Nutritional status and bronchopulmonary dysplasia (BPD)

Joyce P. Chehade

School of Dietetics and Human Nutrition
McGill University, Montreal, Quebec, Canada

July 1994

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements of the degree of Master of Science.

Name Joyce Chehadé

Dissertation Abstracts International is arranged by broad, general subject categories. Please select the one subject which most nearly describes the content of your dissertation. Enter the corresponding four-digit code in the spaces provided.

Nutrition — SUBJECT TERM

0570 — SUBJECT CODE

UMI

Subject Categories

THE HUMANITIES AND SOCIAL SCIENCES

COMMUNICATIONS AND THE ARTS

- Architecture 0729
- Art History 0377
- Cinema 0900
- Dance 0378
- Fine Arts 0357
- Information Science 0723
- Journalism 0391
- Library Science 0399
- Mass Communications 0708
- Music 0413
- Speech Communication 0459
- Theater 0465

EDUCATION

- General 0515
- Administration 0514
- Adult and Continuing 0516
- Agricultural 0517
- Art 0273
- Bilingual and Multicultural 0282
- Business 0688
- Community College 0275
- Curriculum and Instruction 0727
- Early Childhood 0518
- Elementary 0524
- Finance 0277
- Guidance and Counseling 0519
- Health 0680
- Higher 0745
- History of 0520
- Home Economics 0278
- Industrial 0521
- Language and Literature 0279
- Mathematics 0280
- Music 0522
- Philosophy of 0998
- Physical 0523
- Psychology 0525
- Reading 0535
- Religious 0527
- Sciences 0714
- Secondary 0533
- Social Sciences 0534
- Sociology of 0340
- Special 0529
- Teacher Training 0530
- Technology 0710
- Tests and Measurements 0288
- Vocational 0747

LANGUAGE, LITERATURE AND LINGUISTICS

- Language
  - General 0679
  - Ancient 0289
  - Linguistics 0290
  - Modern 0291
- Literature
  - General 0401
  - Classical 0294
  - Comparative 0295
  - Medieval 0297
  - Modern 0298
  - African 0316
  - American 0591
  - Asian 0305
  - Canadian (English) 0352
  - Canadian (French) 0355
  - English 0593
  - Germanic 0311
  - Latin American 0312
  - Middle Eastern 0315
  - Romance 0313
  - Slavic and East European 0314

PHILOSOPHY, RELIGION AND THEOLOGY

- Philosophy 0422
- Religion
  - General 0318
  - Biblical Studies 0321
  - Clergy 0319
  - History of 0320
  - Philosophy of 0322
- Theology 0469

SOCIAL SCIENCES

- American Studies 0323
- Anthropology
  - Archaeology 0324
  - Cultural 0326
  - Physical 0327
- Business Administration
  - General 0310
  - Accounting 0272
  - Banking 0770
  - Management 0454
  - Marketing 0338
- Canadian Studies 0385
- Economics
  - General 0501
  - Agricultural 0503
  - Commerce Business 0505
  - Finance 0508
  - History 0509
  - Labor 0510
  - Theory 0511
- Folklore 0358
- Geography 0366
- Gerontology 0351
- History
  - General 0578
  - Ancient 0579
  - Medieval 0581
  - Modern 0582
  - Black 0328
  - African 0331
  - Asia, Australia and Oceania 0332
  - Canadian 0334
  - European 0335
  - Latin American 0336
  - Middle Eastern 0333
  - United States 0337
- History of Science 0585
- Law 0398
- Political Science
  - General 0615
  - International Law and Relations 0616
  - Public Administration 0617
- Recreation 0814
- Social Work 0452
- Sociology
  - General 0626
  - Criminology and Penology 0627
  - Demography 0938
  - Ethnic and Racial Studies 0631
  - Individual and Family Studies 0628
  - Industrial and Labor Relations 0629
  - Public and Social Welfare 0630
  - Social Structure and Development 0700
  - Theory and Methods 0344
- Transportation 0709
- Urban and Regional Planning 0999
- Women's Studies 0453

