed flask.

The energy content of the residue was determined by bomb calorimetry in triplicate. Daily energy loss was calculated from the known amounts of urine and vomitus collected and the known dilution (for details, see p49-50).

(b) Stool The average weight of napkin-liners was determined by weighing a large number together.

A day's collection of liners was thawed and then weighed collectively, to determine the amount of stool collected. Stool was carefully scraped off liners with a metal spatula, into a pre-weighed plastic container representing a day's collection. This was weighed to determine the amount of stool processed. The stool was freeze-dried in these containers, which were weighed after warming to determine the weight of residue.

The residue was homogenised and its energy content determined by bomb calorimetry in triplicate.

Assuming the daily stool collection to be homogeneous in energy content, daily energy loss in stool was then calculated:

Daily energy loss in stool = **Energy content of residue x wt. of stool collected /wt. of stool processed**

(c) Quality Control

(i) Collection of losses Experiments were carried out to determine the optimum method of processing napkins and bibs.

Experiment 1. To determine the method providing the best energy yield A 1:5 volume dilution of pre-packed milk was prepared. Aliquots of this were freeze-dried for bomb calorimetry to determine energy content. Further aliquots were poured onto batches of pre-weighed napkins and bibs, which were then re-weighed to determine the amounts of milk added. 6 napkins and 3 bibs were used in each batch as the average number used per day on infants during the study. The soiled napkins and bibs were shredded with added water in a basin (the plastic backing was taken off bibs and cleaned of possible residue with water) until they were, subjectively, of a workable consistency. Different batches were then treated in different ways: (a) varying the number of extra washings to each Buchner funnel full of pulp, (b) varying the length of each filtration.

The energy content of the filtrate was determined by bomb calorimetry. Assuming that the filtrate was a homogeneous mixture of 1:5 diluted milk and the water added, the energy content of the original 1:5 diluted milk could be extrapolated, and compared with the value obtained by direct measurement.

Extra 100ml washings per funnel:	Energy in 1:5 diluted milk (kJ/100g) (a) by direct bomb-calorimetry	(b) derived from filtrate after processing
0	60.2	55.5
1	60.2	52.4
2	60.2	53.8
3	60.2	50.9
5	60.2	52.8

There was no clear trend in energy obtained with increasing washings, with a coefficient of variation of 3.2%. There was a mean deficit of 11.8% (range 7.8-15.4%) in energy obtained. Two washings were decided upon.

With one batch of milk-soiled napkins and bibs, portions were subjected to washings of different duration.

2 washings- duration of each washing (min)	Total energy in 1/5 milk derived from filtrate (kJ)
1	42.3
2	50.2
5	53.7
10	52.5
15	53.8

There appeared to be no significant benefit to washings of longer than 2 minutes duration, and this is what was used in the study.

Experiment 2. To ensure the lack of energy contribution from experimental materials

Batches of unused napkins, bibs and filter-papers were homogenised, in turn, in water and processed as above. No visible residue was obtained after freeze-drying the filtrate, and there was no consistent weight rise of the flasks.

Milk of known energy content was added to these flasks and freeze-dried after thorough mixing- there was no significant increase in energy content to suggest contamination.

(ii) Bomb calorimetry Bomb calorimetry was carried out using conventional techniques (Miller and Payne, 1959) using a ballistic bomb calorimeter (Gallenkamp, UK).

Sufficient familiarization with the instrument to obtain reproducible results requires much time and attention to detail; hence the exact technique used here will be described.

The instrument measures the heat of combustion of materials. The material to be tested is placed, suitably prepared, in a crucible inside the sealed bomb calorimeter chamber, and oxygen introduced at 25 atmospheres pressure. The substance is ignited by the burning of a standardized cotton fuse attached to an electronically heated coil, operated by a switch. A thermocouple converts the heat transmitted to the calorimeter casing into an electrical signal displayed on a galvanometer previously calibrated using a standard.

In the present study, a standardized length of single thickness cotton (supplied with the bomb calorimeter) was used as the fuse. Burning the fuse on its own produced a small galvanometer deflection, whose value was derived after burning several of these together. This "fuse deflection" was subtracted from all subsequent measurements.

A single metal crucible was used throughout, since it was found that there was a greater discrepancy between measurements using different crucibles.

The mode of preparation of the sample, leading to optimal combustion, varied for different materials. Optimal combustion was reflected, in practice, by: (i) a smooth rise in chamber pressure after ignition, (ii) no visible unburnt material left in the crucible, (iii) no "spraying" of material outside the crucible, (iv) reproducible results.

Benzoic acid, used as a standard, was melted in a crucible over a Bunsen burner flame - this converted it from a fine powder into a compacted layer in the crucible. The

crucible was weighed empty and after the melted benzoic acid had cooled to room temperature, before combustion. Other materials were prepared as a dry powder by freeze-drying, homogenized, and then packed tightly into the crucible.

The time-course of galvanometer deflection after ignition was studied using different materials. In all cases the deflection reached a definite peak after between 30 and 40 seconds. In many cases a second peak was obtained after about 3 minutes, but this was much less constant. The first peak showed a linear dose-response of sample size to size of deflection intersecting the origin. For the purposes of calculation, the first peak deflection read naked eye on the galvanometer dial was used.

The bomb calorimeter was calibrated daily to allow for the influence of environmental changes. Benzoic acid conforming to a thermochemical standard (Bureau of Analysed Samples Ltd., UK, 26.44 kJ/g) was used. Three successive calibration values showing a standard error of the mean of less than 3% were averaged and a value for kJ/unit deflection calculated (if a wider error was obtained the instrument was checked for leaks, incomplete combustion, etc.). That value was then used for all bomb calorimetry that day.

2.1.5 Energy Expenditure

(i) Methods used

Respiratory gas exchange was measured to provide an indirect estimate of energy expenditure. Attempts were made to develop a system with which infants could be studied over a period of several hours, in different states of activity, without sedation, and with minimal restriction on their normal activity.

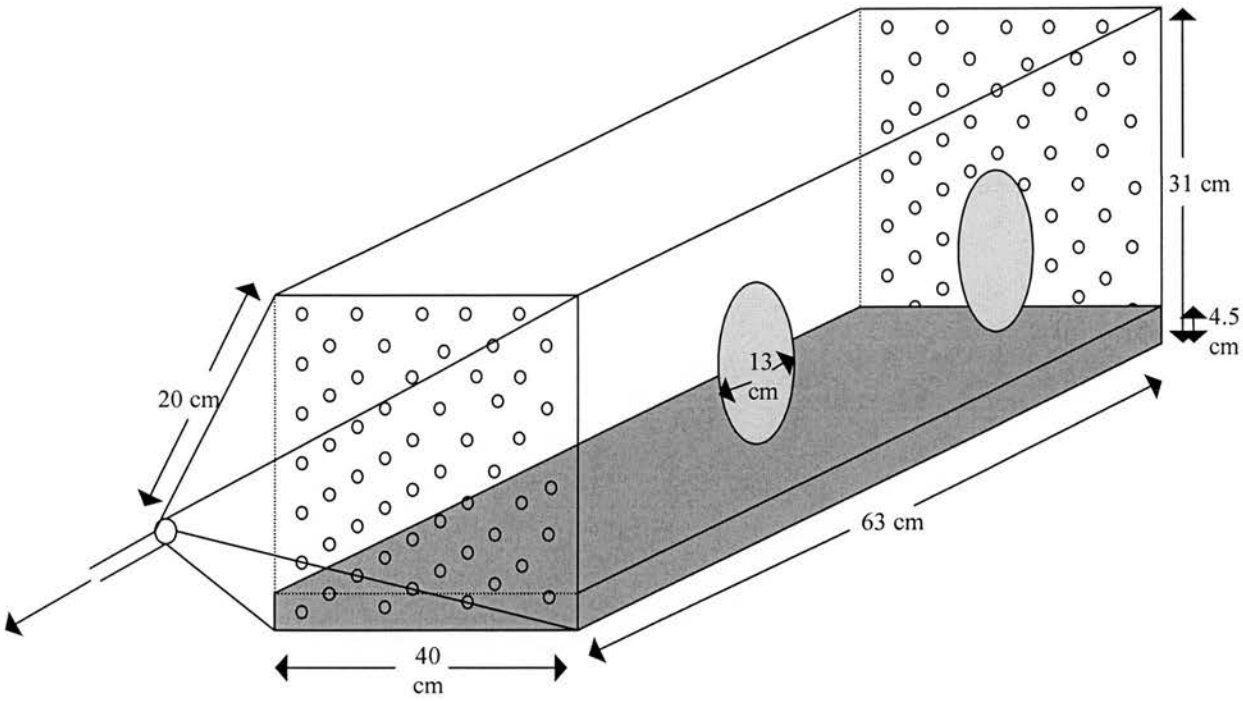

Fig. 2.2. Diagram of metabolic chamber

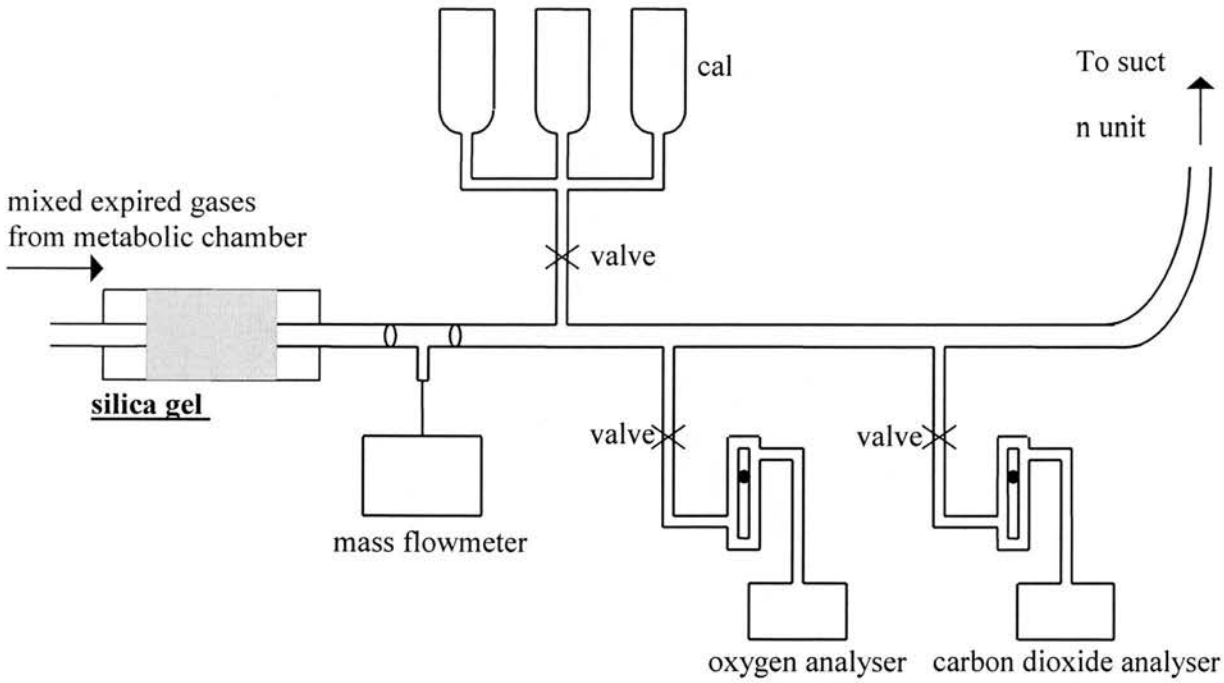

Fig. 2.3. Diagram of circuit for measuring gas exchange

The infant was nursed in a perspex whole-body chamber (see Fig. 2.2), through which room air was drawn by a standard wall-fixed suction apparatus on the ward. A plastic funnel at the "expired" end of the chamber served to mix the expired gases, which were dried by passing through a tube of calcium chloride granules (B.D.H., UK).

The flow rate of mixed expired air was continuously measured using a Hastings linear mass flowmeter (ENALL 10K, Teledyne Hastings-Raydist, USA). Oxygen concentration was measured with a paramagnetic oxygen analyser (OA 272, Taylor-Servomex, UK); carbon dioxide concentration with an infrared analyser (P.K.Morgan 801D, UK) (Fig 2.3). During a study, the gas analysers were intermittently switched to sampling room air (every 30 minutes).

The infant's skin and rectal temperatures, and chamber air temperature were measured by thermistor probes (Yellow Springs Instruments, series 400), attached to an electronic switch-box (YSI model 47).

Flow rates, gas concentrations, and temperatures were recorded on a calibrated, multi-channel chart recorder (Chessels model 302, UK).

Before starting measurements, the flowmeter was electronically zeroed, and the gas analysers calibrated within a narrow scale using two different standardized gases (BOC Certified gases).

The chamber containing the infant was closed and the suction started. A flow rate was used which resulted in a ½-1% difference between inspired and expired gas concentrations.

Each infant was studied over several hours, with frequent records of changes in the state of wakefulness or activity. When necessary, the infant could be quietened or attended to through portholes in the side of the chamber, with little interruption in the measurements.

Using a planimeter (Haff model 315, Gebruder Haff GmbH, W.Germany) to measure areas under curves, averaged inspired-expired concentration differences and flow rates were calculated for short periods (usually 10 minutes).

Then:

$$\begin{array}{c} \textbf{O}_2 \textbf{ uptake} \\ \textbf{or} \\ \textbf{CO}_2 \textbf{ output} \end{array} = \begin{array}{c} \textbf{Flow rate of dry mixed expired air (corr. to STP)} \\ \textbf{X} \\ \textbf{Average inspired-expired conc. difference} \end{array}$$

Energy expenditure was calculated from V_{O2} using the equivalence tables of Zuntz (Harper et al 1979) using the total RQ and ignoring the contribution of protein to metabolism.

For the purposes of comparison between infants, sleeping oxygen uptake and respiratory quotient, and an estimate of resting energy expenditure (REE) derived from at least 3 hours of resting measurement are used here.

In those who had a complete balance study, energy available for deposition in new tissue was estimated by subtracting REE from metabolizable energy intake.

(ii) <u>Development of methodology</u>

An open-circuit indirect calorimetric method with a whole body chamber was used in this study.

Such a method needs to fulfil several basic criteria:

(i) A comfortable subject in a "thermoneutral" environment,

(ii) A rate of airflow through the chamber which avoids stagnation of expired gases and re-breathing of carbon dioxide,

(iii) A net unidirectional flow of air through the circuit with complete entrainment and thorough mixing of expired gases, and no outward leaks,

(iv) The accurate measurement of gas concentrations and flow rate,

(v) Validity of the system as a whole for measuring gas exchange.

The equipment used will now be described in some detail and the above points discussed.

(1) The Chamber

A headbox with a sleeve over the infant's body was tried initially, but this was too restricting in all but the youngest infants, and caused major problems with condensation. It also resulted in a poorly damped trace which showed large minute-by-minute changes in gas exchange.

A prototype whole-body chamber consisted of a perspex box of shoe-box shape. One end wall was moveable, so that the length of the chamber could be adjusted. There was one small entry port and similar exit port for gases at opposite ends. Several modifications subsequently improved the function of the chamber.

The eventual size of the chamber was such as to allow relatively free movement of the largest infant studied, whilst not being so large that it created an unwieldy dead space, slowing the response time of the system. For comfort, the infant lay on a foam mattress 7 cm thick covered with a single sheet.

The pattern of gas flow through the chamber was studied (a) using a smoke generator, (b) by measuring oxygen and carbon dioxide concentrations inside the chamber whilst an infant was being studied. Smoke (which, like carbon dioxide, is denser than air) tended to collect at the base of the chamber. This tendency was lessened by (i) making multiple perforations in the end walls and attaching plastic funnels to each end, (ii) placing obstacles to divert gas flow on the floor of the chamber, thus creating turbulence (the infant may have served this function during studies). The funnel at the exit end, also served to mix expired gases by creating turbulence.

During a gas exchange study, the carbon dioxide concentration was somewhat higher near the base of the chamber. However, the maximum concentration difference between two points was 0.2% and the highest CO_2 concentration was 0.8%.

Two portholes were created on one side of the chamber -to these were attached tightly fitting elasticated sleeves which could be sealed when not in use. These allowed access during a study, for calming a distressed infant, and for nappy-changing or even feeding. A quick-access hatch was provided on top of the chamber for emergency use.

(1) Flowmeter *Hastings linear mass flowmeter, model ENALL 10K (Teledyne Hastings Raydist, USA).* This instrument measures gas flow to 0.01 litres/min between 0 and 10 litres/min, and is calibrated for air.

Its calibration was checked with an Ealing dry gas meter (Scientific and Research instruments, UK), which measures gas volumes with an accuracy of +/-1%. Using room air, and measuring gas volume at a constant flow rate for 10 minutes, the two methods showed a linear correlation in the range 0-10 litres/min.

There was some electrical baseline drift in the first 15 minutes after connecting to mains, but thereafter minimal drift of +/-0.02 litres/min over 18 hours whether connected to gas flow or not.

During gas exchange studies, the flowmeter was measuring dried mixed-expired air, with a higher carbon dioxide and lower oxygen content than room air. When such changes in concentration (up to 1%) were artificially induced by introducing standard gases, there was no measurable effect on the calibration of the flowmeter.

(3) Oxygen analyser *Servomex OA272 paramagnetic oxygen analyser (Taylor Servomex, UK).* This was used with a pump diverting 100ml of dried mixed-expired air into the analyser. The analyser can measure oxygen concentrations between 0 and 100% and has the facility of using an enlarged scale to look at a 25% or 5% oxygen

full scale, anywhere in the range of 0-100% oxygen. In the present studies, a scale of 5% in the range 20-25% oxygen was used, the upper and lower limits of the scale being calibrated using standardized gases (BOC certified gases, British Oxygen Co., UK). (The upper and lower limits can be set separately, and the whole scale can be moved using a "shift" control).

Calibration drift was assessed with the analyser sampling room air over a period of 18 hours. There was some drift in reading around the baseline, which was related to shift in the whole 5% scale rather than an alteration in the upper or lower limit calibration. Drift over 1 hour was up to 0.05%, and during a study this was corrected using the shift control every 30 minutes, after noting on the trace the amount of drift.

(4) Carbon dioxide analyser *PK Morgan model 801D (PK Morgan, UK) infrared carbon dioxide analyser.* This measures carbon dioxide concentrations between 0 and 5%. It has an inbuilt pump, which withdraws 2 litres of dried mixed-expired air. Room air drawn through self-indicating soda lime was used as a zero, and a BOC certified calibration gas with about 2% carbon dioxide for a "high" calibration.

After a 1 hour warm-up period, there was little drift over 18 hours (up to 0.01% CO_2) and negligible drift over 30 minutes in zero and 2% calibrations, provided the soda lime in the instrument was renewed regularly (every 4 hours).

(5) Thermal monitoring and thermal stress *Yellow Springs Instruments (USA) YSI series 400 thermistor probes; YSI model 47 multi-channel temperature monitor.* The air temperature probe was fixed at the expiratory end of the chamber; the skin probe stuck to the skin of a finger or toe with adhesive tape; the rectal probe was used in most cases to obtain intermittent readings. The monitor switched to each of the channels in turn.

It was decided to study infants in thermal conditions as near to those in which they would normally be nursed as possible. Infants were studied lightly clothed with a napkin and a single thin layer of clothing over the trunk at room temperature which was 18 to 22°C. The chamber air temperature did not vary more than 0.5°C from the temperature on the ward. Infants' body temperatures did not vary by more than 0.5°C from 36.5°C (skin) and 37°C (rectal), and the peripheral-core temperature gap was never greater than 0.5°C, suggesting that infants were not under thermal stress.

(6) Chart Recorder *Chessels, model 301, 6-channel chart recorder (Chessell Ltd, UK).* To this was connected the millivolt output of the oxygen and carbon dioxide analysers, flowmeter and thermistor switch box. The recorder was set up so that the full scale represented roughly 1% change in gas concentration and 0.5 litre/min flow rate.

(7) Entrainment of expired gases

No attempt was made to make the chamber completely airtight, as small leaks would be into the chamber, and this would not affect the gas exchange calculations which are based on "expired flow" being measured. However, the more distal part of the circuit needed to be airtight to ensure a complete yield of expiratory gases.

With a constant flow of room air entrained through the chamber, a standardized gas was introduced into the chamber at a constant rate measured by a rotameter (up to 100ml/min) to produce O_2 and CO_2 concentration changes of around 0.5% as in an infant study.

O_2 and CO_2 production (from the cylinder), determined from the measured gas concentrations and flow rate of "mixed expired" gas, were compared with the values calculated from the known composition and flow rate of the cylinder gas. The mean error from calibrations was 4.3% (range 0.5-5.9%).

Flow of 100% CO_2 (ml/min)	Flow of "mixed expired" gas (l/min)	Measured CO_2 conc. in mixed exp. gas (%)	Measured CO_2 production (ml/min)	% Error
10	2	0.47	9.47	-5.3
20	4	0.52	20.94	+4.7
30	6	0.53	31.77	+5.9
40	8	0.48	38.42	-3.95
50	10	0.53	52.75	+5.5
60	6	0.94	56.52	-5.8
70	7	0.98	68.64	-1.95
80	8	1.04	83.44	+4.3
90	9	1.00	90.45	+0.5
100	10	1.05	105.15	+5.15

(8) Calculation of Gas Exchange and Energy Expenditure

This was based on Pettenkofer's principle, illustrated by the equation:

$$\textbf{Oxygen uptake } V_{O_2} = \left[\begin{array}{c}\textbf{Flow into chamber, Vi}\\ \textbf{X}\\ \textbf{O}_2\textbf{ conc. in, Fi}_{O_2}\end{array}\right] - \left[\begin{array}{c}\textbf{Flow out, Ve}\\ \textbf{X}\\ \textbf{O}_2\textbf{ conc. out, Fe}_{O_2}\end{array}\right]$$

The difference between Vi and Ve depends upon the respiratory quotient, the flow rates used, and the volume of gas exchange. In the circumstances of this study the difference amounts to .02 litres/min at the most, and Vi and Ve may be assumed to be the same with little effect on calculated values of gas exchange (up to 5.9% error with the smallest V_{O_2}, the lowest flow rates used in this study, and an RQ of 0.7, see Appendix).