THE SCIENCES AND ENGINEERING

BIOLOGICAL SCIENCES

- Agriculture
  - General 0473
  - Agronomy 0285
  - Animal Culture and Nutrition 0475
  - Animal Pathology 0476
  - Food Science and Technology 0359
  - Forestry and Wildlife 0478
  - Plant Culture 0479
  - Plant Pathology 0480
  - Plant Physiology 0817
  - Range Management 0777
  - Wood Technology 0746
- Biology
  - General 0306
  - Anatomy 0287
  - Biostatistics 0308
  - Botany 0309
  - Cell 0379
  - Ecology 0329
  - Entomology 0353
  - Genetics 0369
  - Limnology 0793
  - Microbiology 0410
  - Molecular 0307
  - Neuroscience 0317
  - Oceanography 0416
  - Physiology 0433
  - Radiation 0821
  - Veterinary Science 0778
  - Zoology 0472
- Biophysics
  - General 0786
  - Medical 0760

EARTH SCIENCES

- Biogeochemistry 0425
- Geochemistry 0996
- Geodesy 0370
- Geology 0372
- Geophysics 0373
- Hydrology 0388
- Mineralogy 0411
- Paleobotany 0345
- Paleoecology 0426
- Paleontology 0418
- Paleozoology 0985
- Palynology 0427
- Physical Geography 0368
- Physical Oceanography 0415

HEALTH AND ENVIRONMENTAL SCIENCES

- Environmental Sciences 0768
- Health Sciences
  - General 0566
  - Audiology 0300
  - Chemotherapy 0992
  - Dentistry 0567
  - Education 0350
  - Hospital Management 0769
  - Human Development 0758
  - Immunology 0982
  - Medicine and Surgery 0564
  - Mental Health 0347
  - Nursing 0569
  - Nutrition 0570
  - Obstetrics and Gynecology 0380
  - Occupational Health and Therapy 0354
  - Ophthalmology 0381
  - Pathology 0571
  - Pharmacology 0419
  - Pharmacy 0572
  - Physical Therapy 0382
  - Public Health 0573
  - Radiology 0574
  - Recreation 0575
  - Speech Pathology 0460
  - Toxicology 0383
- Home Economics 0386

PHYSICAL SCIENCES

Pure Sciences

- Chemistry
  - General 0485
  - Agricultural 0749
  - Analytical 0486
  - Biochemistry 0487
  - Inorganic 0488
  - Nuclear 0738
  - Organic 0490
  - Pharmaceutical 0491
  - Physical 0494
  - Polymer 0495
  - Radiation 0754
- Mathematics 0405
- Physics
  - General 0605
  - Acoustics 0986
  - Astronomy and Astrophysics 0606
  - Atmospheric Science 0608
  - Atomic 0748
  - Electronics and Electricity 0607
  - Elementary Particles and High Energy 0798
  - Fluid and Plasma 0759
  - Molecular 0609
  - Nuclear 0610
  - Optics 0752
  - Radiation 0756
  - Solid State 0611
- Statistics 0463

Applied Sciences

- Applied Mechanics 0346
- Computer Science 0984
- Engineering
  - General 0537
  - Aerospace 0538
  - Agricultural 0539
  - Automotive 0540
  - Biomedical 0541
  - Chemical 0542
  - Civil 0543
  - Electronics and Electrical 0544
  - Heat and Thermodynamics 0348
  - Hydraulic 0545
  - Industrial 0546
  - Marine 0547
  - Materials Science 0794
  - Mechanical 0548
  - Metallurgy 0743
  - Mining 0551
  - Nuclear 0552
  - Packaging 0549
  - Petroleum 0765
  - Sanitary and Municipal 0554
  - System Science 0790
- Geotechnology 0428
- Operations Research 0796
- Plastics Technology 0795
- Textile Technology 0994

PSYCHOLOGY

- General 0621
- Behavioral 0384
- Clinical 0622
- Developmental 0620
- Experimental 0623
- Industrial 0624
- Personality 0625
- Physiological 0989
- Psychobiology 0349
- Psychometrics 0632
- Social 0451