Thus a simplified equation can be used to obviate the need to measure Vi:

$$V_{O_2} = Ve\ (Fi_{O_2} - Fe_{O_2})$$

and $$V_{CO_2} = Ve\ (Fe_{CO_2} - Fe_{CO_2})$$

A planimeter (*Haff, model 315, Gebruder Haff GmBH, W Germany*) was used to measure areas under the curves of Fe_{O_2}, Fe_{CO_2} and Ve, and hence derive averaged values over short periods. It was calibrated before each set of measurements, using known areas

on graph paper. Once calibrated, errors in measuring areas of 100 sq. cm or more were less than 0.1% as suggested by the manufacturer.

In calculating energy expenditure, it was chosen to ignore the protein contribution to metabolism, and treat the total RQ as the non-protein RQ. The maximum possible error in energy expenditure measurements arising from this assumption is 3.2% (see Appendix).

2.1.6 Growth

Anthropometric measurements were made by the author, on at least 3 occasions during the balance period, and where possible at follow-up. Each measurement was made at the same time of day in a particular infant, and weights were always measured immediately before a feed.

Weight: on Avery baby weighing scales to 5g.

Length: using a standard technique with a neonatal stadiometer (Holtain Ltd, UK) to 1 mm.

Skinfold thickness: using skinfold callipers (Harpenden Ltd, UK), taking the mean of three measurements at each site, always on the left side to 0.1mm.

Biceps and Triceps: at the mid-upper-arm, midway between acromion olecranon processes.

Subscapular: immediately below the angle of the scapula.

Suprailiac: immediately above the posterior superior iliac spine.

Head circumference: the largest occipitofrontal circumference measurement of 3 obtained using a paper, non-elastic tape measure.

Mid-upper arm circumference: the arm circumference midway between the acromion and olecranon processes, with the same tape measure.

Foot length:	calcaneum to tips of toes	measured using a miniature perspex gauge made for the purpose (based on James et al, 1979).
Forearm length:	olecranon to fingertips	

2.1.7 Statistical analysis

Statistical analysis was done using Statview software for Apple Macintosh computers (SAS Institute Inc, USA) and SPSS software for PC (SPSS Inc, USA).

Correlation between two variables, and the proportion of variability explained, was tested using the regression coefficient for linear and quadratic regression. The difference between 2 groups was assessed using the non-parametric Mann Whitney U test. Deviation of anthropometric data from the population norm was computed from British Growth Reference data (Cole et al 1998) and software to calculate the z-score (Child Growth Foundation, London).

2.2 BRONCHOPULMONARY DYSPLASIA

For Results, see 3.2
For Discussion, see 4.2

These studies were carried out in the 3 years from 1989-1992. They were carried out in the neonatal unit of the Royal Infirmary of Edinburgh, which is associated with a maternity unit delivering about 6000 babies per annum, and providing a regional referral service for perinatal care and neonatal intensive care for the South-East of Scotland.

2.2.1 Ethical approval and consent

The studies were approved by the Edinburgh Reproductive Medicine Ethics Committee. Parents or guardians were fully informed about the study, and written consent was obtained.

2.2.2 Study design

A prospective survey of the nutrient intake and growth of a consecutive cohort of infants of birthweight less than 1500g, in one centre, was carried out over a 2 year period.

During the first part of this period, methodology for measurement of energy balance and bone mineral content was developed. Studies were subsequently carried out on retrospectively selected nested subgroups of these infants in order to compare specific aspects of nutrition in infants with and without BPD (see Fig. 2.4 below).

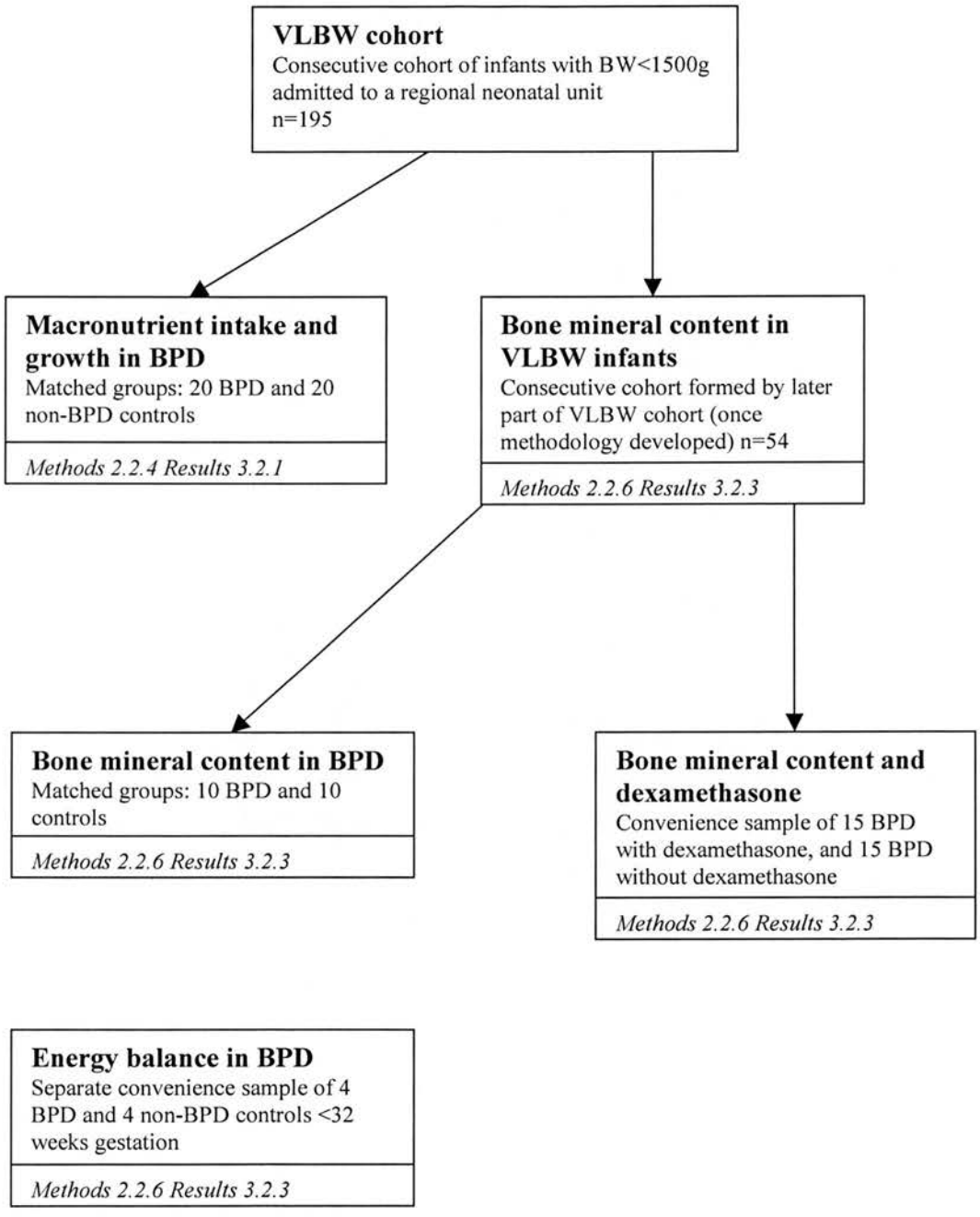

Fig. 2.4. Studies of BPD: study design

2.2.3 Selection of cases

Criteria for inclusion were absence of major congenital malformation or other diagnosis which might affect nutritional status, and the availability of 3 weeks' nutritional data and data on respiratory outcome. Babies with BPD were identified by the need for supplementary oxygen at 28 days postnatal age to maintain an arterial oxygen partial pressure of 6-10kPa or arterial oxygen saturation of 88-93% with X ray changes in keeping with the diagnosis, and the absence of other respiratory diagnosis. Of the 195 babies, there were 29 deaths before 28 days, 7 babies for whom consent was not obtained for any collection of data, and 50 babies for whom there was insufficient data because of transfer to another hospital. Of the remaining 109 babies, 53 had BPD, and 56 did not.

2.2.4 Macronutrient intake and growth in bronchopulmonary dysplasia

All fluid intake was recorded prospectively in 195 consecutive very low birth weight (VLBW, birthweight less than 1500g) infants daily for the first week and then weekly until full term equivalent, or as long as the infant was still in the neonatal unit. Fluid given (rather than that prescribed) was charted as volume of milk administered measured by syringe, and volume of intravenous fluids delivered indicated on volumetric pumps. No direct measurement was made of losses by vomiting or by leakage or extravasation from intravenous lines. Nutrient intake was calculated from manufacturers' data on nutrient composition and published data on the composition of expressed preterm breast milk (Lemons et al 1982). The main reason for incompleteness of data was early discharge to another unit after intensive care.

Babies had weekly detailed anthropometric measurement. This comprised weight (to nearest 5g with averaging electronic scales [Mettler 515, Mettler Electronics Corp. USA], with subtraction of known weights of equipment attached to the baby, e.g. nasogastric tube, endotracheal tube and fixation), crown-heel length (to nearest

millimetre with Holtain neonatal stadiometer), head circumference (to nearest mm with paper tape measure), forearm and lower leg length (measured olecranon to distal ulnar styloid and tibial tuberosity to medial malleolus, respectively, to nearest 0.1mm using RS vernier callipers, RS components, UK, see Bishop et al 1990) and four skinfold thicknesses (biceps, triceps, subscapular and suprailiac, to nearest 0.1mm with Harpenden skinfold callipers). These measurements were done provided the baby was judged sufficiently stable by medical and nursing staff responsible for their clinical care.

Babies were divided into those with and without BPD on the basis of continuing requirement for supplemental oxygen, following neonatal respiratory disease, at 28 days postnatal age. Each baby with BPD was then matched as closely as possible for gestation and birthweight with control infants. Babies with a match for whom there was nutritional data available for at least 3 weeks and data on respiratory outcome were included.

Feeding was by established protocols for clinical care and in individual babies was decided by medical and nursing staff clinically responsible for them. Soon after birth bigger babies who were in good condition (no respiratory distress, hypoglycaemia, asphyxia or circulatory problems) were started on enteral feeds via nasogastric tube. Most were initially given intravenous dextrose, later supplemented with electrolytes and calcium when the need arose. Enteral feeds (as boluses via nasogastric tube) were started as soon as the infant's general condition allowed, and provided there were no specific contraindications (e.g. paralysis, high-placed umbilical artery catheter). Mothers were encouraged to provide expressed breast milk for their own infant (this was given fresh or after storage for up to 24 hours in a -4^0C refrigerator or for up to 3 months in a -20^0C freezer), and when this was unavailable or insufficient, a preterm formula (SMA low birth weight or Prematil) was given. Parenteral feeding was given when enteral feeding had not been established in the first few days, earliest in the smallest infants. This

comprised Primene (Clintec, UK) as the protein source (McIntosh and Mitchell 1990), given to a maximum of 3.5g/kg/day and 10% Intralipid (Kabi Pharmacia, UK) giving up to 3g/kg/day of fat. Fluid intake was increased from 75ml/kg/d by daily increases of 25ml/kg/d up to 150ml/kg/day, as allowed by clinical and biochemical assessment of hydration, and provided there was no clinical problem related to the ductus arteriosus. Further increases were made if needed to allow weight gain at about 15g/kg/day.

The macronutrient composition of formula feeds and parenteral nutrition solutions was calculated from manufacturers' data and of preterm expressed breast milk from data in Lemons et al (1982) and were as follows:

Preparation	Energy (kJ/100 ml)	Protein (g/100 ml)	Fat (g/100ml)
10% dextrose	167	0	0
Amino acid	188	2.4	0
10% Intralipid	460	0	10
Expressed breast milk	305	1.4	3.4
Prematil	293	2	3.5
SMA gold cap	272	1.5	3.6
Aptamil	280	1.5	3.6
SMA LBW formula	335	2	4.4

2.2.5 Energy balance and growth in bronchopulmonary dysplasia

Energy balance methodology was available for only the latter part of the research period. Energy intake and losses, and body growth were studied in 4 babies with BPD and 4 controls who were selected as a convenience sample of preterm infants less than 32 weeks gestation, distinct from the VLBW cohort. All studies were done at a postnatal age of 3-8 weeks when the baby was on full enteral feeds and clinically stable. All BPD babies were still receiving supplemental oxygen via ventilator, headbox or nasal cannula.

The methods used were essentially the same as those used for the study of babies with congenital heart disease.

Although attempts were made to devise a system to measure energy expenditure by indirect calorimetry in babies receiving supplemental oxygen or artificial ventilation, technical problems highlighted by other workers (Kalhan and Denne 1990), precluded this.

2.2.6 Bone mineral content in bronchopulmonary dysplasia

Bone mineral content was studied longitudinally in a subset of 54 babies within the original cohort of VLBW infants (described in 2.2.4). The method used was dual energy radiographic densitometry of the forearm using a portable X-ray machine (Williams et al 1994, modified from Lyon et al 1989). Using this technique, weekly measurements were taken of total bone mineral and bone mineral per unit length of the ulna, when possible of the same forearm. In phantom studies, this technique has a coefficient of variation of 7%.

Of the cohort of babies studied, it was possible to match 10 infants with BPD with control infants, for gestation and birthweight, thus providing a nested cohort study. The pattern of postnatal change in bone mineral content was compared in the matched groups.

The effect of systemic corticosteroid treatment used for the treatment of BPD on bone mineral content and linear growth was examined by comparing their postnatal course in BPD babies separated into 2 groups depending on whether or not they received dexamethasone. This was an exploratory study of the possible effect of dexamethasone on bone mineral, and no sample size calculation was done. No attempt was made to match cases and controls for this study, except for the diagnosis of BPD. Dexamethasone was given to babies with BPD on clinical indication. The usual practice was to give it to babies who were still ventilator-dependent at around 1 month of age, to help ventilator weaning. It was started at a dose of 0.5 mg/kg/day and reduced over about 30 days (Avery

et al 1985), the schedule being influenced by the severity of lung disease, clinical response to steroid treatment, and the severity of any adverse effects.

In the group given steroids data were analysed for the 4 weeks before treatment, and also during and up to 4 weeks after treatment. In the control group, the "treatment" period was derived from the median times of starting and stopping treatment in the steroid group. There were 15 infants with sufficient data (at least 2 measurements before starting, and 2 during treatment) in each group.

2.2.7 Statistical analysis

Statistical analysis was done using Statview software for Apple Macintosh computers (SAS Institute Inc, USA) and SPSS software for PC (SPSS Inc, USA).

Nutritional outcome variables were assumed to be distributed in a non-Gaussian way and described using median and range or quartiles and groups compared using the Mann Whitney U test. For comparison of longitudinal data between groups, separate significance tests were carried out at each time point and the P value corrected for repeated measures. Multiple logistic regression analysis was used to study the independent predictive value of explanatory variables on particular outcomes. Deviation of anthropometric data from the population norm was computed from British Growth Reference data (Cole et al 1998) and software to calculate the z-score (Child Growth Foundation, London).

Chapter 3 Results

3.1 CONGENITAL HEART DISEASE

For Methods, see 2.1
For Discussion, see 4.1

3.1.1 Subjects

Following an initial period of development and validation of methodology, case recruitment was over a period of 18 months for the studies of intake and losses, and 1 year for energy expenditure. Hence, only a limited number of infants had a complete energy balance study performed. A requirement for selection for the energy balance studies was that infants were in hospital for at least 3 days, in a stable clinical condition. This necessarily selected infants with certain types of heart disease. Gas exchange measurements required a shorter period of study. Control infants were considerably more difficult to recruit, as they had to be hospitalized, but well, infants. Most were previously healthy infants recovering from some minor illness on a children's ward.

30 infants were included in the study; 21 with congenital heart disease, 9 controls.

Table 1. Energy balance in CHD: Studies and number of subjects

	Total no of infants	Energy intake + losses alone (EI+EL)	Gas exchange alone (EE)	Both parts of study (All)
CHD	21	7	3	11
Control	9	0	4	5

The infants are described in Table 2, together with diagnoses and some of the clinical features in cardiac infants. Several of the cardiac infants had cardiac catheterization around the time of the study.

During the studies, none of the infants was acidotic or had serum electrolyte abnormality. All remained clinically and biochemically stable except one infant with ventricular septal defect who required increased drug treatment because of worsening heart failure - this study had to be repeated.

Table 2. Description of cardiac and control infants studied

	Post-natal age at study (days)	Post-term age at study (days)	Diagnosis	Heart failure	Cyanosis	Pulmonary hypertension	Studies
(a) Cardiac infants							
1	113	113	VSD	+	0	+	EI+EL
2	126	126	VSD, ASD	+	0	0	EI+EL
3	21	14	VSD, ASD	+	0	+	EI+EL
4	121	121	VSD, PDA ligated, PA banded	+	0	+	EI+EL
5	12	5	TGA, Rashkind	0	+	0	EI+EL
6	62	55	Tricuspid atresia, PDA	0	+	0	EI+EL
7	43	22	Fallot's tetralogy	0	+	0	EI+EL
8	18	18	VSD	+	0	0	All
9	103	103	Coarctation repaired, mitral stenosis	+	0	+	All
10	59	59	VSD	+	0	0	All
11	75	75	Coarctation repaired, VSD	+	0	+	All
12	50	43	TGA, Rashkind. VSD, PDA	0	+	0	All
13	44	30	VSD	+	0	0	All
14	246	246	VSD, aortic stenosis	+	0	+	All
15	5	-9	TGA, Rashkind. VSD, PDA	+	+	0	All
16	61	61	VSD	+	0	?	All
17	104	69	VSD	+	0	+	All
18	44	44	Tricuspid atresia, VSD	+	+	?	All
19	34	34	PDA	+	0	0	EE
20	49	49	Tricuspid atresia, VSD	+	+	0	EE
21	30	30	Coarctation repaired, PDA, double inlet RV	+	0	0	EE
(a) Control infants							
1	56	49	Upper respiratory tract infection				All
2	42	0	Ex-preterm 34 weeks gestation				All
3	86	86	Feeding problem				All
4	68	68	Upper respiratory tract infection				All
5	56	-14	Ex-prem 30 weeks gestation; URTI				All
6	73	73	URTI				EE
7	35	35	Breath-holding attacks				EE
8	5	5	Normal newborn				EE
9	14	14	?seizure				EE

3.1.2 Birthweight and Gestational age

Birthweight data were available from the birth records for the 5 hospitalized controls who had complete energy balance studies, and for all except 2 of the CHD infants.

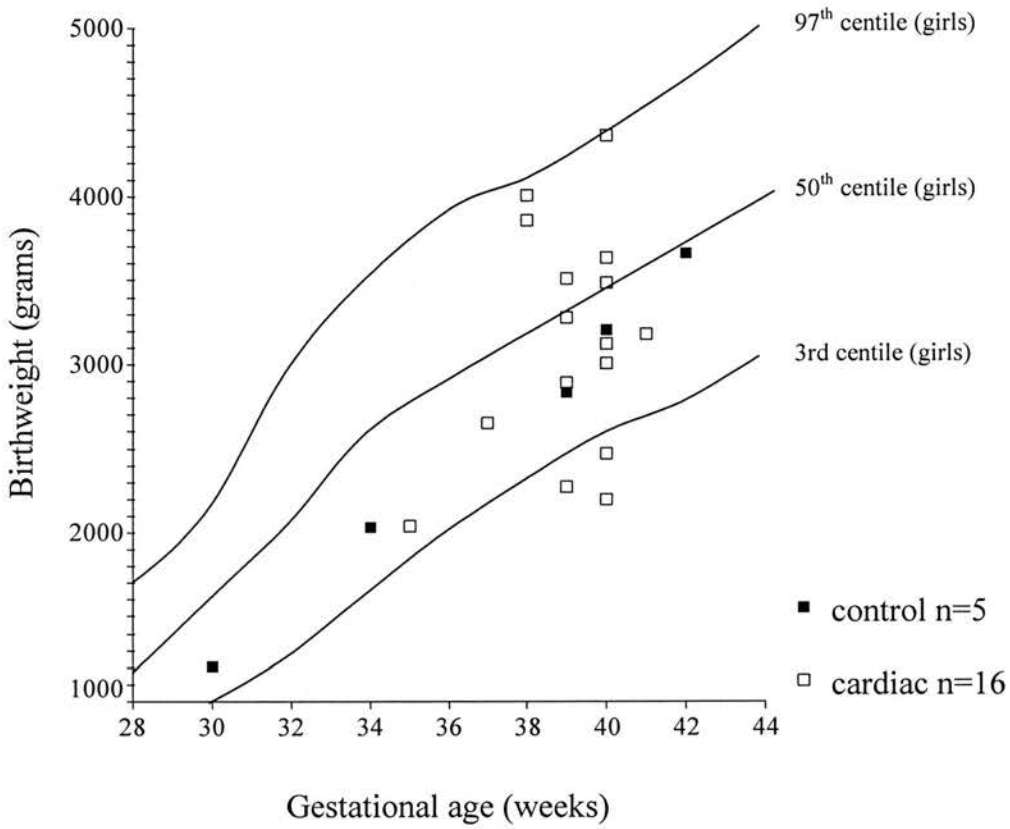

Fig. 1. Gestational age and birthweight of study and control infants

All the control infants had birthweights between the 10th and 50th percentiles for gestation. Of the cardiac infants, 3 were at about the 97th centile, and 3 were below the 3rd centile, the others being between these extremes.

3.1.3 Age at study

Babies were studied at a postnatal age between 5 and 246 days, with a post-term age of –14 to 246 days. Gestation at birth varied from 30 to 41 weeks.

Table 3. Post-term age of babies studied

median and range	**All babies**	**Energy intake + losses**	**Gas exchange**	**Both parts of study**
CHD	49 (-9 to 246)	57 (-9 to 246)	46.5 (-9 to 246)	59 (-9 to 246)
Control	35 (-14 to 86)	49 (-14 to 86)	35 (-14 to 86)	49 (-14 to 86)

3.1.4 Weight as a proportion of expected weight, with age, in cardiac infants

There was a steady fall in study weight standard deviation score (SDS) with age for cardiac infants.