ABSTRACT

NUTRITIONAL STATUS AND BRONCHOPULMONARY DYSPLASIA

The present study was performed to determine whether ongoing oxidative stress in some BPD infants contributes to their increased energy expenditure leading to growth failure. The study consisted of two parts. The first is a descriptive census of BPD infants (n=38) followed at the outpatient clinics at The Montreal Children's Hospital (MCH). The second is a cross-sectional study of fifteen patients wherein anthropometric parameters, energy intake, and oxidative stress measures (red cell glutathione (GSH) and plasma malondialdehyde (MDA)) were assessed. Nine infants with growth failure were compared to six thriving infants with respect to their nutritional and oxidative stress status. Growth failure was defined as weight for age and weight for height for age less than the tenth percentile (z score $\leq$ -1). Results revealed that the prevalence of growth failure in the BPD infants followed at MCH ranged between 45% and 55%. The mean (± SD) energy intakes for thriving and failing to thrive infants expressed as a percent of the recommended nutrient intake were 104 ± 46% and 133 ± 35% respectively. Six infants had reduced mean (± SD) blood glutathione per hemoglobin (3.63 ± 0.37 umol/g) compared to adult controls (6.57 ± 1.04 umol/g). Four of the six infants had growth failure while two were thriving. Fourteen infants including all failing to thrive infants had elevated mean (± SD) plasma MDA levels compared to adult controls (129 ± 48 vs 55 ± 3 nmol/l). Differences in oxidative stress markers were not observed between the two groups. These results suggest that growth failure is associated with an increase in caloric consumption and not with a decrease in caloric intake. The preliminary findings on oxidative stress markers suggest a depletion of the GSH antioxidant in some infants and marked lipid peroxidation in the BPD population.

RÉSUMÉ

ÉTAT NUTRITIONNEL ET DYSPLASIE BRONCHOPULMONAIRE

Cette étude a été réalisée pour déterminer si le stress oxydatif chez certains bébés atteints de dysplasie bronchopulmonaire (DBP) contribue à la dépense d'énergie plus grande observée et entraînerait un retard de croissance. Cette étude était divisée en deux parties. La première était un recensement descriptif des bébés atteints de DBP (n = 38) traités aux cliniques externes de L'Hôpital de Montréal pour Enfants (HME). La deuxième était une étude clinique transversale menée auprès de 15 patients et au cours de laquelle ont été évalués les mesures anthropométriques, l'apport énergétique et les paramètres du stress oxydatif (gluthation érythrocytaire (GSH) et malondialdéhyde plasmatique (MDA)). Neuf bébés présentant un retard de croissance et six bébés dont la croissance était normale ont été comparés sur les plans de l'état nutritionnel et du stress oxydatif. Le retard de croissance était défini par une masse corporelle (MC) en fonction de l'âge et une MC en fonction de la taille et de l'âge inférieures au dixième percentile (cote $z \leq -1$). Les résultats montrent que la prévalence d'un retard de croissance chez les bébés atteints de DBP suivis à l'HME était entre 45% et 55%. Dans le cas des enfants de croissance normale et de ceux présentant un retard de croissance, les valeurs de l'apport énergétique moyen (± écart-type de la moyenne), exprimé en pourcentage de l'apport nutritif recommandé, ont été de 104 ± 46% et de 133 ± 35%, respectivement. Six bébés présentaient une concentration moyenne (± É-T) de gluthation sanguin par hémoglobine (3.63 ± 0.37 µmol/g) par rapport aux adultes-témoins (6.57 ± 1.04 µmol/g). Quatre de ces six bébés présentaient un retard de croissance et deux, une croissance normale. Quatorze bébés, y compris tous les bébés touchés par un retard de croissance, présentaient des concentrations moyennes (± É-T) élevées de MDA plasmatique par rapport aux adultes-témoins (129 ± 48 vs 55 ± 3 nmol/L). Aucune différence n'a été observée entre les deux groupes sur le plan des marqueurs de stress oxydatif. Ces résultats suggèrent que le retard de croissance est associé à une augmentation de la consommation de calories et non à une diminution de l'apport calorique. Les résultats préliminaires sur les marqueurs de stress oxydatif suggèrent une carence en antioxydant GSH chez certains bébés et une peroxydation importante des lipides dans la population de bébés atteints de DBP.

Acknowledgements

I would like to extend my sincere thanks and appreciation to my supervisor, Dr. Hinda Kopelman for her support, guidance and encouragement during the course of my study. I also appreciate the support provided by committee members, Dr. Katherine Gray-Donald and Dr. Errol Marliss especially for their constructive comments. Sincere thanks go to the BPD clinic team members at the Montreal Children's Hospital for their assistance in the recruitment process, notably Drs. Pat Reilly, Louise Auger, Michael Davis, Michelle Pepin and Donna Johnston (Home Care nurse). I would like to especially thank Dr. Michael Davis for his valuable assistance and advice. The technical assistance provided by Ginette Viau, Claude Gauthier and Joseé Champagne in the handling and preparation of samples is most appreciated. The use of the equipment and computer facilities at the Dietetics Department is highly recognized. I am, in particular, grateful to the dieticians, Natalie Beland and Lynne McCauley, for their anthropometric assessment training and their guidance in the use of the nutritional assessment computer software. I am also indebted to my collaborators, Dr. Sylvain Baruchel and Dr. Guy Lepage, for their measurement of the oxidative stress markers, and valuable comments. I wish to extend my gratitude to the Starr Foundation for its financial support of my training and work to attain this degree. Finally, I am most grateful to my family, Sari Gedeon and Amy Ing for their patience and great support.

NUTRITIONAL STATUS AND BRONCHOPULMONARY DYSPLASIA

TABLE OF CONTENTS **PAGE**

LIST OF TABLES

LIST OF FIGURES

SECTION ONE INTRODUCTION

Bronchopulmonary dysplasia (BPD) is a common sequela for infants born prematurely with respiratory distress syndrome treated with supplemental oxygen and mechanically assisted ventilation. With the increasing number of preterm infants surviving mechanical ventilation, BPD has become one of the most common sequalae of neonatal intensive care (Bancalari et al, 1986). It accounts for the prolonged hospitalization of 10% to 20% of infants who survive mechanical ventilatory support (Goetzman, 1986).

Growth failure is a major problem in infants with BPD but the cause is unclear. Growth failure has been associated with decreased caloric intake and an increased energy expenditure. The cause for the elevated metabolic rates in infants with BPD remains to be determined.

Because premature infants are born with inadequate nutrient stores and an immature pulmonary antioxidant defense system in the context of increased oxidant damage, these infants may be at risk for ongoing oxidative stress. Oxidative stress occurs as a result of an imbalance between the protective activities of antioxidants and the toxicity of oxidants. Oxidative stress in turn may be associated with energy expenditure as the cellular and molecular damage caused

by oxidative stress must be repaired. The cost of the repair process may suggest a role for oxidative stress in the increase in energy expenditure seen in BPD.

The present study is undertaken to examine the association between oxidative stress, energy expenditure and growth failure. The objectives of the study are threefold. Firstly, to determine the prevalence of growth failure in infants with BPD followed at the Montreal Children's Hospital. Secondly, to examine whether growth failure is associated with decreased energy intake and/or increased energy expenditure. Thirdly, to determine whether oxidative stress exists in the BPD population and to examine whether differences in oxidative stress exist between thriving and non thriving BPD infants.

SECTION TWO LITERATURE REVIEW

A. Bronchopulmonary Dysplasia

Bronchopulmonary dysplasia is an iatrogenic chronic lung disease occurring largely in premature infants treated with mechanical ventilation after respiratory distress syndrome. It is clinically characterized by tachypnea, dyspnea, hypoxemia, hypercarbia, and occasionally, signs of pulmonary hypertension with cor pulmonale (Monin and Vert, 1987).

Because of the increased survival of extremely premature infants, the incidence of BPD is rising (Monin and Vert, 1987). This finding was recently confirmed by Parker et al. (1992) who reported an increase in the incidence of BPD from 10.6% in 1976 through 1980, to 21.7% (1981 through 1985), and to 32.9% from 1986 through 1990 in very low birth weight neonates defined as infants weighing less than or equal to 1500g at birth. Parallel to this increase, a decline in the incidence of neonatal deaths was observed during the same periods (26.4%, 18.3%, and 15.9%, respectively). BPD has become the most common form of chronic lung disease in infancy (Northway, 1990).

The incidence of BPD appears to be inversely related to birth weight and gestational age (Bancalari, 1988). It is

estimated that 40 to 70 % of infants weighing less than 1000g at birth and require respiratory support, develop BPD (Bergman and Farrell, 1992). Although medical advances have improved the survival rate of infants with chronic neonatal lung disease, these infants are nevertheless at severely high risk during the first years of life, with a 40 % mortality rate during the first year of life (O'Brodovich and Mellins, 1985). BPD accounts for a major proportion of the physical, financial and emotional morbidity in survivors of neonatal intensive care due to prolonged hospitalization, frequent need for home oxygen administration and repeat hospitalization (Sinkin and Phelps, 1987).