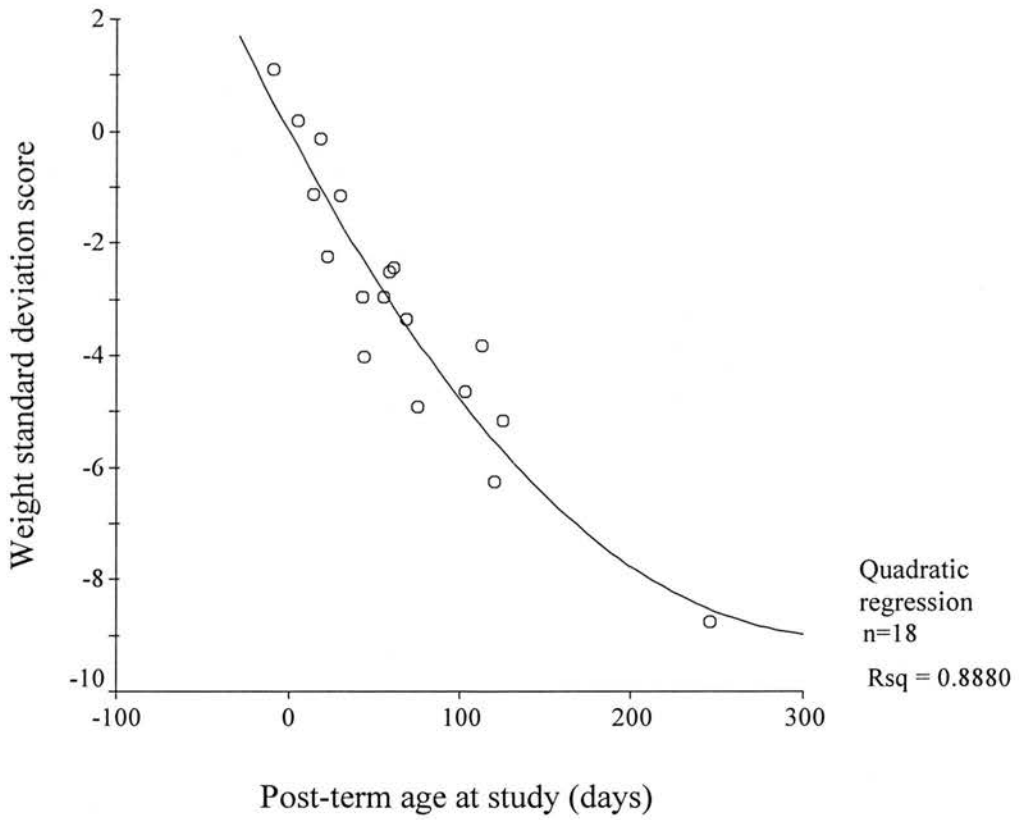

Fig. 2. Study weight standard deviation score and post-term age: cardiac infants

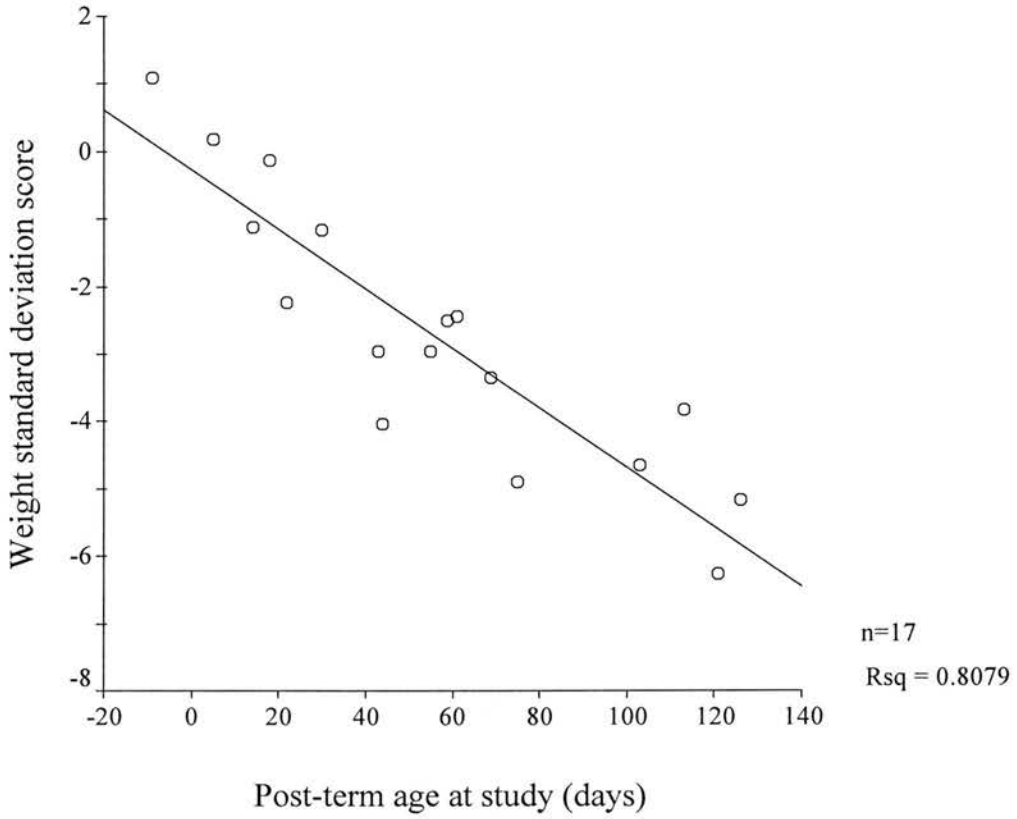

Fig. 3. Body weight standard deviation score and post-term age: cardiac infants without outlier

This cross-sectional data suggests a 1SD drop, compared to population norms every 21 days in body weight. By 120 days of age, babies were, on average, only 50% of their expected weight, although only 3 of the babies were studied after this age. The worst affected child was more than 8SD below the mean for weight. In Fig 3, an outlier of 246 days has been excluded. More than 80% of the variability of weight SDS was explained by age.

3.1.5 Body length and head circumference with age in cardiac infants

Only those infants who were hospitalized for long enough to have EI and EL measurements (18 CHD and 5 control infants) had complete anthropometry, hence this information is available for only a proportion of the infants.

A postnatal fall off in centile level was also seen in these parameters, but to a lesser extent than in weight. There was a 1SD reduction compared to population norms in

length every 43 days, and head circumference every 37 days. However, only around 60% of the variability in these measurements was explained by age.

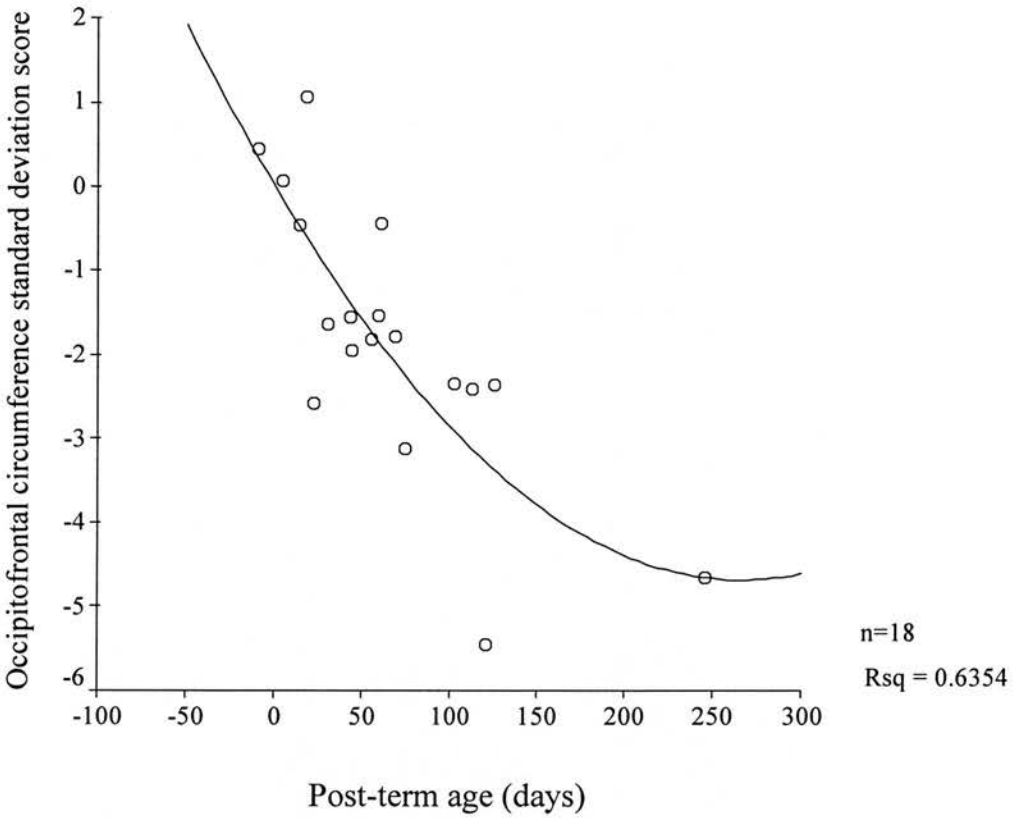

Fig. 4. Head circumference standard deviation score and post-term age: cardiac infants

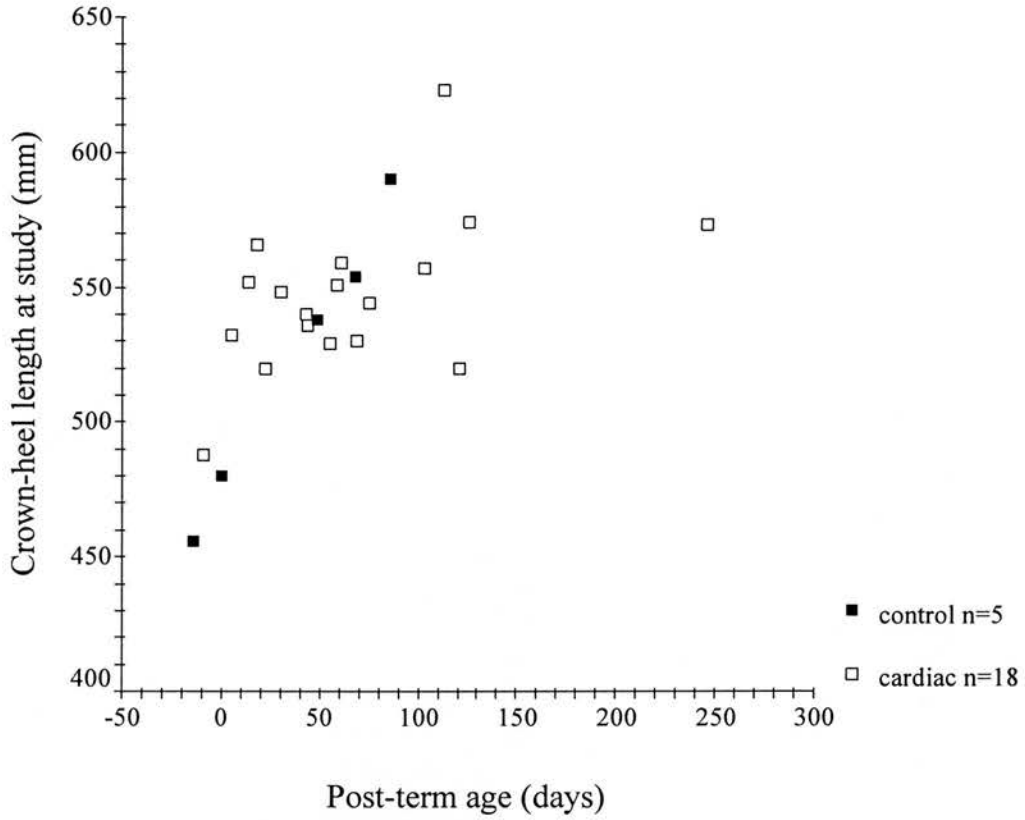

Fig. 5. Body length with age, comparing cardiac and control infants

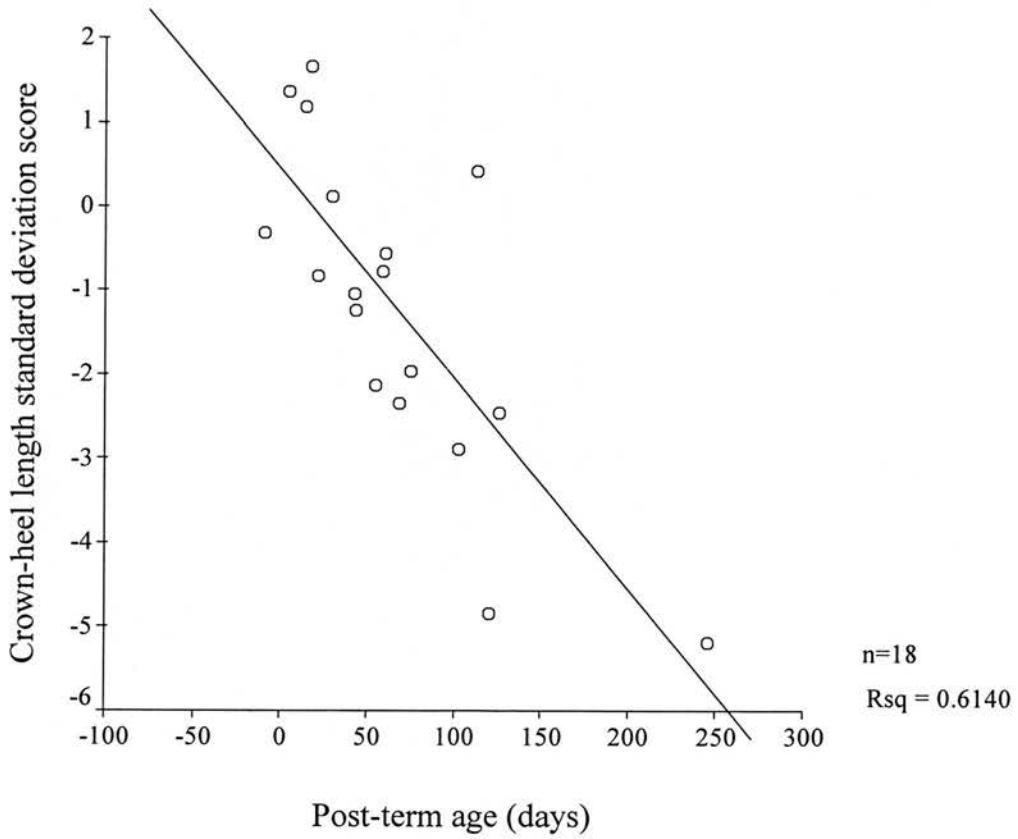

Fig. 6. Body length standard deviation score and post-term age: cardiac infants

3.1.6 Weight gain during balance study

The daily weight gain [median (quartiles)] of the cardiac infants [11.0g (2.5-16.7)] was less than that of the control infants [39.0g (20.0-47.5)], and that expected for age. Some cardiac infants gained no weight. The difference was statistically significant, P=0.0034, using the Mann-Whitney U test. There was a similar difference between the two groups in weight gain/expected gain for age (Fomon et al 1971): cardiac 0.41 (0.10-0.54) and control 1.11 (0.75-2.06), P=0.0017. 2 outliers in age in the cardiac group aged 5 days and 246 days have been excluded from the analysis.

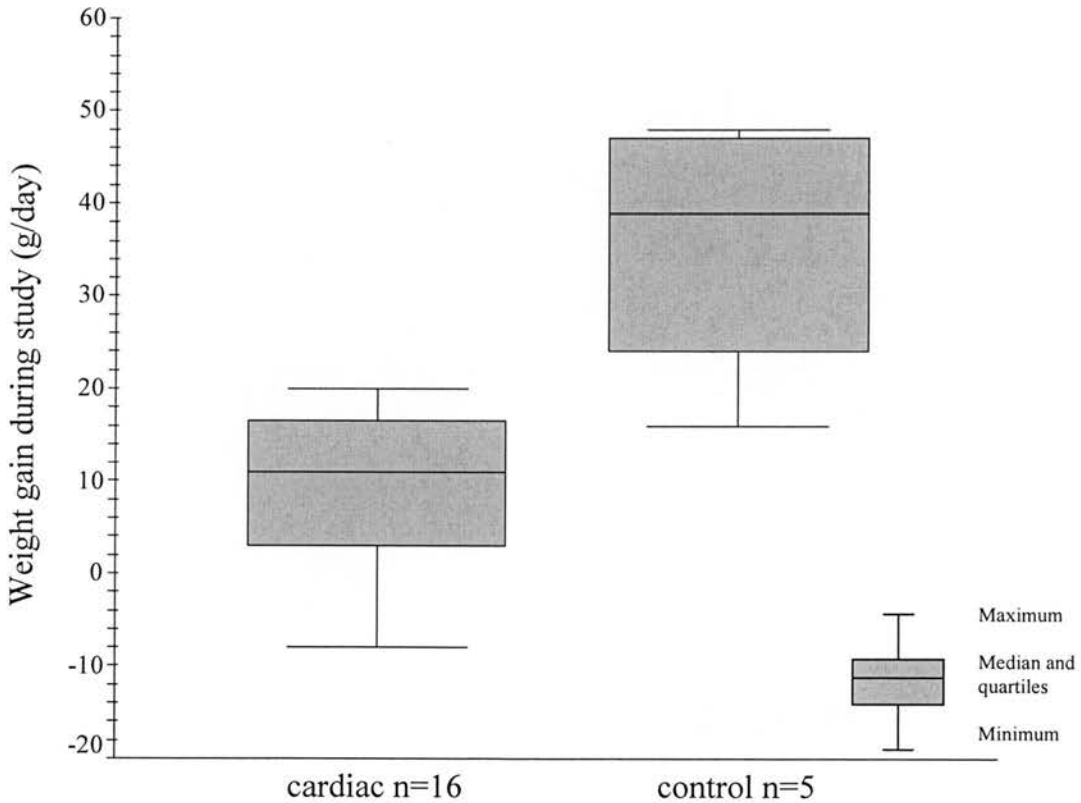

Fig. 7. Weight gain during balance study, comparing cardiac and control infants

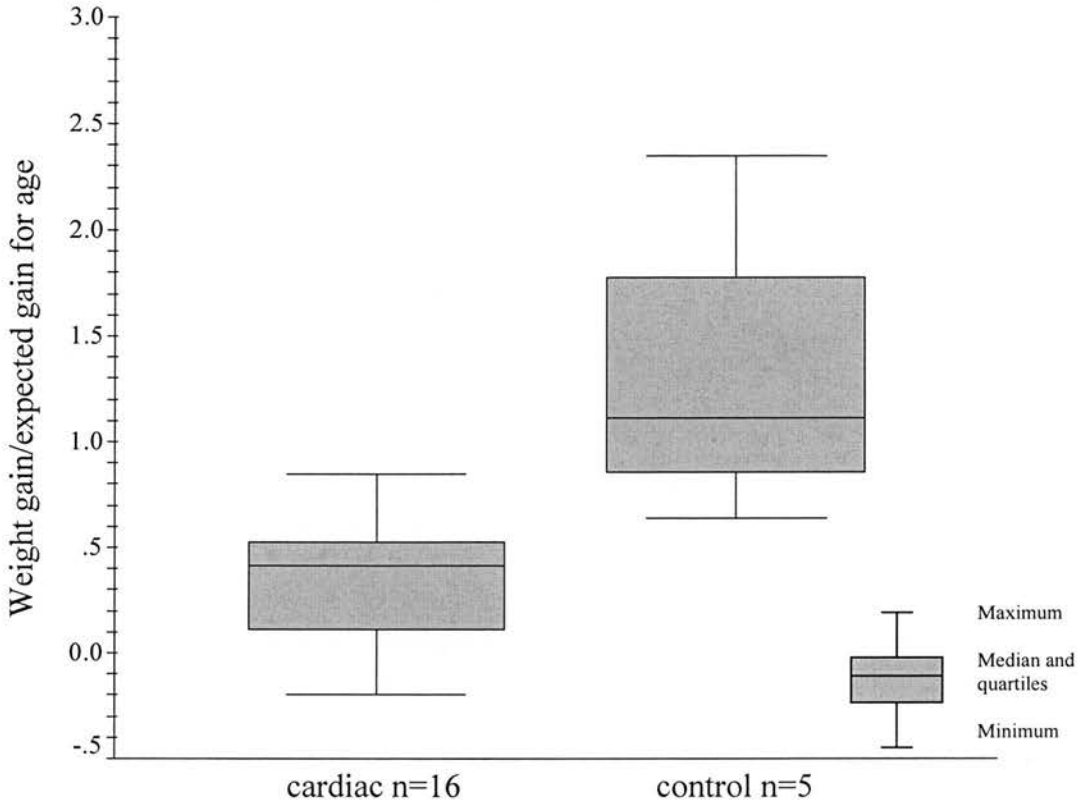

Fig. 8. Weight gain during balance study as a proportion of expected gain, comparing cardiac and control infants

3.1.7 Energy value of milks

The values obtained for heat of combustion by bomb calorimetry are shown in Table 4, with, for comparison, values provided by manufacturers which are calculated as the metabolizable energy of the constituents (Widdowson et al 1960).

Different batches of the same formula showed no significant difference in energy content.

Table 4. Energy value of milks

Brand name	Heat of combustion (kJ/100g)	Calculated metabolizable energy (kJ/100ml)
Cow and Gate premium	305	285
Cow and Gate plus	305	272
Osterfeed	292	285
Ostermilk comp.	275	272
SMA goldcap	292	272
Cow and Gate prematalac	365	331
Nenatal	332	318
Milumil	281	289

3.1.8 Daily intake of milk volume and energy

There was a wide spread of values with a large degree of overlap between the two groups: for volume, Mann-Whitney U test P = 0.06; for energy intake, Mann-Whitney U test P = 0.14 (Fig. 9, Table 5). When energy intake was expressed per kg 50th centile weight for age, there appeared to be a bigger difference, but this did not reach statistical significance: Mann-Whitney U test P = 0.09 (Fig. 10, Table 5).