BPD is usually diagnosed in infants who have been ventilated during the first days of life, and who have developed radiological abnormalities of the lungs and still need respiratory support (supplementary oxygen treatment and/or mechanical ventilation) at 28 days postnatal age (Bancalari, 1986). Blood gases usually show hypercapnia and hypoxemia. Minute ventilation is elevated secondary to higher respiratory rate. These infants have increased airway resistance which is present early in the course of respiratory distress syndrome (Burchfield et al, 1993).

Four principal factors have been identified in the pathogenesis of BPD: a) premature birth b) early respiratory

failure requiring ventilation c) oxygen toxicity and d) barotrauma (Northway, 1990).

Premature birth is associated with pulmonary immaturity, lack of surfactant, and inadequate respiratory drive all of which lead to respiratory failure. The latter is critical in the pathogenesis as it requires treatment with supplemental oxygen and mechanical ventilation. It is the interaction of the concentration and duration of supplemental oxygen and the pulmonary immaturity that determines in part the development of BPD. Positive pressure ventilation plays an important role in the pathogenesis because it allows the delivery of supplemental oxygen and injures the lung by barotrauma (trauma associated with mechanical ventilation) (Northway, 1990). Thus, the combined effect of high oxygen concentrations and barotrauma damage the small airways and cells lining the alveoli. This causes increased capillary permeability and leakage of plasma proteins and fluid into the alveoli and interstitium. Consequently, any surfactant that is present is inactivated (Foster, 1989).

A number of factors that prolong artificial ventilation and supplemental oxygen therapy contribute to the severity of BPD such as pulmonary edema (due to patent ductus arteriosus, cor pulmonale, congestive heart failure, and fluid overload), pulmonary air leak (due to interstitial emphysema and

pneumothorax) and pulmonary infection (Northway, 1990).

Frank and Sosenko (1988) hypothesized that undernutrition is a major contributing factor in the pathogenesis of BPD. Undernutrition can compromise the ability to resist hyperoxia, barotrauma, and infections, limit the capacity to repair ongoing lung injury and prevent normal growth.

B. Growth Failure

In broadest terms, growth failure refers to infants and children who fail to gain weight and grow at the expected rate for their age and sex (Frank and Zeisel, 1988). Although it has been agreed that failure to thrive (FTT) should be diagnosed on the basis of anthropometric criteria, there are no universally accepted criteria for both clinical and research purposes (Frank and Zeisel, 1988). Two types of criteria have been used in the literature: a) growth velocity for both weight and height below standard and b) attained growth at a single point in time below a specified percentile on the growth chart (Frank and Zeisel, 1988). A definition of FTT based on the former requires a decrease in growth velocity of two or more major percentile categories over three months (Peterson et al, 1984). Published velocity criteria have differed in the period for which a low rate of weight gain is

considered as FTT. They have varied from 10 or more days to 56 days for children under 5 months of age or 90 days for children over 5 months of age. The definition of FTT based on attained growth at a single point in time expresses the weight and height and weight for height relative to standard norms to identify the chronicity of growth failure. These definitions have also varied among researchers and clinicians. Failure to thrive has been defined as a weight persistently below the third percentile (Berwick, 1980) or fifth percentile for age (Bithoney et al, 1992), or weight less than 80% of ideal weight for age (Berwick, 1980). Goldbloom (1982) found it insufficient to label a child below the third percentile for weight as failing to thrive and instead suggested that weight and length values be extrapolated horizontally to the 50th percentile line on the growth chart. The latter would define the infant's height age and weight age offering an insight on the weight/height relationship. This method allows for the determination of weight as a percent of ideal weight for actual height for age which in turn defines the severity of protein-energy malnutrition using the standards of McLaren and Reed. Bithony and Rathburn (1983) reported that weight for height measurement is the key in the evaluation of FTT and that a weight for height value less than the 5th percentile indicates nutritional deficiency.

One way of expressing the attained anthropometric value

at a certain point in time is using the z score. The z score shows how far anthropometric measures lie from the median of the National Center for Health Statistics percentiles (NCHS), expressed in standard deviation units. Z scores eliminate the age bias inherent in percent of median malnutrition criteria where the latter express individual measurements as a percentage of fiftieth percentile values in the reference population. At different ages, a given percentage of median does not correspond to the same proportion of standard deviation from the median. With increasing age, a static percent of median corresponds to a progressively greater deviation than the median (Peterson et al, 1984). Percentage of median is thus an age-biased approximation of standard deviation from the median. In contrast, z scores of different aged children can be pooled without incurring an age bias. It has been concluded that z scores provide the most accurate technique for classifying growth deficits for research purposes (Peterson et al, 1984). Using z scores, FTT has been defined as weight more than 2 standard deviations below the median (z=-2) of a normal population or weight less than the fifth percentile (z= -1.96) (Peterson et al, 1984). Alternatively, the World Health Organization (1983) suggested a cutoff of 1 standard deviation (SD) below the median (z=-1) or a multiple of this SD (-1.5, -2) be used to define growth failure.