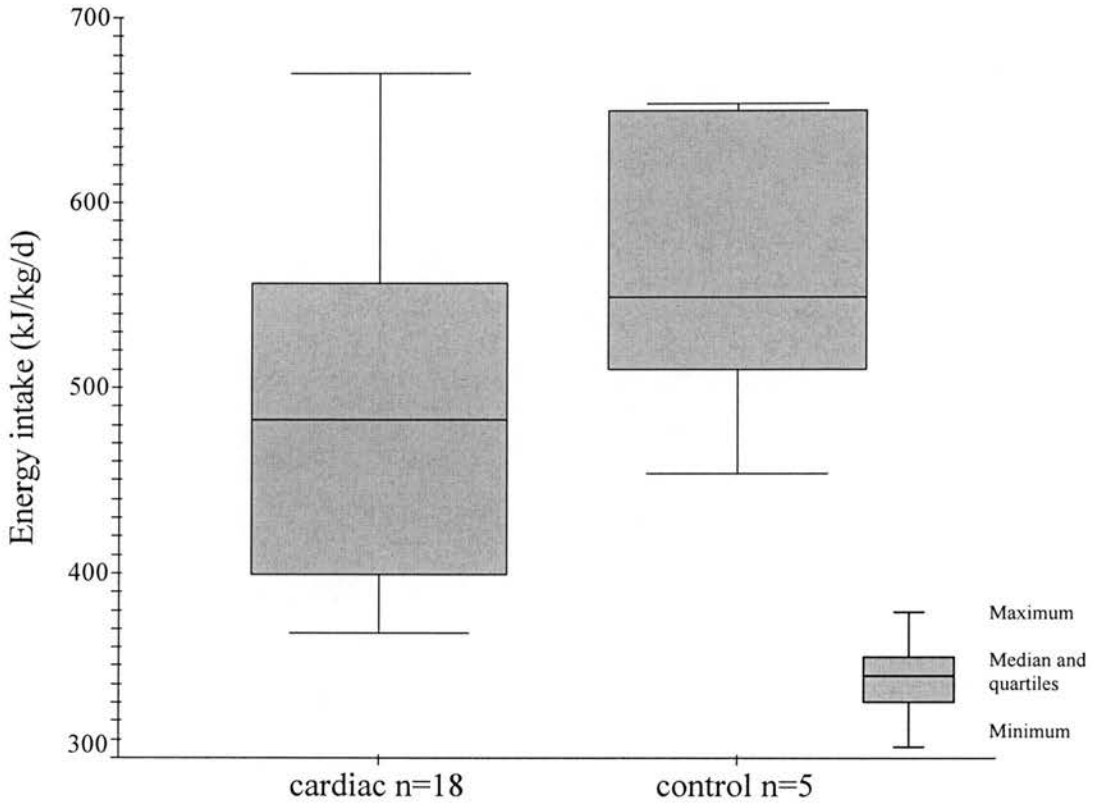

Fig. 9. Daily energy intake, comparing cardiac infants with controls

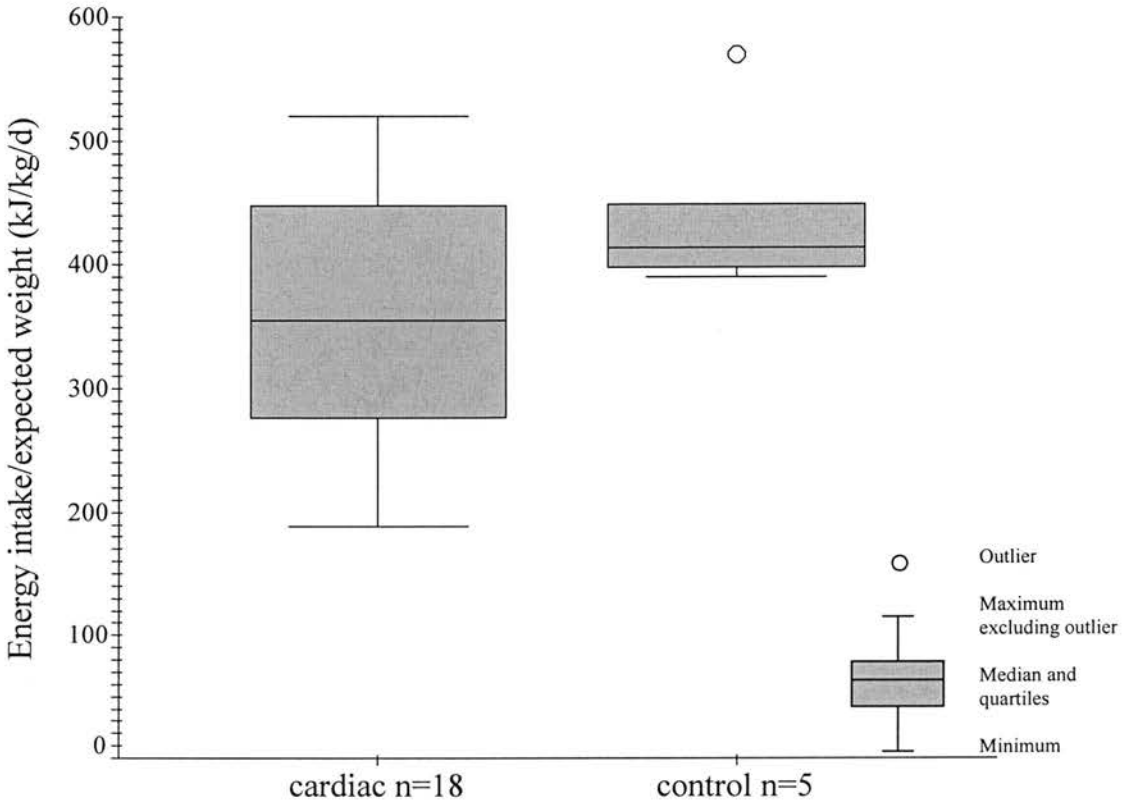

Fig. 10. Daily energy intake for expected weight, comparing cardiac infants with controls

3.1.9 Energy losses in urine and vomit

There was a wide inter-individual variation, with no significant difference between the 2 groups: [median (quartiles)]: cardiac 27.4 kJ/kg/day (19.4-37.1), control 22.6 kJ/kg/day (18.9-31.7); Mann-Whitney U test, P = 0.54. One cardiac infant vomited 11% of feed intake, and 9/18 cardiac infants and 2/5 controls vomited 5-10% of intake. If allowance is made for the systematic shortfall in energy yield by processing nappies and bibs (11.8%), the largest correction to MEI would be about 6kJ/kg/day.

3.1.10 Energy losses in Stool

Energy losses in stool tended to decrease in both groups with age. Values varied from 11.5 to 108.4 kJ/kg/d and 2.9 to 19.7% of energy intake. There was a large overlap between cardiac infants and controls [median (quartiles)]: cardiac 45.2 kJ/kg/day (33.3-62.2), control 73.6 kJ/kg/day (30.2-98.2); Mann-Whitney U test, P = 0.23.

3.1.11 Metabolizable energy intake (MEI)

MEI was, in general, lower in cardiac infants, especially as a proportion of that expected, but the difference did not reach statistical significance [values are medians (quartiles)]: for cardiacs 417kJ/kg/d (348-451), for controls 455kJ/kg/d (405-548), Mann Whitney U test P=0.22 (Table 5 and Fig.11).

Table 5. Energy intake and losses, and metabolizable energy in cardiac and control infants

Baby	Daily energy intake (kJ/kg/d)	Daily energy intake for expected weight (kJ/kg/d)	Energy loss in stool (kJ/kg/d)	Energy loss in urine and vomit (kJ/kg/d)	Metabolizable energy intake for body weight (kJ/kg/d)
Cardiac					
1	400	260	11.5	10.5	378
2	670	376	36.2	19.8	615
3	557	476	50.3	28.1	418
4	391	179	34.7	17.8	337
5	471	469	61.8	53.5	357
6	637	435	81.9	26.4	529
7	510	342	53.4	37.0	420
8	501	520	37.3	29.9	435
9	418	236	34.1	41.1	345
10	383	292	31.1	38.0	315
11	593	325	52.4	36.6	492
12	556	377	24.5	18.3	513
13	368	305	57.2	20.7	307
14	481	188	19.1	15.2	445
15	397	418	78.3	26.0	291
16	516	402	63.6	35.2	416
17	401	234	40.1	26.7	422
18	484	282	65.7	37.3	381
Median	482	355	45.2	27.4	417
Control					
1	649	552	39.2	22.6	590
2	549	392	73.6	18.4	455
3	510	445	88	19.4	403
4	454	414	21.2	24.1	408
5	654	398	108.4	39.3	506
Median	548	414	73.6	22.6	455

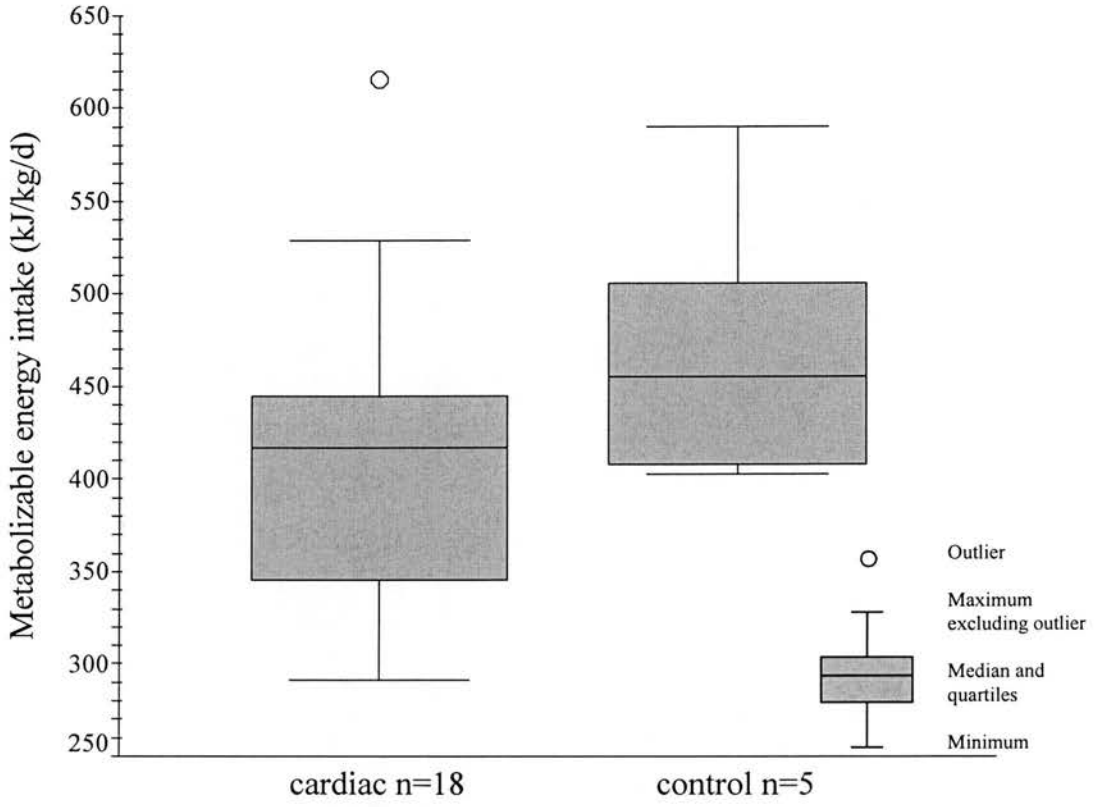

Fig. 11. Metabolizable energy intake, comparing cardiac infants with controls

MEI as a percentage of recommended dietary allowance for age (RDA, DHSS, 1979) showed a positive correlation with weight gain during the study (Fig. 12); this was statistically significant for cardiac infants, although MEI explained less than 30% of the variability in weight gain (Fig. 12, $r^2 = 0.28$; $P = 0.024$).

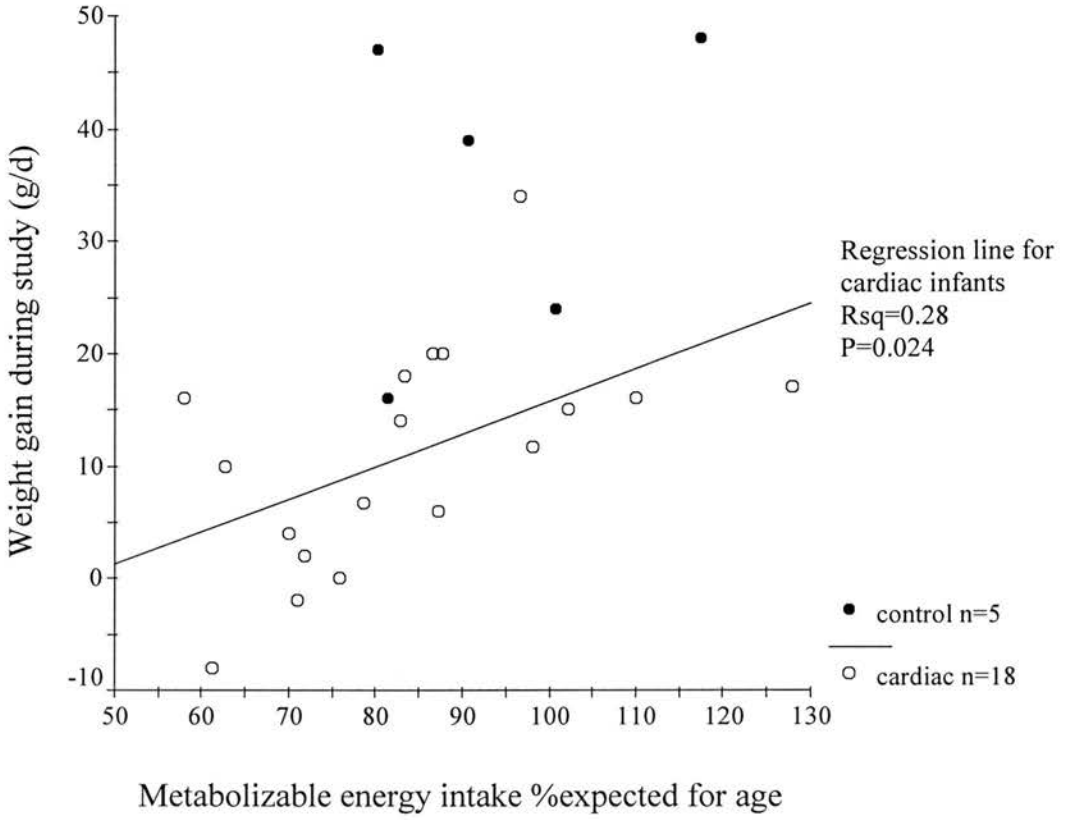

Fig. 12. Metabolizable energy intake as % expected for age, and weight gain

On analysis of covariance, MEI %RDA and the presence of congenital heart disease showed significant independent correlations with weight gain.

Table 6. Independent predictors of weight gain during balance study

Independent correlations with weight gain	Coefficient	Standard error	P value
MEI %RDA	0.29	0.121	0.0262
CHD	-20.663	5.122	0.0006

3.1.12 Sleeping oxygen uptake (SV_{O2}), and resting energy expenditure

Measurements used for analysis of SV_{O2} were for a minimum period of 30 minutes, and were all at least 1 hour after a feed. Resting energy expenditure (REE) was calculated from a cumulative period of resting measurement of between 3 and 4 hours.

Table 7. Sleeping oxygen uptake and respiratory quotient, and estimated values for daily energy expenditure and energy available for deposition: cardiac and control infants

Baby	Postnatal age (days)	SV_{O2} (ml/kg//min)	Mean sleeping RQ	MEI (kJ/d)	Resting energy expenditure (REE, kJ/d)	Energy available for deposition (MEI minus REE, kJ/d)
Cardiac						
8	18	7.49	.81	1761	1044	717
9	103	12.90	.81	1226	1721	-495
10	59	9.10	.84	1142	1038	104
11	75	13.88	.84	1418	1353	65
12	50	10.57	.78	1653	1031	622
13	44	11.09	.86	1172	1202	-30
14	246	12.70	.79	1464	1236	228
15	5	7.95	.84	1075	904	171
16	61	10.16	.80	1556	1131	425
17	104	9.45	.81	1452	986	466
18	44	10.01	.73	1021	779	242
19	34	9.86	.72			
20	49	9.83	.77			
21	30	7.70	.84			
Control						
1	56	9.23	.89	2330	1276	1054
2	42	9.14	.82	1138	728	410
3	86	10.75	.77	1912	1377	535
4	68	8.75	.81	1845	1685	160
5	56	11.66	.81	1017	670	347
6	73	9.27	.85			
7	35	9.89	.82			
8	5	7.50	.82			
9	14	7.75	.82			

SV_{O2} (mls/min) and SV_{O2} per kg body weight tended to increase with age. It was particularly high in 3 of 4 infants with persistent cardiac failure and pulmonary hypertension (pht, see Fig. 13). SV_{O2}/kg correlated inversely with measures of body fat (summed skinfold thicknesses, SSFT, and body mass index, BMI). SSFT showed an exponential relationship to SV_{O2}/kg and log SSFT showed a stronger correlation with SV_{O2}/kg: r^2=0.671; P=0.0001 (Fig. 14). SV_{O2} correlated with estimated metabolic body size (weight$^{3/4}$ and weight$^{1/2}$) for control infants (r= 0.86, P<0.01), but not for cardiacs (r=0.37).

On multiple regression analysis, SV_{O2} for all babies correlated independently with weight, estimated metabolic weight, and log (summed skinfold thicknesses). In this analysis, postnatal age and the presence of CHD were not independent predictors of SV_{O2} (Table 8).

Table 8. Independent predictors of sleeping oxygen uptake in cardiac and control infants

Independent correlations with SV_{O2}	coefficient	Standard error	P value
Study weight	0.011	0.002	0.0001
log SSFT	-34.5	9.67	0.0044
Postnatal age	0.025	0.021	0.27
CHD	0.94	1.89	0.63

Sleeping respiratory quotients (mean +/-sd) were similar in the 2 groups (CHD 0.81 +/- 0.04; control 0.83 +/- 0.03). Resting energy expenditure showed a large overlap between cardiac and control infants (Table 7).

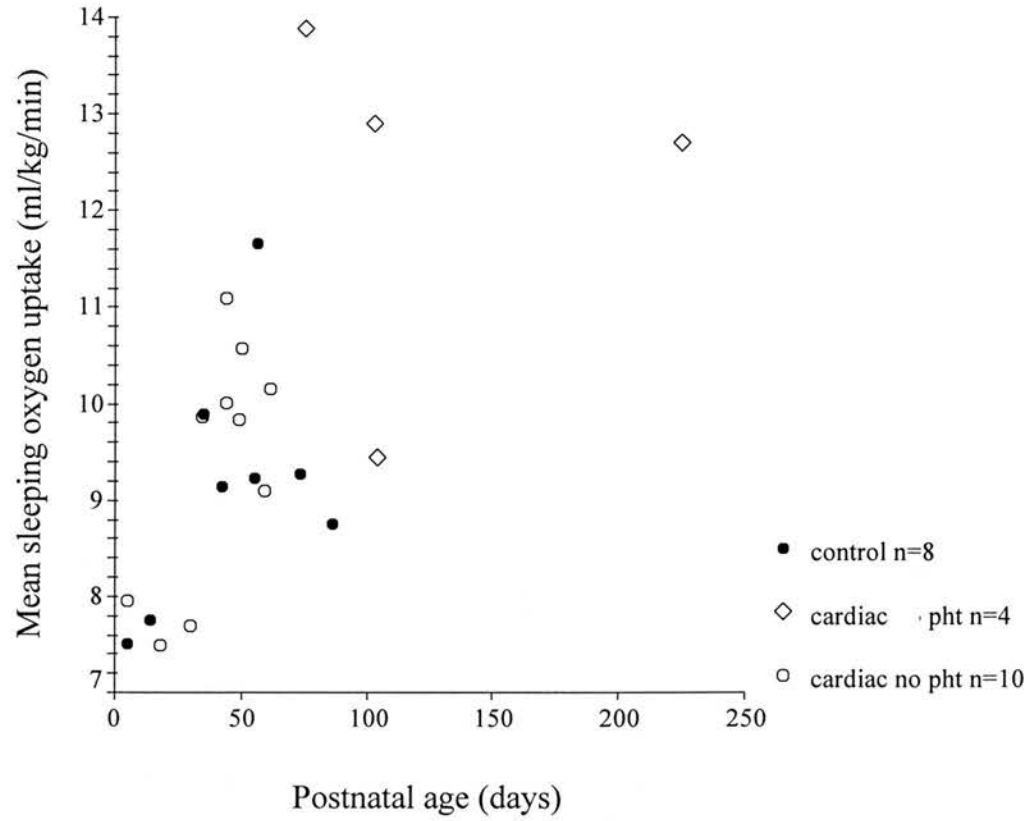

Fig. 13. Change in sleeping oxygen uptake with age

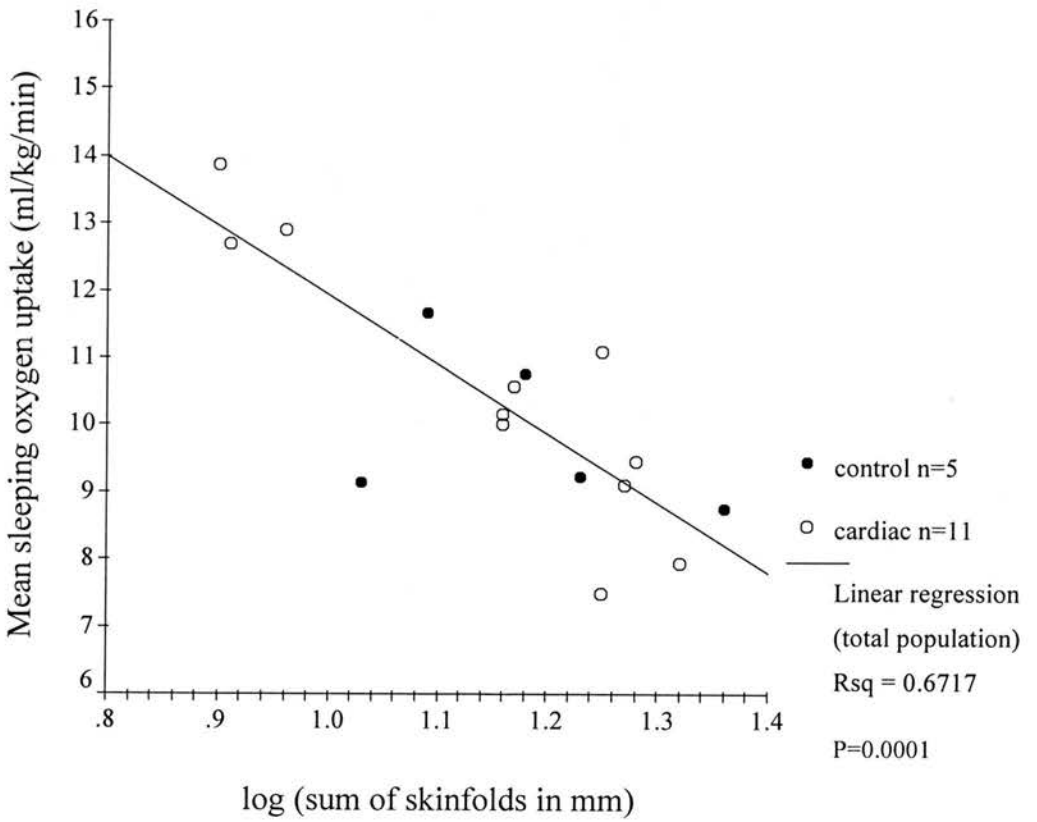

Fig. 14. Relationship between sleeping oxygen uptake and skinfold thicknesses

3.1.13 Energy available for deposition (EAD)

EAD, which is a component of the energy cost of growth, was extrapolated for those infants who had a complete study performed, as: (MEI - REE). It should be noted that REE will be lower than total energy expenditure, and therefore EAD is likely to be an overestimate. EAD showed a large inter-individual variation with considerable overlap between study and control groups, but there was a positive correlation between EAD and weight gain. Note that the intercept of the regression line is significantly different from zero (Fig. 15). There appears to be an outlier, with a negative value for EAD and no growth, influencing the regression line, which has thus been redrawn excluding this individual.

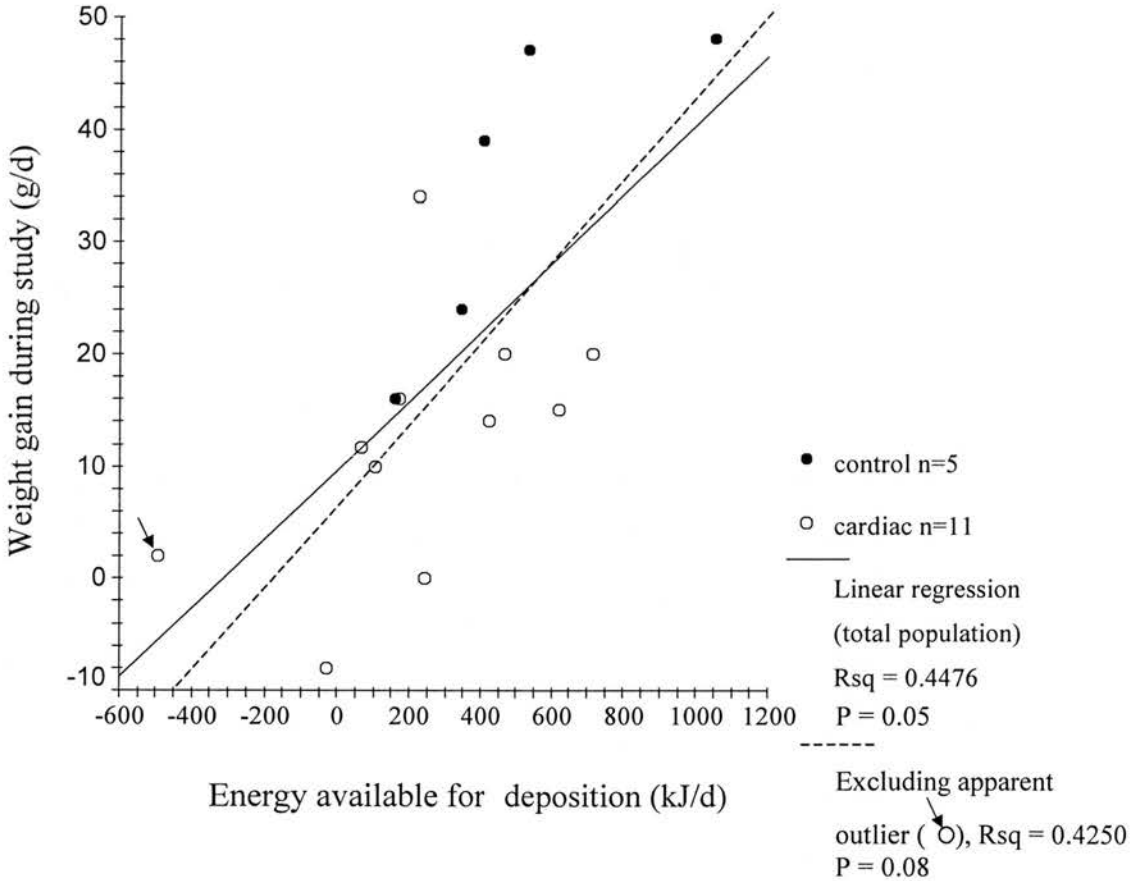

Fig. 15. Relationship between energy available for deposition and weight gain

3.2 BRONCHOPULMONARY DYSPLASIA

For Methods, see 2.2
For Discussion, see 4.2

3.2.1 Macronutrient intake and growth

(i) Subjects

Of the 195 infants in the consecutive VLBW cohort, 49 babies developed BPD. There were 20 infants with BPD and 20 matched controls with sufficient data.

Table 9. Description of BPD and control infants

median and range	BPD (n=20)	Control (n=20)
Gestation (weeks)	29 (26-30)	29 (26-31)
Birthweight (g)	1140 (905-1426)	1225 (825-1485)
Duration of ventilation (days)	15 (2-55)	1.5 (0-5)
Duration of supplemental oxygen requirement (days)	53 (31-110)	2.5 (0-7)
Postnatal steroid therapy	6	0
Post-conceptional age at discharge (weeks)	39.4 (33.0-40.9)	35.5 (30.0-40.3)

(ii) Macro-nutrient intake

The total intakes of energy, fat, and protein from enteral and parenteral sources were compared in the 2 groups for the 24 hours at the end of each week (for the first 8 weeks) after birth. Although data was available for longer in some babies, only 8 weeks of data have been analysed since after this time an increasing amount of data is missing because of discharge of babies. Mean daily intake for the 1st week was also compared between groups. The results are summarized in the Tables 10-13 and shown graphically in Figs 16-21.

(1) Energy intake

Energy intake was lower in BPD infants in the first 2 weeks of life, most strikingly for total 1st week intake, for which the difference between medians was 85.8 kJ/kg/day.

Table 10. Daily energy intake (enteral and parenteral) in BPD and control infants

ENERGY INTAKE (kJ/kg/d) *median and quartiles*	BPD n=20	Control n=20	Mann-Whitney U test: P value (with Bonferroni correction for 10 tests)
1st week daily mean	262 (210-282)	347 (293-372)	0.0003 (0.003)
end of week 1	408 (310-448)	494 (435-519)	0.0001 (0.001)
end of week 2	465 (391-559)	561 (506-604)	0.013 (0.13)

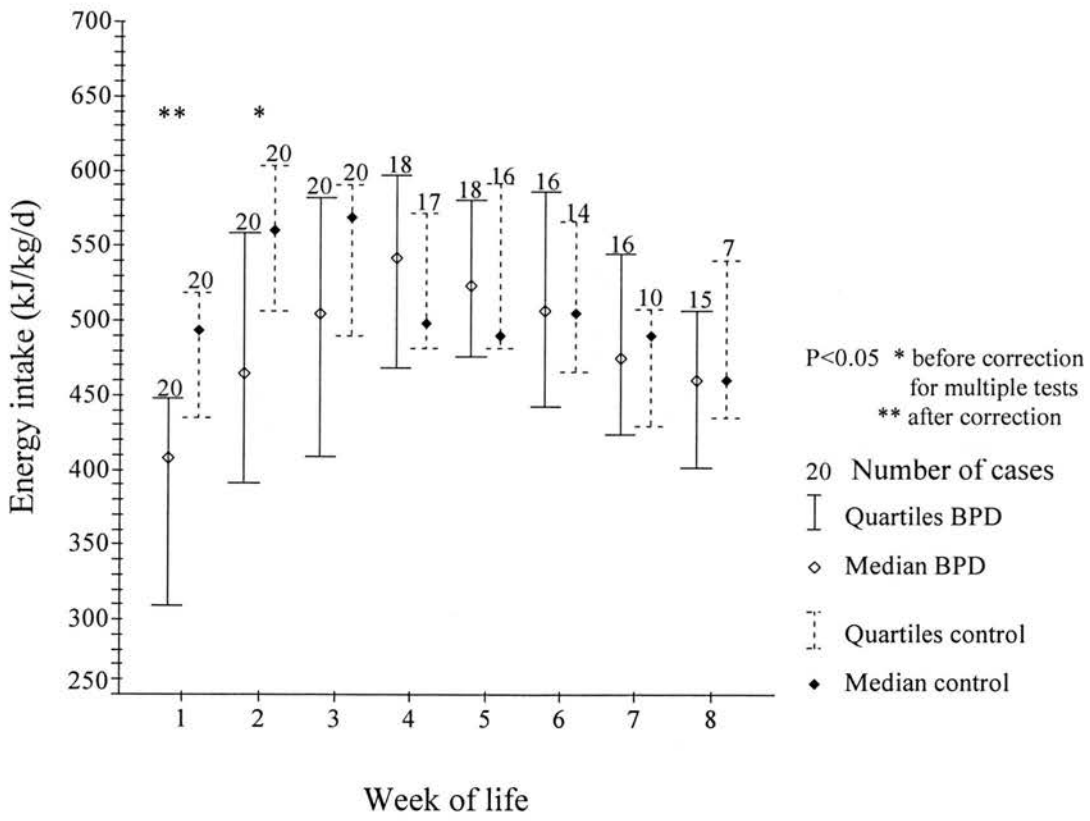

Fig. 16. Energy intake and postnatal age, comparing BPD and control infants

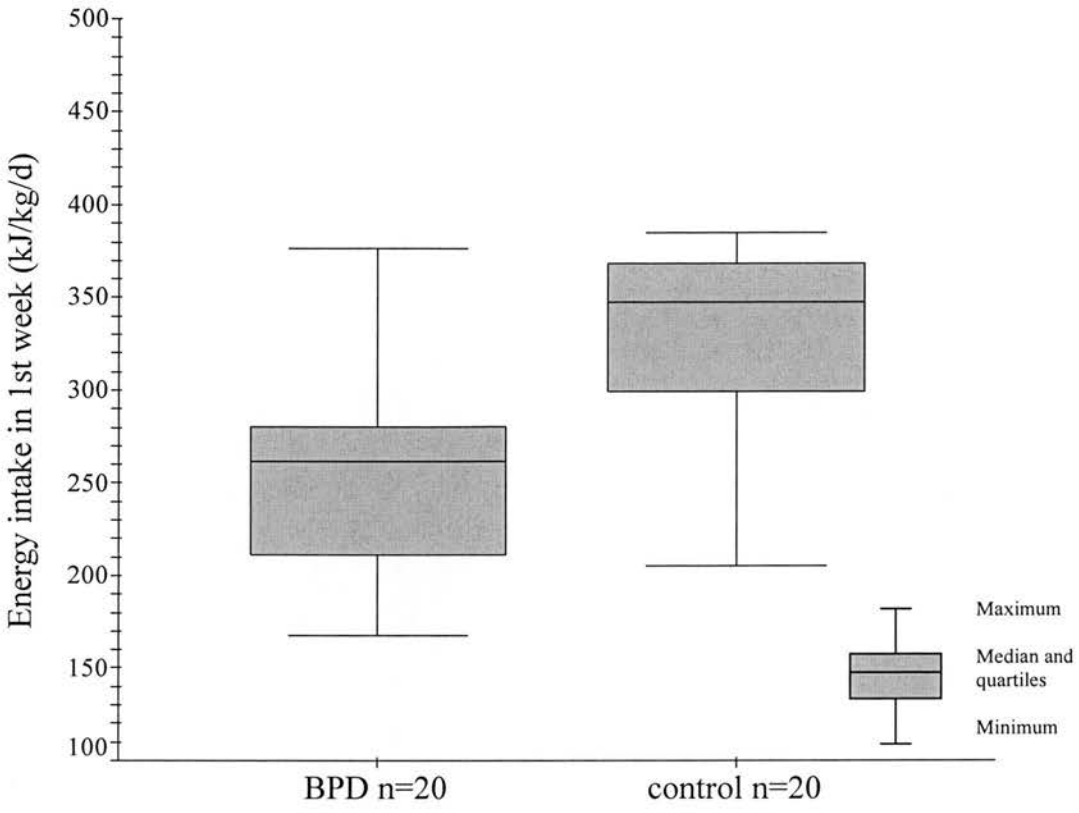

Fig. 17. Mean daily energy intake in first week, comparing BPD and control infants

(2) Fat intake

Fat intake was markedly lower in BPD infants when analysed for total intake in the first week and lower (though not reaching statistical significance after correction for multiple tests) at the end of the first week. Differences between medians were 2.15 and 1.36g/kg/d respectively.

Table 11. Daily fat intake (enteral and parenteral) in BPD and control infants

FAT INTAKE (g/kg/d) *median and quartiles*	BPD n=20	Control n=20	Mann-Whitney U test: P value (with Bonferroni correction for 10 tests)
1st week daily mean	1.73 (0.92-2.58)	3.88 (3.03-4.16)	0.0001 (0.001)
end of week 1	4.66 (1.45-5.86)	6.02 (5.22-6.95)	0.009 (0.09)

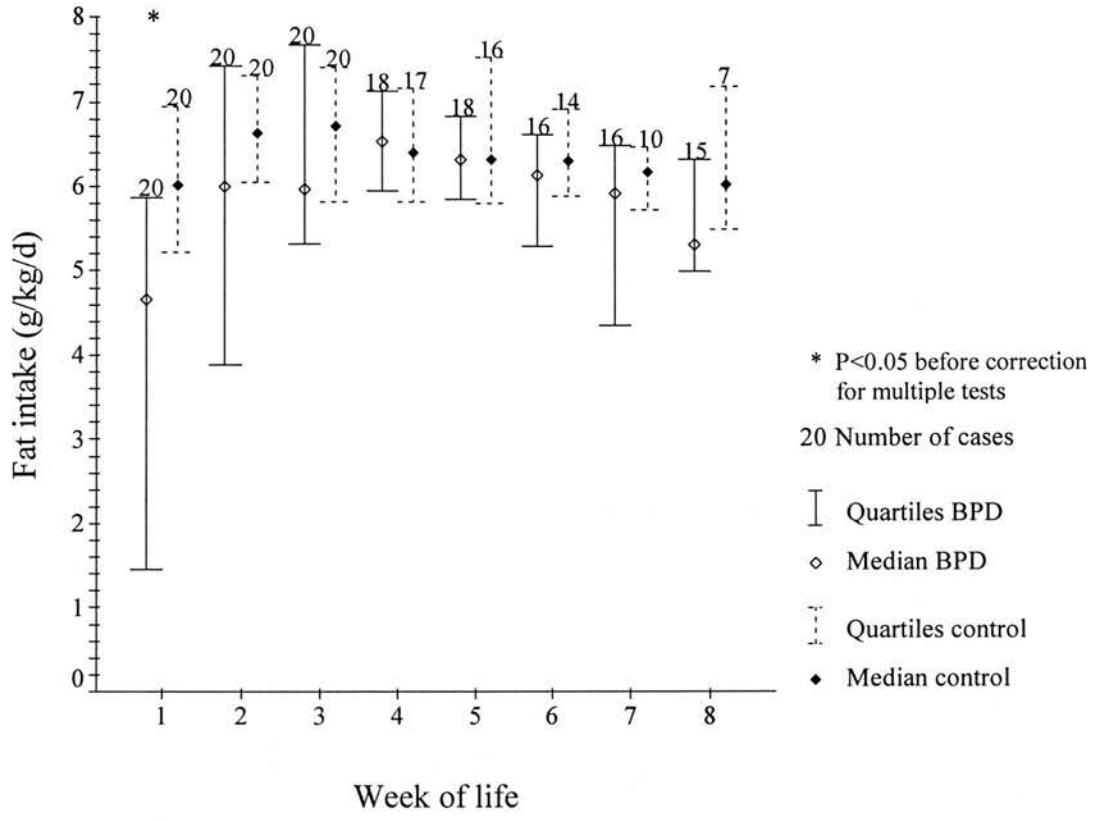

Fig. 18. Fat intake and postnatal age, comparing BPD and control infants

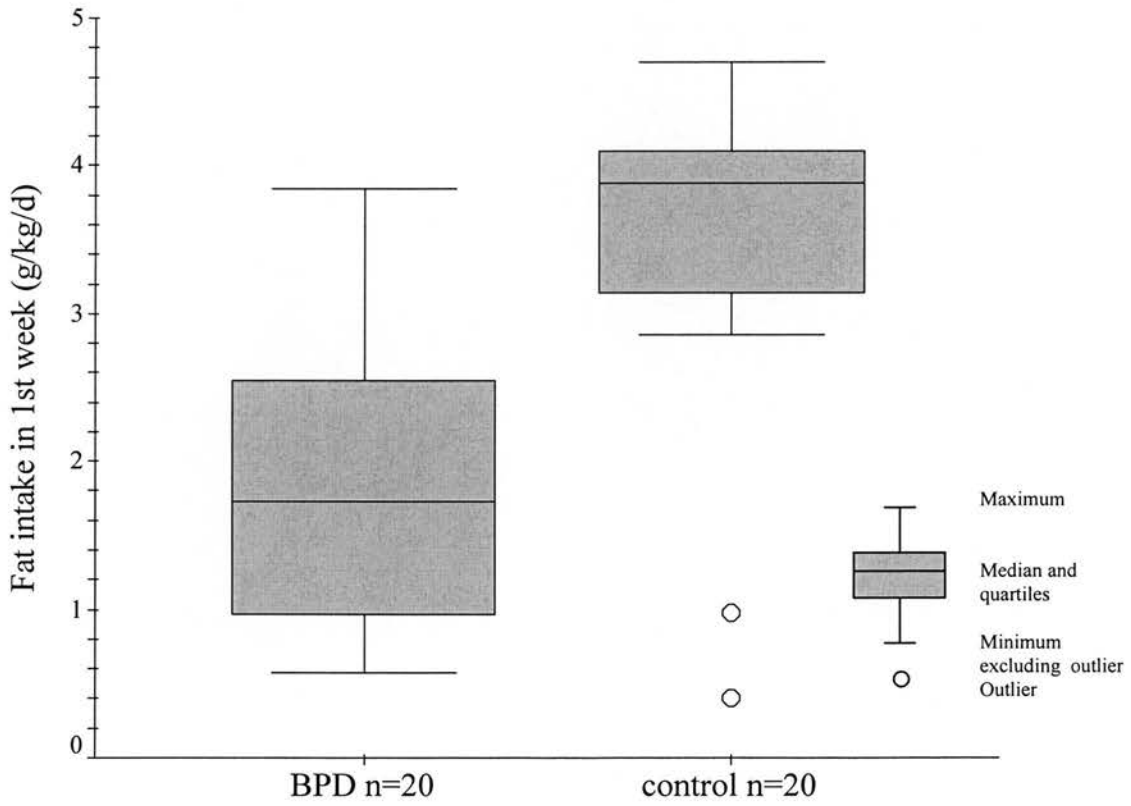

Fig. 19. Mean daily fat intake in first week, comparing BPD and control infants

(3) Protein intake

The only significant difference in protein intake (before correction for multiple tests) was in the total first week intake, with a difference between medians of 0.42g/kg/d.