a) Growth Failure in Infants with BPD:

Data from a number of studies, in addition to clinical experience, reveal that a significant number of BPD infants fail to thrive. Markstead and Fitzhardinge (1981) found that in their population of 20 infants with BPD, the average weight and height at 40 weeks postconception were at or below the third percentile. At 2 years' postterm, the mean weight reached the third to tenth percentile for both sexes. Brown (1993) reported that 45% of 33 BPD survivors were growth retarded and estimated that in general only about 7% of survivors are at or above the 50% percentile in height and weight at 2 years of age. Yu and coworkers (1983) reported that the mean weight of 16 infants with BPD at 2 years of age was at the tenth percentile, the mean height at the 10-25th percentile and the mean head circumference at the 50th percentile suggesting growth failure of weight and height development compared to head circumference.

The growth failure in BPD has been attributed by some to to the severity of the neonatal course BPD per se, and by others, to BPD per se. Vohr et al. (1982) found that a significant number of BPD infants were below the third percentile at 4 and 12 months compared to preterm infants who did not have BPD. Meisels and coworkers (1986) compared the growth outcome at 2 years between preterm infants who had had

respiratory distress syndrome (RDS) but did not develop BPD to infants who had developed BPD. Similiar to the physical growth findings of Markstead and Fitzhardinge, 67% of the infants with BPD had weights below the tenth percentile in the second year of life as opposed to 35% of infants with RDS alone; 53% of infants with BPD had their lengths below the tenth percentile versus only 25% of RDS infants. These findings suggest that BPD and not just prematurity confers some growth failure. Davidson et al. (1990) studied the growth outcome of 71 ventilated very low birth weight infants with (n=30) and without (n=41) BPD. Male and female infants without BPD had better growth outcomes than those with BPD. Female infants without BPD had their mean weight at both 12 and 21 months of life (corrected age) above the tenth percentile while BPD females remained below the tenth percentile. Male infants without BPD had greater weight and length measurements compared to BPD infants but the difference did not reach statistical significance. Recently, Bilbrey and coworkers (1993) evaluated the growth of infants with mild, moderate, and severe BPD. Using one way analysis of variance, the height percentile was the only anthropometric variable that differed across the groups with lower values found in the severe group. The authors concluded that linear growth delay is related to disease severity.

These findings clearly demonstrate that infants with BPD

have growth delays in the first years of life and these appear to be due to BPD and not simply premature birth.

b) Significance of Growth Failure in BPD:

Growth failure in BPD has been suggested to result in dual consequences: slower recovery from pulmonary disease and compromised developmental outcome (Niermeyer, 1988). Optimal growth of infants with BPD is desirable so that new lung will develop normally in order to reduce the dependence of these infants on respiratory support (Kalhan, 1990).

Markstead and Fizhardinge (1981) associated growth retardation (height < third percentile) with prolonged respiratory dysfunction. Georgieff et al. (1989) observed that infants who continued to be small at 12 months corrected age (chronological age - number of weeks of prematurity based on 40 weeks gestation) had more neurological abnormalities at 4 months and had poorer developmental status at 12 months. Hack et al. (1984) has shown that poor catch-up growth in VLBW infants is associated with severity of neonatal complications, poor head growth, and chronic physical and neurologic outcome. Whether growth retardation is the cause or simply a concomitant complication of disease severity is not clear from these studies. However, Ross et al. (1983) found that infants with good neurobehavioral outcome (defined by a mean Bayley

score greater or equal to 85) had a significantly larger head circumference than infants with poor neurobehavioral outcome from 3 months postterm throughout the first year ($p<0.05$), suggesting that growth parameters might be an important predictor of later development. Similiar differences were noted for length and weight from 3 months postterm throughout the first year ($p<0.05$). Hack and coworkers (1982) followed a sample of 192 very low birth weight infants up to 8 months and observed that appropriate for gestational age (AGA) defined as birth weight less than the 10^{th} percentile for gestational age and small for gestational age (SGA) infants defined as birth weight between the 10^{th} and 90^{th} percentile for gestational age who failed to thrive during infancy or failed to catch-up in weight by 8 months had lower mean Bayley developmental quotients ($p<0.005$), smaller head circumferences ($p<0.005$) and a high rate of neurosensory impairment than AGA infants with normal postnatal growth. Gross et al. (1974) considered occipital-frontal circumference percentile at birth and head growth by 6 weeks as strong predictors of early developmental outcome in VLBW infants.