Table 12. Daily protein intake (enteral and parenteral) in BPD and control infants

PROTEIN INTAKE (g/kg/d) *median and quartiles*	BPD n=20	Control n=20	Mann-Whitney U test: P value (with Bonferroni correction for 10 tests)
1st week daily mean	1.54 (1.09-1.82)	1.96 (1.64-2.21)	0.016 (0.16)

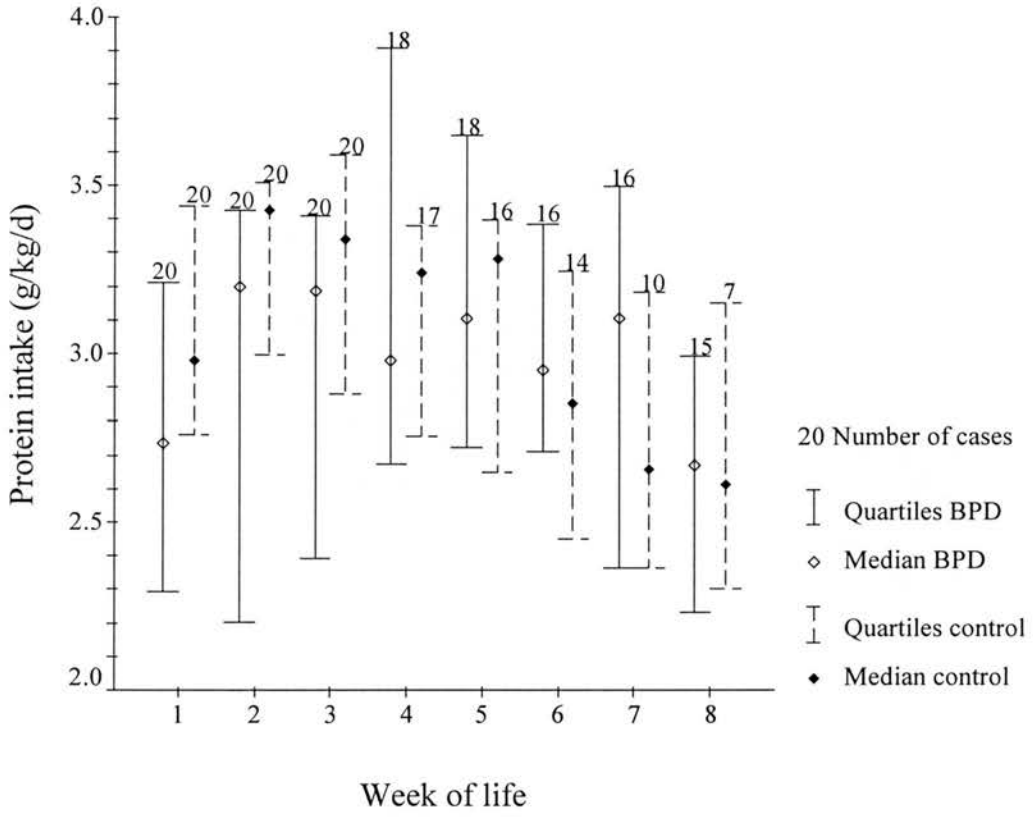

Fig. 20. Protein intake and postnatal age, comparing BPD and control infants

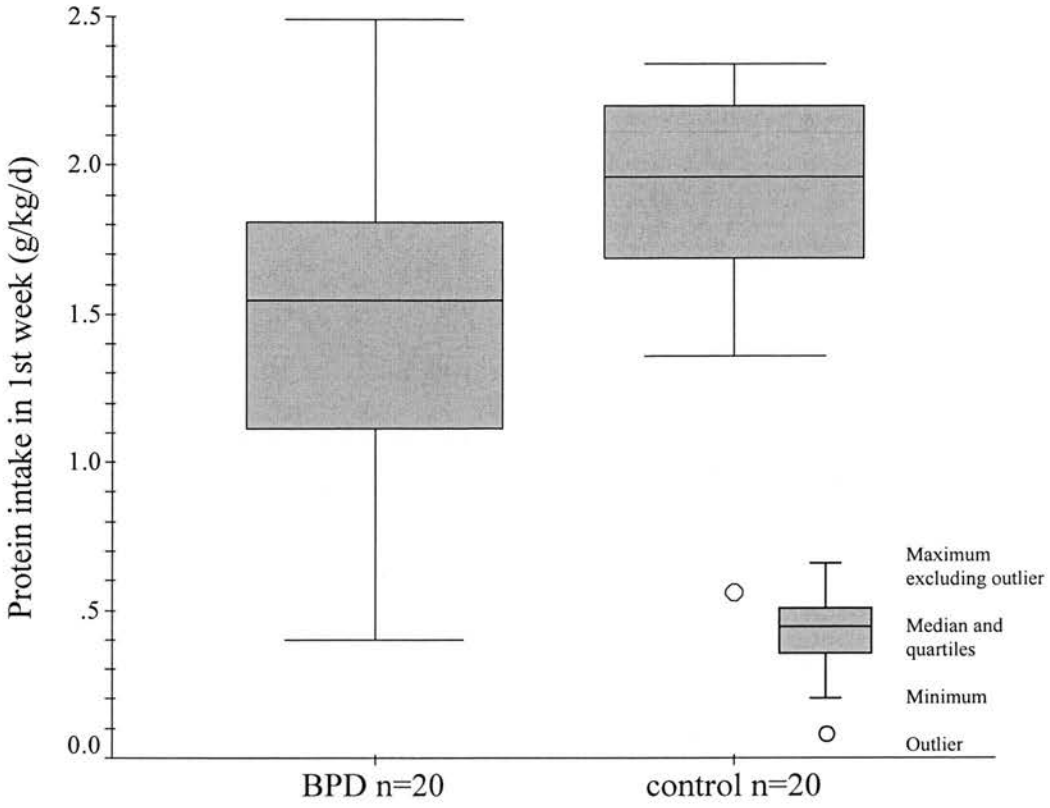

Fig. 21. Mean daily protein intake in first week, comparing BPD and control infants

(4) Enteral feeds

Enteral intake contributed significantly less to total energy intake in the first two weeks of life in infants who later developed BPD. At the end of the second week of life all control infants and 16 of 20 BPD babies were receiving full enteral feeds.

Table 13. Enteral energy as a proportion of total intake in BPD and control infants

ENTERAL ENERGY INTAKE AS PERCENTAGE OF TOTAL *Median and quartiles*	BPD n=20	Control n=20	Mann-Whitney U test: P value
1st week	68 (31.3-79.2)	91 (78.0-93.0)	0.0025
end week 1	94 (43.5-100)	100 (100-100)	0.0019
end week 2	100 (90.0-100)	100 (100-100)	0.032

(iii) Growth pattern in neonatal period

BPD infants gained weight at a slower rate after the first week than controls. Growth rates (slopes and 95% CI) were 151.5 g/week (135.5-167.6) for babies with BPD and 192.2 g/week (178.5-205.9) for controls, $P<0.05$.

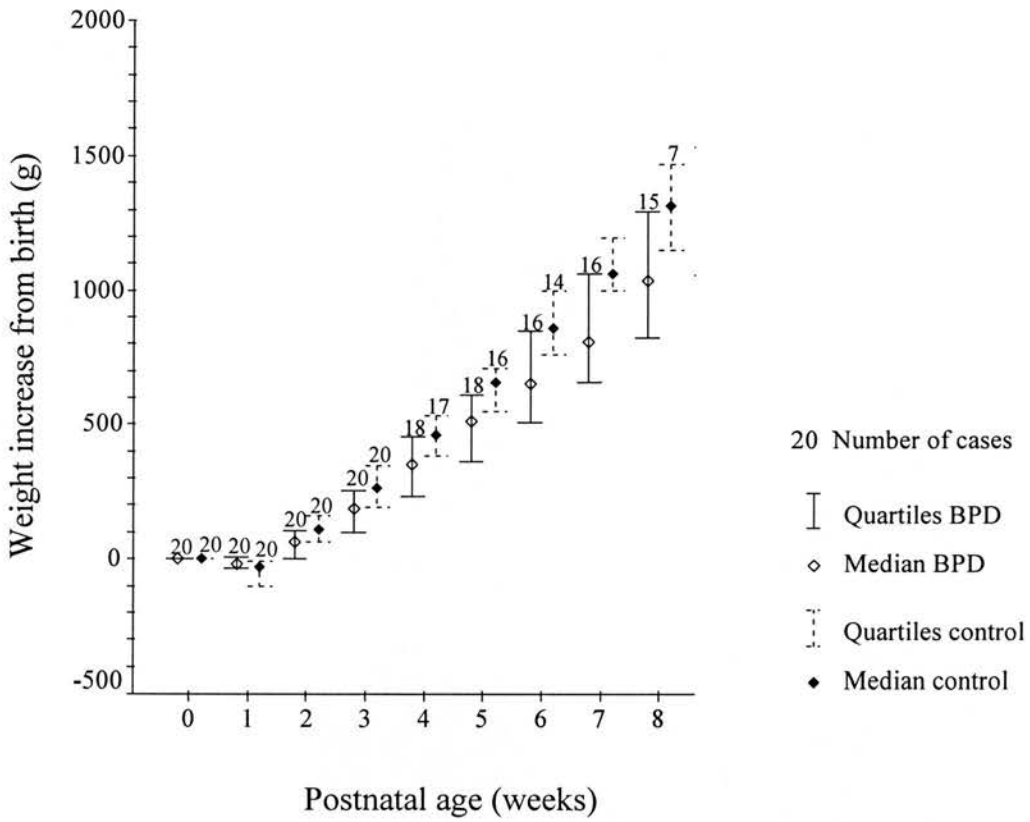

Fig. 22. Weight gain with age, comparing BPD and control infants

There was no apparent difference between the groups in growth in forearm length.

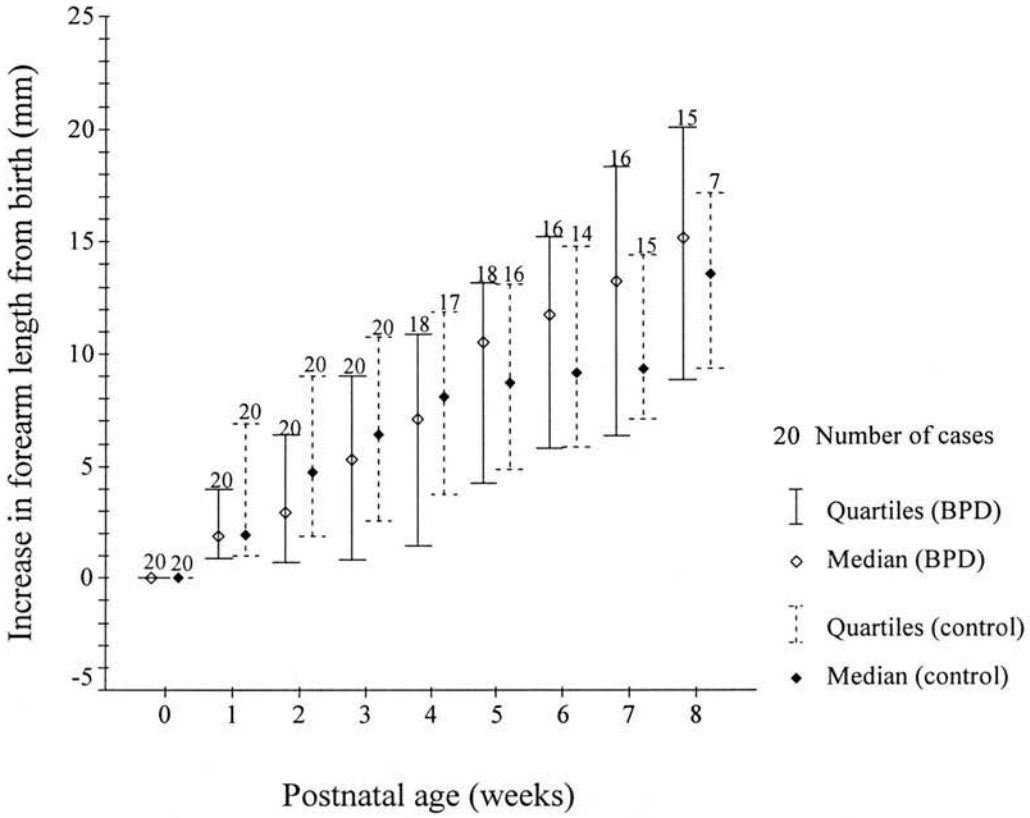

Fig. 23. Gain in forearm length with age, comparing BPD and control infants

(iv) <u>Growth attainment at discharge</u>

There was a large overlap between the groups in weight standard deviation score at discharge [median (quartiles)]: BPD = -0.71 (-1.20 to -0.11) and control -0.87 (-1.89 to -0.37).

Crown-heel length, head circumference and body mass index (weight in grams/[height in mm]^2 x 100) at discharge increased as expected with post-conceptional age at discharge, with no major difference between BPD and control infants.

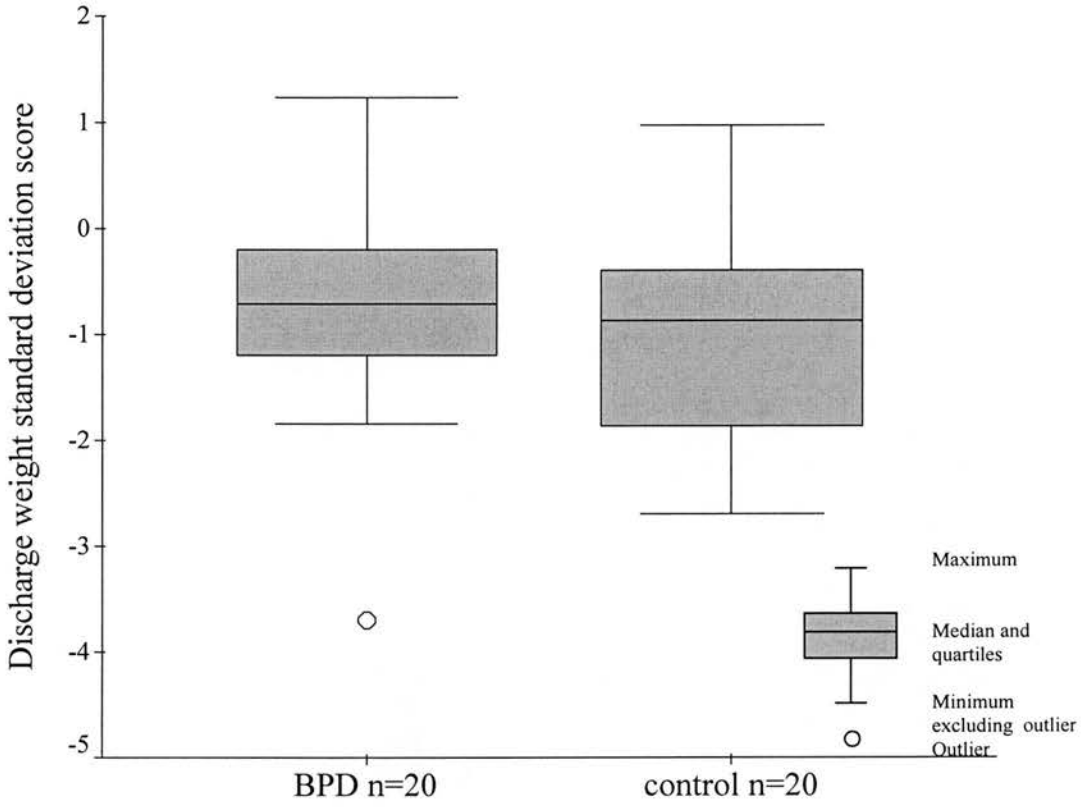

Fig. 24. Discharge weight standard deviation score: BPD and control infants

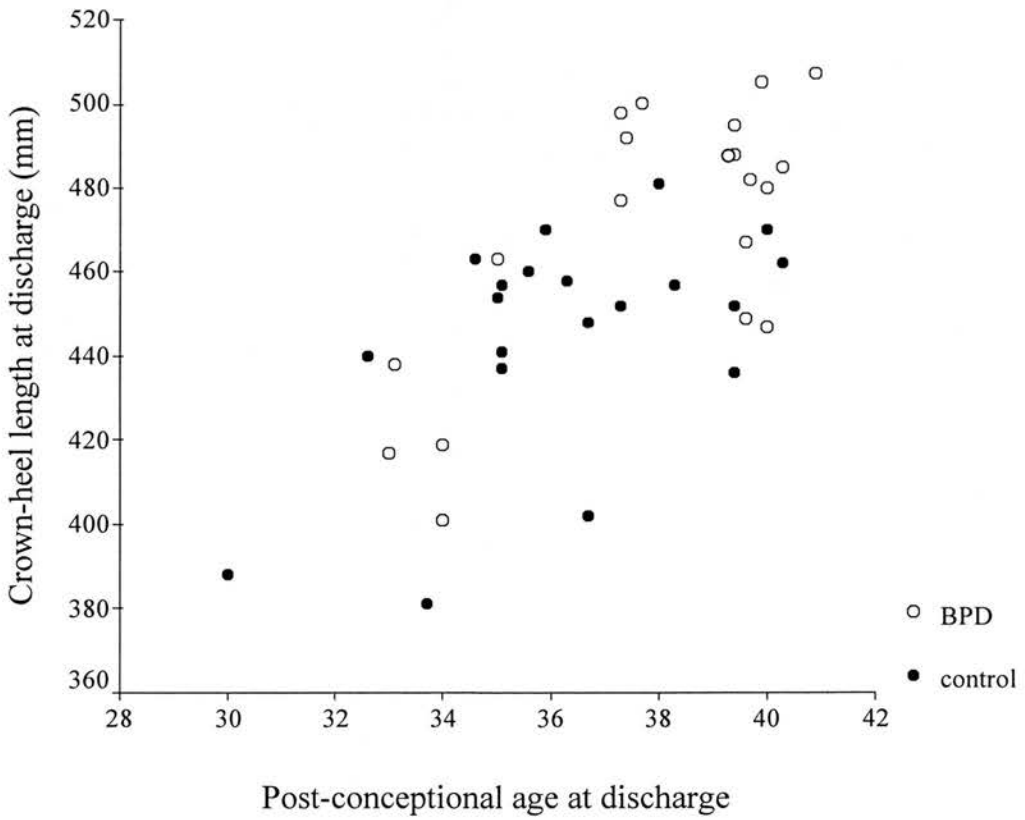

Fig. 25. Body length and post-conceptional age at discharge

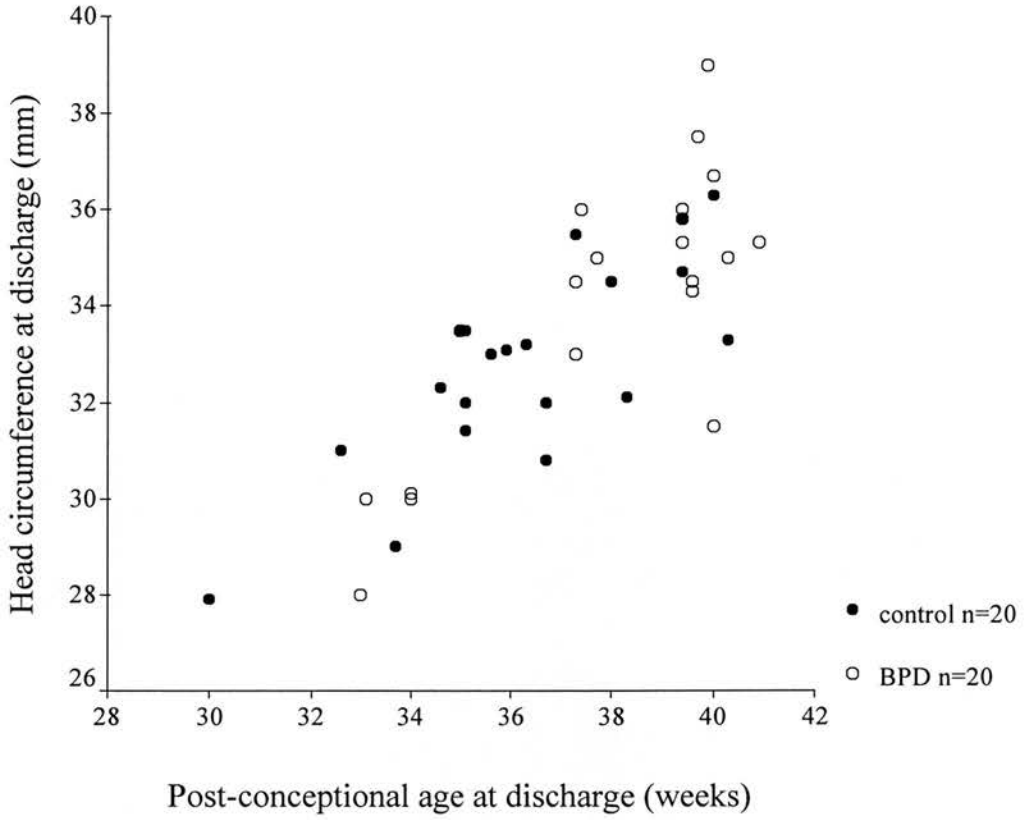

Fig. 26. Head circumference and postconceptional age at discharge

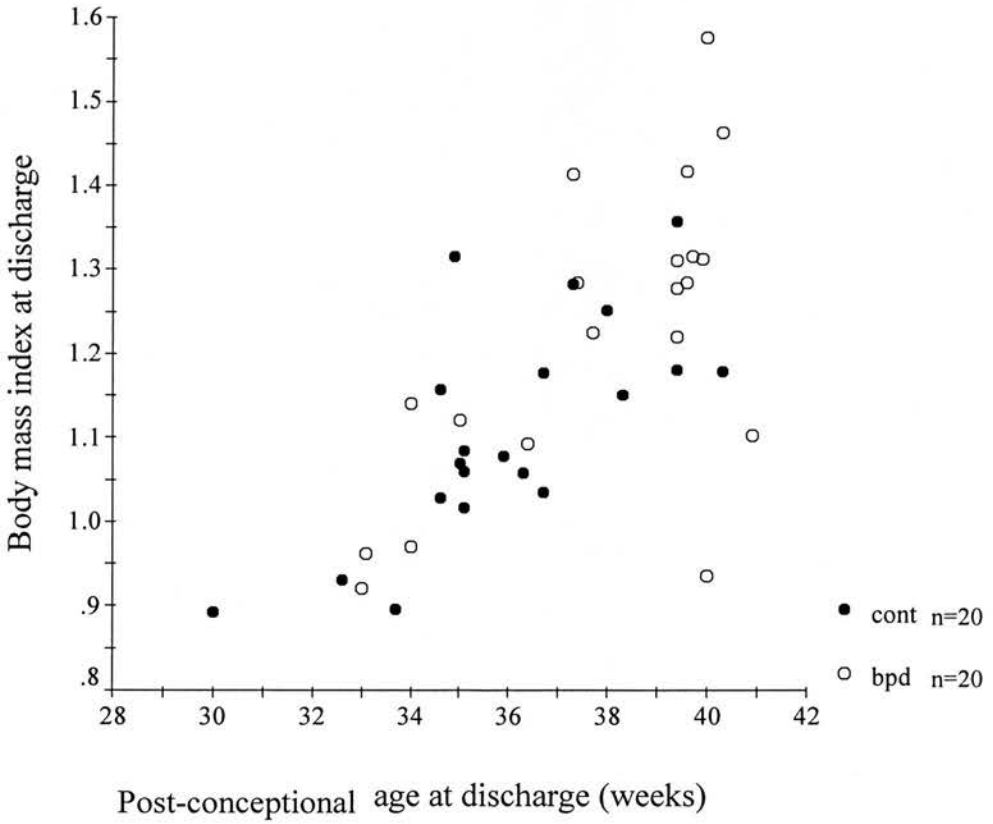

Fig. 27. Body mass index and post-conceptional age at discharge

3.2.2 Energy balance

(i) Subjects

Table 15. Energy balance in BPD: description of infants

	Gestation (weeks)	Birthweight (grams)	Birthweight z-score	Gender	Age at study (days)	Weight at study (grams)	Weight z-score at study
BPD							
1	30	1715	1.42	f	50	2519	-0.75
2	27	900	-0.47	f	44	1314	-2.72
3	29	765	-2.32	f	92	2539	-0.18
4	23	712	1.86	f	46	1221	-0.84
Median BPD	28	832	0.48		48	1916	-0.79
Control							
1	24	745	0.42	m	44	1055	-2.31
2	28	905	-1.01	f	29	1532	-2.52
3	29	825	-2.27	m	23	1156	-4.15
4	28	1255	0.81	f	34	2128	-0.74
Median control	28	865	-0.32		32	1344	-2.41

There was a difference in postnatal age between BPD babies and controls. This is because, although babies were identified at 28 days as having BPD, an attempt was made to study these babies once they had significant established chronic lung disease. It was unusual for control infants to still be hospitalized at this stage. None of the babies with BPD received steroid treatment.

(ii) Energy intake and losses There was no significant difference between groups in energy intake, energy losses in stools, urine or vomitus, or in metabolizable energy intake (Mann-Whitney U test).

Table 16. Energy balance in BPD: energy intake and losses, and weight gain

median and range	BPD	Control
Daily weight gain (grams)	35.1 (17.7-39)	26.5 (15-55)
Energy intake (kJ/kg/day)	633 (502-812)	647 (611-808)
Energy losses in stool (kJ/kg/d)	47 (21-59)	63 (50-67)
Energy losses in urine and vomitus (kJ/kg/d)	15 (12-20)	17 (13-21)
Metabolizable energy intake (kJ/kg/d)	592 (425-741)	565 (527-737)

3.2.3 Bone mineral content

(i) Postnatal course

For the cohort of babies who had bone mineral measurements (n=54), BMC at birth [median (quartiles)] was 1.79mg/mm (1.57-2.03), with a fall in the first 5 weeks of 0.23mg/mm (0.09-0.41), followed by a rise, with a value at 10 weeks of 1.99mg/mm (1.69-2.16). In Fig. 28, the postnatal course of BMC is shown as a change from the value at birth in each baby.

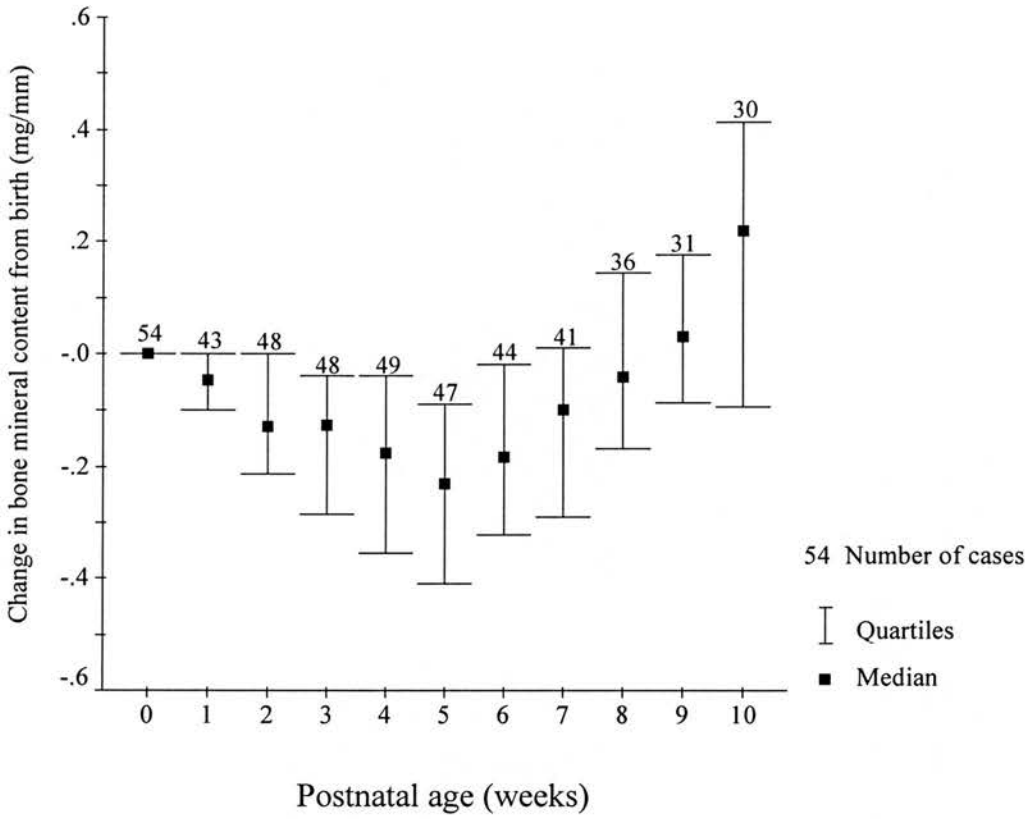

Fig. 28. Change in bone mineral content with age: all babies

(ii) Effect of BPD

Bone mineral content (BMC, mg/mm of bone length) was compared in 10 BPD and 10 control infants matched as closely as possible for gestation and birthweight.

Table 17. Bone mineral content in BPD: description of infants

Subjects	BPD	Control
Gestation (weeks) median and range	29 (28-30)	29 (28-30)
Birthweight (g) median and range	1179 (980-1253)	1187 (1140-1263)
Sex	4 f; 6 m	7 f; 3 m
Steroids	3 infants	0

The postnatal course of bone mineralization in the two groups is shown in Fig. 29. There was a steady drop in BMC to about 6 weeks of age, followed by a more rapid rise.

There was no significant difference between the groups in the postnatal course of bone mineralization or linear growth.

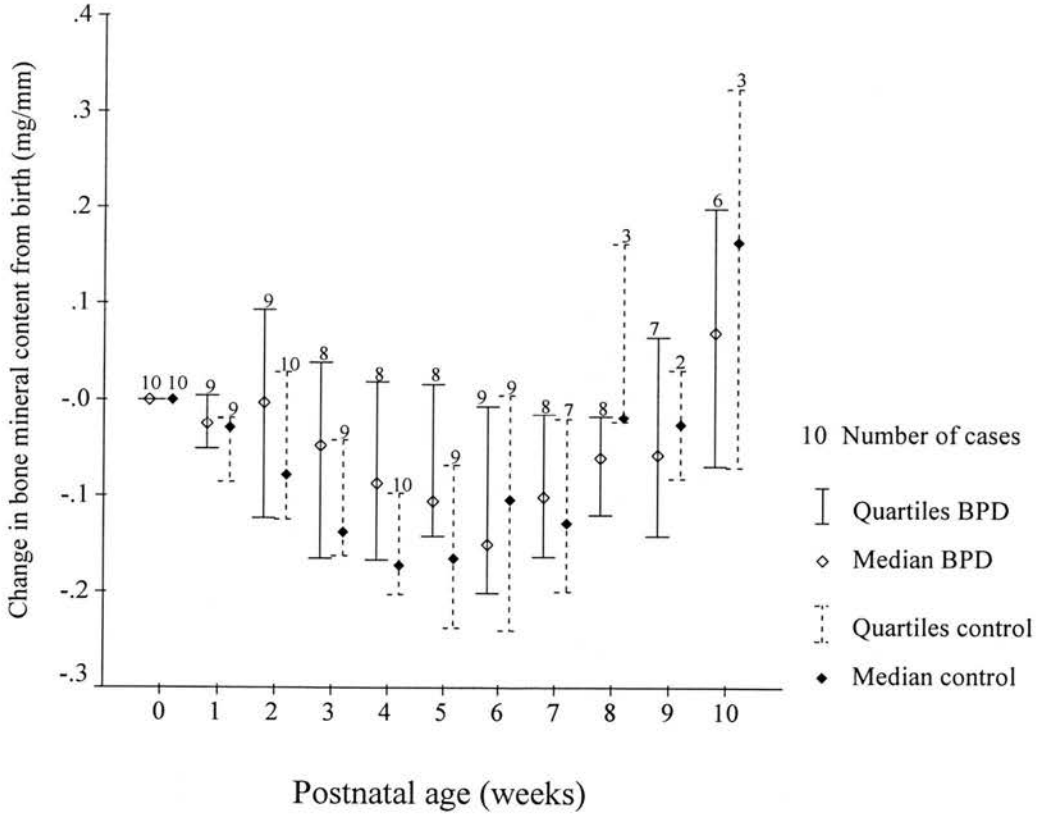

Fig. 29. Change in bone mineral content with age, comparing BPD and control infants

(iii) Effect of Dexamethasone

15 infants treated with dexamethasone for bronchopulmonary dysplasia (treatment given for clinical indications to help weaning from ventilator support, Avery et al 1985), median (range) gestation 27 (26-29) weeks were compared with 15 other infants with BPD, median (range) gestation 27 (25-31) weeks. Some measurements were not done when a baby was considered too unstable, or later because of discharge from the study hospital.

Pre-treatment values were:

Table 18. Bone mineral content in BPD: pre-treatment values

median and range	Steroid-treated	Control	P value
Bone mineral content (mg/mm)	n=9 1.51 (.98-3.18)	n=9 1.65 (1.27-1.88)	NS
Forearm length (mm)	n=14 50.9 (40.6-68.3)	n=14 49.6 (42-67.1)	NS

Subsequent values were expressed as a change from immediate pre-treatment values (change in bone mineral content, dBMC; change in forearm length, dFAL).

There was no difference in dBMC between the groups at any stage (Fig. 30).

There was an increasing difference in dFAL between the BPD and control groups which just reached statistical significance in the week after completion of steroid treatment (Mann Whitney U test, see Fig. 31 and Table 19).

Table 19. Bone mineral content in BPD: change in bone mineral content and forearm length with dexamethasone treatment

median and range	Steroid-treated	Control	Mann-Whitney U test: P value (with Bonferroni correction for 9 tests)
dBMC (mg/mm)	n=10 +0.22 (0.02-0.69)	n=6 +0.24 (0.11-0.44)	NS
dFAL (mm)	n=13 +7.54 (0.6-17.6)	n=11 +11.6 (5.4-24.5)	0.019 (0.171)

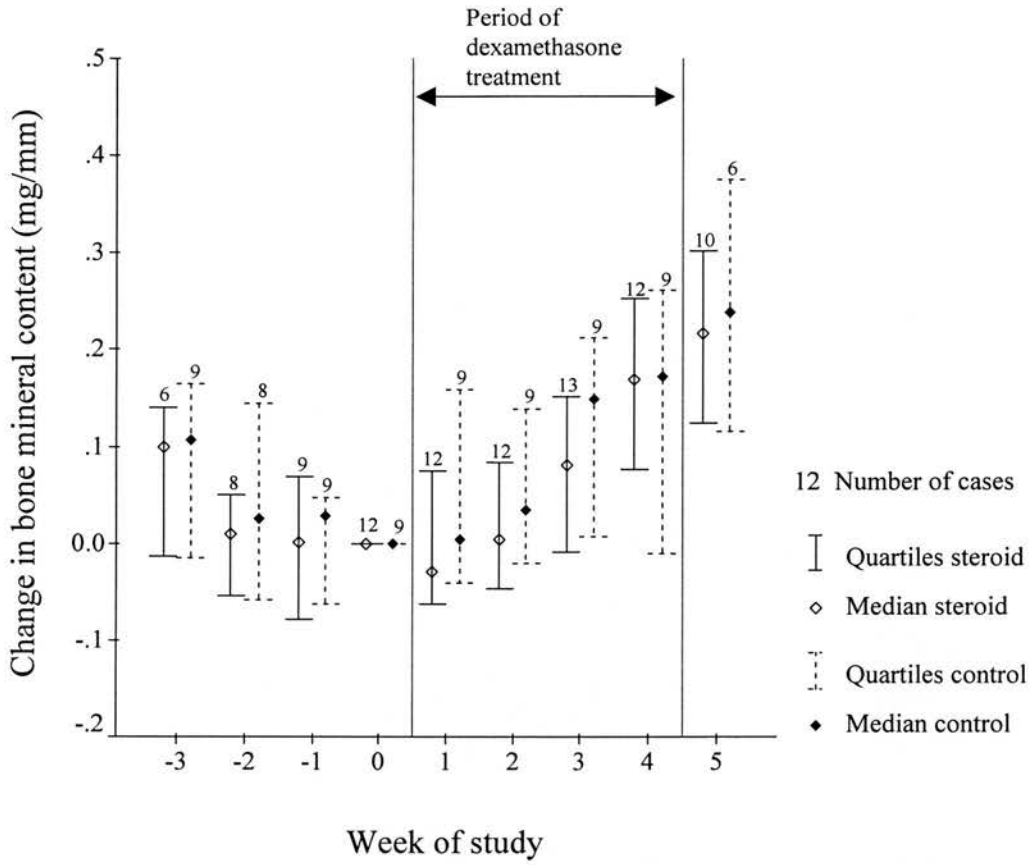

Fig. 30. Change in bone mineral content with age, with and without dexamethasone treatment

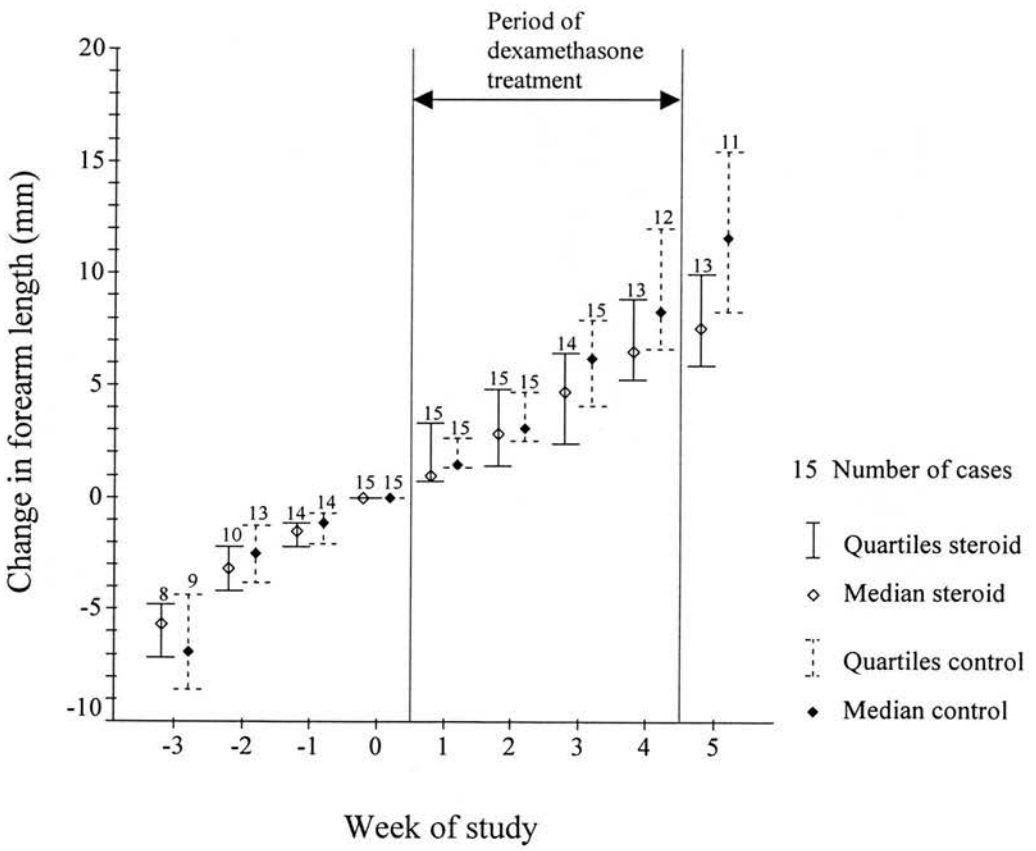

Fig. 31. Change in forearm length with age, with and without dexamethasone treatment

Chapter 4 Discussion

4.1 CONGENITAL HEART DISEASE

For Methods see 2.1
For Results see 3.1

4.1.1 Summary of findings

The current work comprises descriptive studies of hospitalized children with congenital heart disease in comparison with controls. It suggests that these children are within the normal range in size at birth, but subsequently accumulate a deficit in weight, length and head size. In association with this, infants with CHD appear to have a lower range of total and metabolizable energy intakes (although this was not statistically significant), with a positive correlation between MEI as a percentage of RDA (a measure of the appropriateness of energy retention for age) and weight gain in infants with CHD. There is increased resting energy expenditure (when expressed per kg body weight) in some infants with CHD, and REE is inversely related to measures of body fat.

4.1.2 Implications for clinical practice

The pattern of increasing postnatal deficit in body size suggests an external influence, probably undernutrition, although some basic abnormality of metabolic adaptation taking effect postnatally is not ruled out by the current work. The correlation demonstrated between (metabolizable) energy intake and growth rate suggests that retention of energy is a limiting factor for growth in infants under 8 months of age with CHD. Some infants are in "double jeopardy" because an elevation in energy expenditure means an even greater intake needed to provide for growth.

Although there is no randomised controlled study looking at the influence of aggressive nutrition on important clinical outcomes in CHD, there is considerable indirect evidence of benefit and lack of harm. Good nutritional intake and optimal growth should be major goals in the care of infants with CHD. Early consideration should be given to fortification of feeds and supplementary intragastric feeding in infants who have

demonstrated a significant fall in centile level, the target being catch-up growth. This may be particularly important in infants with cardiac failure because of the temptation to restrict fluids. Research following on from the current work, in the same centre, suggests that increased energy intake can result in a useful improvement in energy retention, although resulting in less catch-up growth than would be expected from the increased metabolizable energy. This suggests either that (a) this additional intake is metabolised as a fuel source, perhaps because insufficient protein was given to allow utilization of energy for growth, or that (b) diet-induced thermogenesis was increased, or that (c) these infants had a higher energy cost of weight gain because of a different tissue composition (Jackson and Poskitt 1991).

4.1.3 Critique of current work

The present study is important because it provides quantitative and detailed information about all components of energy balance and relates this to short-term growth in infants with congenital heart disease within a narrow age range. Another strength is that an attempt has been made to make comparison with healthy controls using the same methodology. It appears to have been the first study to achieve these aims, at least in a limited way. Although modern stable isotope methods enable the assessment of energy requirements from accurate measurements of TEE, energy balance cannot be so fully compartmentalized as with the methods used here.

The limitations of this work are that (1) it is a descriptive study without calculation of sample size, (2) the number of cases is relatively small, with a particular shortage of control infants; thus there is a greater possibility of results representing the effect of chance or insufficient numbers, with the resultant risk of drawing erroneous conclusions, (3) the sample selected is probably not representative of (that is, generally

sicker than) the total population if infants with CHD, thus limiting the generalizability of the results.

In common with studies of nutrient balance, in general, there appears to be a systematic shortfall in energy retrieved from collection of losses. However, correcting for the underestimation measured in the quality control experiments has an insignificant effect on MEI, and does not affect the conclusions drawn here.

There are particular problems with the interpretation of energy expenditure measurements: (1) There are errors in extrapolating total energy expenditure from measurements of resting oxygen consumption. The energy expenditure of an individual varies greatly with time, and even 24-hour measurements may not adequately represent typical values. In addition, the energy consumed during feeding has not been measured in this work, and there may be a significant difference between the groups. (2) There is a large biological interindividual variability in energy expenditure measurements even in an apparently homogeneous group of infants under standard conditions (Denne and Kalhan 1986). This reduces the likelihood of detecting an important difference between groups unless large sample sizes are used.

It should be remembered that, where energy intake has been related to expected intake, recommended dietary allowances (RDA) have been used which have been revised since these studies were carried out. The current values for RDA, being about 10% lower, imply a smaller shortfall of MEI in the subjects of the current study than implied by the results as presented here. However, this does not significantly affect the relationship between the CHD and control groups, or the correlation between MEI%RDA and weight gain.

The strong inverse correlation between sleeping V_{O_2} and measures of fatness might be explained in one of several ways: (1) lack of body fat in thin infants is a direct

consequence of increased energy expenditure not being matched by a commensurate increase in intake, (2) when energy expenditure is expressed in relation to body weight, a larger proportion of this weight is metabolically active lean tissue in undernourished infants with the highest expenditure, (3) in thin infants, hypermetabolism may merely be an adaptation to greater heat loss. Recent studies showing a dramatic drop in total energy expenditure soon after corrective or palliative surgery suggest that physiological changes linked to the basic anatomical abnormality, rather than a basic metabolic defect, are to blame for the increased metabolic rate in these infants (Mitchell et al 1994). The less good correlation, in infants with CHD than in controls in the present study, between SV_{O2} and measures of metabolic body size again suggests that in CHD REE is strongly influenced by factors other than body size. There is, of course, a question mark over how well derived values (such as weight$^{3/4}$, Kleiber 1961 and weight$^{1/2}$, Davies et al 1989) represent metabolic body size in infants with CHD, because they have not been validated in this group, and also because of the possibly greater water content of lean body tissue in some infants with CHD (Mitchell et al 1995).

An attempt has been made to derive the energy available for deposition in the current study. Apart from errors in the extrapolation of total energy expenditure, comparing the energy available for deposition in different populations assumes that the composition of tissue gain, and hence the energy cost of growth, is always the same. This may not be true in clinically different populations, at different ages, and at different rates of weight gain as (a) the stored energy values of protein and fat are very different (5.65 vs 9.25kcal/g) and (b) the energy required for tissue accretion is greater for protein than for fat (7.8 vs 1.6kcal/g) (Roberts and Young 1988).

Can the explanations for altered energy balance in other chronic diseases such as cystic fibrosis help to elucidate what is happening in CHD? In cystic fibrosis, as appears

to be the case in CHD, the insufficiency of intake to maintain a sufficiently positive energy balance for growth is contributed to both by a limitation of intake and by an increase in expenditure. In both conditions, the raised REE is probably partly related to increased respiratory and cardiac work. A chronic inflammatory process is certainly a feature of chronically ill children with cystic fibrosis, and evidence for this has been found in adults with chronic cardiac failure, with a correlation between markers of inflammation and energy expenditure. No published work has been found looking at the inflammatory process in CHD. Interestingly, in cystic fibrosis and in acquired immune deficiency syndrome, despite a raised REE, TEE is not increased because of a reduction in energy expended in activity. No conclusion can be reached about activity in CHD because neither TEE nor any measure of activity was obtained in this study. However, it is unlikely that changes in activity level, which contributes a very small proportion of daily energy use at this age, could fully compensate for the hypermetabolism seen in some babies in this study.

4.1.4 Suggestions for further research

In order to try to improve the nutritional outcome for infants with CHD (that is to improve nutritional status until surgery can be performed) more detailed studies of energy and protein turnover need to be carried out, probably using stable isotope technology. Nutritional interventions need to provide the optimal balance of protein and energy. The quality of weight gain should be studied using stable isotope and other methods of studying body composition, having derived validation equations for this particular population of sick children.

Most useful will be studies looking at major outcomes (survival, time to surgery, and incidence of postoperative complications including infection) with aggressive compared with conventional nutrition. The data in the present study, suggesting

progressive postnatal undernutrition of early onset, may justify starting such an intervention at diagnosis in the most at-risk patients.

Systemic inflammatory responses should be studied in infants with CHD since there appears to be a strong association between inflammatory markers and nutritional impairment in adults with cardiac failure, and this may generate the possibility of therapeutic interventions.

4.2 BRONCHOPULMONARY DYSPLASIA

For Methods, see 2.2
For Results, see 3.2

4.2.1 Summary of findings

In the current work several measures of nutritional status were compared in babies who went on to develop BPD and controls who did not. In BPD babies, prior to development of established chronic lung disease, macronutrient intake was significantly less in the first week for energy, fat and protein, and a greater proportion of nutritional intake in the first two weeks was by the parenteral route. This was associated with a significantly slower weight gain in these infants in the first few weeks. There was no detectable difference in energy intake or losses or in MEI between babies with established BPD and control infants at 3-8 weeks postnatal age. There was a trend for all babies to accumulate a postnatal deficit in bone mineral content for the first 5 weeks with a subsequent slow increase, but leaving a large deficit at full term equivalent.

The postnatal trend of forearm bone mineral content and linear growth were not demonstrably affected by the presence of BPD. The use of dexamethasone in BPD appeared to slow linear growth of the forearm without any measurable effect on bone mineral.

4.2.2 Implications for clinical practice

Because of the association between early undernutrition and BPD and the

plausibility of an aetiological link, clinicians should aim to maximize nutritional intake as part of the routine early management of babies at high risk of developing BPD. This should include consideration of the early use of parenteral nutrition with lipid, and the avoidance of unnecessary delay in introducing milk feeds, unless there are specific contraindications. There is little rationale in limiting fluid intake routinely in BPD, unless a baby is very sick, and in that case powerful diuretics are likely to be used. There is also a case for early treatment of persistent ductus arteriosus with its tendency to increase lung fluid, thus obviating the need for symptomatic treatment such as fluid (and hence nutritional) restriction or the chronic use of diuretics. The significant deficit in bone mineral accumulated by the VLBW babies by full term equivalent in this study, although not shown to be different in babies with BPD, may contribute to respiratory difficulty in all babies by creating inefficiency of chest wall movement. Insufficient data are available at present to justify the chronic use of diuretic treatment, which has the potential for creating further mineral depletion (Brion et al 2002).

4.2.3 Critique of current work

The current work is informative in providing some insight into the possible aetiological link between early undernutrition and BPD, which has previously been strongly suggested by animal studies. It also contributes to the literature on nutritional status in established BPD. The limitations are that (1) the studies are retrospective and observational, increasing the likelihood of confounding factors influencing the results, and (2) the sample size in all these studies is relatively small (particularly the study of energy balance) with no power calculation, raising the possibility of a Type II statistical error.

An early nutritional deficit is associated with poor early weight gain in BPD, although in the current work there is no evidence that body size attainment at discharge is

affected. Early undernutrition may contribute to the pathogenesis of BPD because of increased oxygen toxicity and slow lung healing. Differences in energy and fat intake may, in this situation, merely be markers for more important differences in micro-nutrient intake, for example of the fat-soluble vitamins A and E which are thought to be important in lung protection and healing. The current results are consistent with the findings of Wilson et al (1991) who performed a similar case-control study matching for gestation alone, and found, in addition, that babies with BPD were smaller for gestation at birth.

Babies with BPD appear to demonstrate poor weight gain for the first few weeks without an obvious ongoing shortfall in intake, although there is no evidence that this results in a deficit in size at discharge. In contrast to these results, Embleton et al (2001) showed that in their unit infants of gestation less than 32 weeks continue to accumulate a deficit in energy and protein intake (compared with recommended dietary intakes) for several weeks postnatally, in association with a sustained deficit in weight z score. The nutrient deficit explained 45% of the variation in weight. Other possible explanations for ongoing growth failure are (a) a persistent inflammatory process fuelling a hypermetabolic state with a resultant requirement for a greater than normal energy intake (b) chronic hypoxaemia.

The greater proportion of feeds given parenterally in BPD babies may purely be the reflection of the approach to management of a sicker group. However, could it be a reflection of a beneficial effect of nutrients in milk on lung protection (because of better bioavailability of protective agents)? Conversely, could it be an adverse effect of the use of parenteral nutrition? One candidate for the latter is lipid toxicity; the other is the greater likelihood of nosocomial infection (with its association with BPD, Gonzales et al 1996) in babies fed parenterally.

In the small comparative study reported here of energy balance in infants with established BPD and controls, there are limitations in the comparability of the groups. The control infants were smaller for gestation at birth, and younger and smaller for age when studied. There was no detectable difference in energy intake or energy retention. Thus the explanation for poor growth, when seen in babies with BPD, may be more likely to lie either in hypermetabolism related to the disease process or a difference in the energy needs for tissue deposition. A recent study (Boehm et al 1996) showed increased fat excretion in the stool (with a reduction in duodenal lipase production) and reduced weight gain in infants with BPD compared to other preterm infants. They found, as in the present study, a greater use of the parenteral route for nutrition in babies who developed BPD, and suggested that this could have led to a slower maturation of pancreatic lipase production and less bile acid release because of cholestasis, resulting in a greater degree of fat malabsorption. The authors also point out that steroids enhance pancreatic function –none of the babies in this study received steroids during or prior to energy balance measurements. In the babies studied here, the range of stool energy was, if anything, lower in babies with BPD. This could have been an effect of age as the BPD babies were older than the controls in this small group.

It was not possible to accurately measure energy expenditure by indirect calorimetry in babies needing supplemental oxygen with the technology available at the time of this study. The alternative would have been to perform these measurements once oxygen therapy was discontinued. Such a study would have shown whether there was a persistent hypermetabolic state in BPD, but left considerable uncertainty about energy expenditure during the period when lung disease was at its worst and when growth is most impaired.

The one intervention study which has looked at the influence on clinical outcomes of aggressive nutritional input did not show any effect on the incidence of chronic lung disease (Wilson et al 1997). However, there are several reasons why this should not be thought of as a definitive answer to the question of nutrition in the aetiology of chronic lung disease: (1) chronic lung disease was not defined as the main outcome in this study; (2) it may not have been sufficiently focussed on a group at significant risk of chronic lung disease -the definition used was the need for added oxygen at 28 days postnatal age, an endpoint which was probably too crude and potentially influenced by many inexact clinical factors: it occurred in nearly half the babies in both groups; (3) the intervention was complicated and arguably not aggressive enough in terms of macronutrient intake, only achieving 100kcal/kg/day total intake after 7 days, and in terms of enteral intake, taking a median of 21 or 22 days (and a minimum of 13 days) to reach full enteral feeds (compared with a median of 9 days in our unit during this study); (4) there is the possibility that the higher intravenous lipid intake in the intervention group had the effect of worsening pulmonary outcome, thus confounding the results; (5) the higher illness severity of babies in the intervention group may have predisposed this group to adverse outcomes, obscuring a beneficial effect of aggressive nutrition.

In our VLBW subjects, a large deficit in BMC continues to be accumulated for about the first 6 weeks, long after these babies have established a constant enteral intake. This, together with a slow subsequent rate of accretion, resulted in a mean BMC similar at full term equivalent to that at birth. This leaves a substantial deficit since 80% of foetal bone mineral accretion normally occurs in the 3rd trimester. Others have described a similar deficit (Lyon et al 1989), although there have been reports of achievement of intrauterine rates of mineralization with supplemented feeds (Chan et al 1988). In theory,

osteopenia might be more likely in babies with BPD because of poor early intake exacerbated by the use of loop diuretics and steroids. The lack of any detectable difference in the postnatal course of bone mineralization in infants with and without BPD is in agreement with the results of Greer et al (1987).

Dexamethasone was being used commonly in our unit at the time of this study. Although there are theoretical reasons for concern about bone growth and mineralization with steroid treatment, only a slight slowing of linear growth was demonstrated.

There is the possibility that confounding factors associated with the occurrence of BPD and with the use of steroids influenced the results of the studies of BMC. However, confounders such as poor early nutritional intake would have been expected to act to reduce bone mineral in the study group compared with the controls, as the BPD babies in the first study and the steroid-treated babies in the second are likely to have been sicker and at greater risk of undernutrition. However, it is possible that the limits of sensitivity of the technique for measuring bone mineral content, as well as inter-individual variation, may have masked a real difference between the groups in these studies. Other reasons for caution in interpreting these results are (1) it has become accepted practice, since this work was done, to correct values of total body BMC for body size because this has a strong independent influence on bone mineral content (Prentice et al 1994) –correction for body size in the current study would have led to relatively higher values in BPD (smaller size) than in controls (2) only localized assessment of BMC was made (there were not the facilities for measuring total body BMC), and these may not be representative.

Even though none of the steroid-treated babies developed acute complications such as overt glucose intolerance, significant hypertension, or systemic sepsis, caution should be used in interpreting the present results as suggesting that dexamethasone is a

safe drug. There are metabolic concerns about linear growth, and protein catabolism (Bloomfield et al 2001). In addition, the more recent discovery of an association between the use of dexamethasone and adverse neurodevelopmental outcome has led to serious questioning of the place of neonatal steroid treatment (Barrington 2001).

Since the current work was done, the incidence of severe ("old") BPD appears to have reduced considerably. This is probably the result of better obstetric and early neonatal care, including the more routine use of antenatal steroids in preparation for preterm delivery and the early use of surfactant, reducing the need for damaging respiratory support. However, there is now the phenomenon of "new" BPD, seen predominantly in babies of less than 1kg, who have had only modest early lung disease. This may be partly caused by nutritional deficiency, a possible model for this being the small for gestation preterm infant who has experienced intrauterine undernutrition and has a higher than gestation-related risk of chronic lung disease which is not associated with early severe lung disease (Lal et al 2003).

4.2.4 Suggestions for further research

There is good reason to believe that poor early nutritional intake is an aetiological factor in BPD. The published evidence suggests that feeding practice in preterm infants is very variable. Extremes of current approach are likely to pose serious but conflicting threats. Further well-designed randomized controlled clinical trials are needed, comparing different feeding strategies, with sufficient statistical power to detect differences in important outcomes. Two areas should be a priority: (1) studies of aggressive compared with cautious use of parenteral nutrition, looking at chronic lung disease as the main outcome in babies of <1kg birthweight, and (2) studies of rapid compared to slow introduction of enteral feeds, with necrotizing enterocolitis and nosocomial (especially line-related) infection as end points.

Interventions aimed at reducing the postnatal deficit in bone mineral should be assessed, including mineral supplementation, and studies of the clinical risks and benefits of diuretic use in BPD.

Longer term nutritional interventions should be studied, aimed at normalizing post-term growth rate and body composition. Studies of dietary supplementation should be accompanied by energy and fat balances, and include long term follow up.

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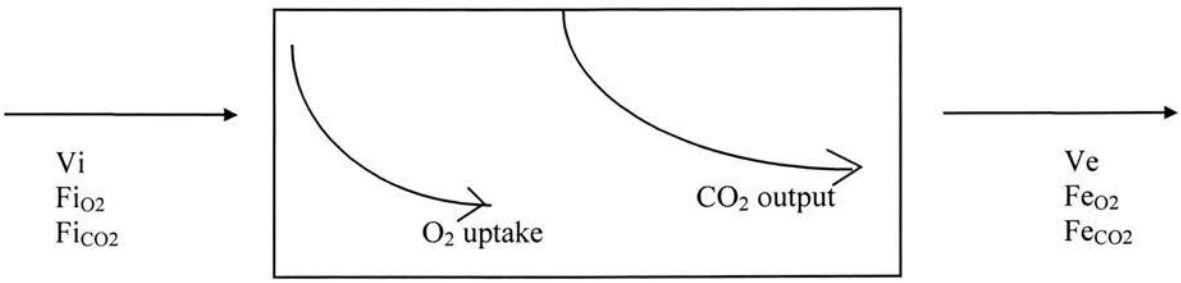
Appendix

THE EFFECT OF ASSUMING VI=VE



$$Ve = Vi - O_2 \text{ uptake} + CO_2 \text{ output}$$

$$\frac{O_2 \text{ uptake}}{CO_2 \text{ output}} = RQ$$

The greatest difference between Vi and Ve will be when there is an RQ furthest from 1, i.e. 0.7, and when the flow rate is at its lowest. The lowest flow rate (Vi) used is one resulting in an mixed-expired CO_2 concentration of 0.5%.

Take the extremes of V_{O2} in our studies, with an RQ of 0.7.

True values		Measured	Calculated from RQ	Assuming Ve=Vi	
V_{O2} (ml/min)	V_{CO2} (ml/min)	Ve (l/min)	Vi (l/min)	V_{O2} (ml/min) error (%)	V_{CO2} (ml/min) error (%)
70	49	9.8	9.82	69.58 0.6	49.0 negligible
25	17.5	6	6.007	23.52 5.9	17.5 negligible

Accurately, $V_{CO2} = Ve\ Fe_{CO2} - ViFi_{CO2}$

$$Ve = \frac{V_{CO2} + ViFi_{CO2}}{Fe_{CO2}}$$

and $Vi = Ve + V_{O2} - V_{CO2}$ (from above)

Assuming $ViFi_{CO2}$ to be negligible (Fi_{CO2} v. low), Ve and Vi can be calculated (see table). V_{O2} and V_{CO2} (assuming Vi = Ve) can then be calculated, and the error involved ascertained.

Fe_{O2} is calculated from:

$$Fe_{O2} = \frac{ViFi_{O2} - (V_{CO2}/0.7)}{Ve}$$

THE EFFECT OF IGNORING THE PROTEIN CONTRIBUTION TO OXIDATIVE METABOLISM ON THE CALCULATION OF ENERGY EXPENDITURE

The non-protein RQ allows for the protein contribution to oxidative metabolism when calculating energy expenditure. When the total RQ is used instead, we are assuming that the protein contribution is 0.

Total RQ = V_{CO2} / V_{O2}

Non-protein RQ = $V_{CO2} - V_{CO2}^{prot} / V_{O2} . V_{O2}^{prot}$

V_{CO2}^{prot} and V_{O2}^{prot} are derived from urinary protein excretion.

When protein contribution = 0, non-protein RQ = total RQ.

Example Baby with V_{O2} 50ml/min

Calculate RQ from proportions and kcal equivalents using Weir's formula for protein and non-protein components.

	O% protein energy		**12.5% protein energy**		**25% protein energy**		**Diff. in kcal/min between 0 and 25% protein energy**
	tot RQ	**kcal/min**	**tot RQ**	**kcal/min**	**tot RQ**	**kcal/min**	
No CHO	0.707	0.2343	0.714	0.2326	0.732	0.2348	0.21%
CHO+fat 50:50	0.859	0.2430	0.852	0.2379	0.844	0.2379	2.14%
No fat	1.00	0.2524	0.973	0.2485	0.947	0.2445	3.23%

For column *25% protein energy*

25% protein energy ≈ 26.8% protein O2

For row *No CHO* Fat RQ = 0.707 Protein RQ = 0.802

Total RQ = .732

V_{O2} =50ml/min; V_{CO2} = 50 x 0.732 = 36.6ml

V_{O2}^{prot} = 26.8 x 50/100 = 13.4ml

Since RQ^{prot} = 0.802, V_{CO2}^{prot} = 13.4 x 0.802 = 10.74ml

Non-prot V_{O2} = 50 - 13.4 = 36.6ml

Non-prot V_{CO2} = 36.35 - 10.74 = 25.76ml

Non-prot RQ = 25.76ml / 36.6ml = 0.7038

≡ 4.68kcal/l O2 (Zuntz table)

≡ 4.68x 36.6ml = 0.1712kcal non-prot energy/min

Add protein contribution = 13.4 x 4.46 = 0.0597kcal

TOTAL Energy Expenditure = 0.1712kcal + 0.0597kcal

= 0.2348kcal/min

For row *CHO+fat 50:50*

RQ 50:50 CHO:fat =.859; RQ protein =.802

Total RQ = .844

V_{O2} =50ml/min; V_{CO2} = 50 x 0.844 = 42.2ml

V_{O2}^{prot} = 26.8 x 50/100 = 13.4ml

Since RQ^{prot} = 0.802, V_{CO2}^{prot} = 13.4 x 0.802 = 10.746ml

Non-prot V_{O2} = 50 - 13.4 = 36.6ml

Non-prot V_{CO2} = 42.2ml - 10.74 = 31.46ml

Non-prot RQ = 31.46ml / 36.6ml = 0.859

≡ 4.87kcal/l O2

≡ 4.87 x 36.6ml = 0.1782kcal non-prot energy/min

Add protein contribution = 13.4 x 4.46 = 0.0597kcal

TOTAL Energy Expenditure = 0.1782kcal + 0.0597kcal

= 0.2379kcal/min

For row *No fat* CHO RQ = 1.000 Protein RQ = 0.802

Total RQ = 0.947

V_{O2} = 50ml/min; V_{CO2} = 50 x 0.947 = 47.34ml

V_{O2}^{prot} = 26.8 x 50/100 = 13.4ml

Since RQ^{prot} = 0.802, V_{CO2}^{prot} = 13.4 x 0.802 = 10.74ml

Non-prot V_{O2} = 50 - 13.4 = 36.6ml

Non-prot V_{CO2} = 47.34 - 10.74 = 36.60ml

Non-prot RQ = 36.6ml / 36.6ml = 1.000

≡ 5.05kcal/l O2 (Zuntz table)

≡ 5.05x 36.6ml = 0.1848kcal non-protein energy/min

Add protein contribution = 13.4 x 4.46 = 0.0597kcal

TOTAL Energy Expenditure = 0.1848kcal + 0.0597kcal

= 0.2445kcal/min

<u>For column *12.5% protein energy*</u>

12.5% protein energy ≈ 13.4% protein O2

For row No CHO Fat RQ = 0.707 Protein RQ = 0.802

Total RQ = 0.720

V_{O2} =50ml/min; V_{CO2} = 50 x 0.720= 35.98ml

V_{O2}^{prot} = 13.4 x 50/100 = 6.7ml

Since RQ^{prot} = 0.802, V_{CO2}^{prot} = 6.7 x 0.802 = 5.37ml

Non-prot V_{O2} = 50 - 6.7 = 43.3ml

Non-prot V_{CO2} = 35.98 - 5.37 = 30.61ml

Non-prot RQ = 30.61ml / 43.3ml = 0.7069

≡ 4.686kcal/l O2 (Zuntz table)

≡ 4.686 x 43.3ml = 0.2029kcal non-prot energy/min

Add protein contribution = 6.7 x 4.46 = 0.0298kcal

TOTAL Energy Expenditure = 0.2029kcal+0.0298kcal

= 0.2328kcal/min

Declaration

The work reported in this dissertation was carried out in two parts:

1. Liverpool Sept. 1981- Aug. 1983 as Senior Research Assistant.

The balance collections and measurements of energy expenditure were performed in the wards of the Royal Liverpool Children's Hospital, Myrtle Street. The processing of collections, bomb calorimetry, and validation studies were performed in the Institute of Child Health, Alder Hey Children's Hospital.

I was supervised by Dr Elizabeth Poskitt, Senior Lecturer in Child Health in Liverpool. Apart from help from nursing staff in the collections for the balance studies which I supervised, all practical work was carried out by me.

The studies were undertaken after approval by the Liverpool Paediatric Sector Ethical Committee.

This work has been published in part: Menon G and Poskitt EME. Why does congenital heart disease cause failure to thrive? Arch Dis Child 1985, **60**:1134-39.

2. Edinburgh Aug. 1989- Dec. 1992 as Lecturer in Child Life and Health.

The patient studies were all carried out on the Neonatal Unit in the Simpson Memorial Maternity Pavilion. The processing of balance collections and bomb calorimetry were performed in the laboratories of the Department of Child Life and Health, University of Edinburgh. The analysis of arm X-rays for bone mineral content was done in the Department of Medical Physics and Medical Engineering, Western General Hospital.

This work was supervised by Professor N McIntosh, Head of Department of Child Life and Health, University of Edinburgh.

In performing anthropometry, supervising arm X-rays, and collecting data on nutrient intake, I was helped by Research Sisters Val Morgan and Breda McLoughlin. Dr Jerry R Williams, Physicist in the Dept of Medical Physics and Medical Engineering, developed with us the technique for measurement of bone mineral content, and performed the computerized analysis of bone mineral content.

The studies had the approval of the Lothian Health Board Paediatric/ Reproductive Medicine Ethical Committee.

The studies of bone mineral content have been published in part: (i) Williams JR, Davidson F, Menon G and McIntosh N (1994) A portable dual energy X-ray densitometry technique for the measurement of bone mineral in preterm infants. Pediatr Res, **36**: 3, 351-7. (ii) Menon G, Williams JR, McLoughlin B, Davidson F and McIntosh N (1993) Bone mineral content and linear growth in infants treated with dexamethasone for bronchopulmonary dysplasia. Pediatr Res (abstract). (iii) Menon G, Williams JR, McLoughlin B, Davidson F, Morgan V and McIntosh N (1993) Postnatal changes in bone mineral content in preterm infants. Proc Nutr Soc **52**: 223A.

This thesis was composed solely by myself and has not been submitted in candidature for any other degree, diploma or professional qualification.

Gopi Menon