These findings suggest that early growth attainment is an important predictor of later developmental outcome. If growth failure does negatively impact on developmental outcome as has been suggested (Georgieff, 1989) , then it is essential to begin to understand the causes of growth failure in order to

alleviate this problem and attempt to achieve better outcomes.

c) Etiology of Growth Failure:

Several hypotheses have been proposed to explain the mechanisms contributing to the failure to thrive in infants with BPD. These include chronic hypoxia, emotional deprivation from prolonged hospitalisation (Yu et al, 1983), heart failure, poor gastrointestinal absorption (Vohr et al, 1982; Oh, 1986), high energy expenditure, and inadequate caloric intake due to anorexia, respiratory distress or iatrogenic limitation of fluids (Vohr et al, 1982; Oh, 1986).

Yeh and coworkers (1989) reported significantly lower energy intake ($p<0.01$) in infants with BPD compared to controls matched for birth-weight, gestational age and postnatal age. Weight gain was significantly less in BPD infants than in controls ($p<0.05$). The authors attributed growth failure to inadequate energy intake. The finding of a lowered energy intake in BPD infants in this study is consistent with results of Wilson and colleagues (1991) who observed significantly lower energy intakes from day 7 to day 56 in infants with BPD compared to infants matched for gestational age but without BPD. Kurzner et al. (1988) compared energy intake in thriving and non thriving BPD

infants. Growth failure was defined as weight and length less than the tenth percentile of the Babson growth curve. No statistically significant difference in total energy, protein or fat intake was observed between BPD infants with normal growth and those with growth failure, indicating that growth failure in this study was not associated with poor nutritional intake.

With the availability of indirect calorimetry, measurements of resting energy expenditure (REE) have been performed in BPD infants. To date, six groups of investigators have reported an increase in resting energy expenditure in BPD infants.

Yeh and co-workers (1989) reported 33% higher total energy expenditure in 5 BPD infants receiving oxygen when compared to five non BPD infants matched for birth weight, gestational age and postnatal age. Earlier data from Weinstein and Oh (1981) were also consistent with the findings of Yeh and collegues, reporting a 25% increase in resting oxygen consumption (VO_2), a measure of energy expenditure, in 8 BPD infants receiving supplemental oxygen at more than 4 weeks of age when compared to seven infants who had no major medical problems at the time of the study. Infants were matched for gestational age, postnatal age and birth weight. These investigators postulated that the increase in work of

breathing was responsible for the increase in resting VO_2.

Kurzner et al. (1988a) compared resting VO_2 between thirteen infants with BPD and twelve healthy term infants. The BPD group was comprised of seven infants who were failing to thrive and six thriving infants. Seven infants of the control group were size matched to the BPD infants with growth failure while five controls were age matched to thriving BPD infants. Results revealed that VO_2 in BPD infants with growth failure was markedly elevated compared to thriving BPD infants and healthy term infants. In a subsequent study, the same authors compared pulmonary function between BPD infants with growth failure and normally growing BPD infants (Kurzner et al, 1988b). There was no statistically significant difference between the two groups with respect to work of breathing and respiratory rate; however, dynamic compliance (distensibility of lungs) was significantly lower in the group with growth failure. The authors concluded that work of breathing and pulmonary mechanics may explain some but not all of the increase in resting energy expenditure seen in BPD.

In order to determine whether an increased VO_2 in infants with BPD is caused by increased work of breathing, Kao and coworkers (1988) conducted a double-blind cross over study of sixteen infants with BPD treated with theophylline, a bronchodilator and/or diuretics. Results revealed that infants

in the placebo group had elevated VO_2 (7.65 ml/kg/min), increased airway resistance and increased mechanical power of breathing (work of breathing * respiratory rate) compared to reported control data (Kurzner et al, 1988b; Kao et al, 1983; Thibeault et al, 1966). Treatment with theophlline, diuretics, or theophylline plus diuretics resulted in a significant improvement in pulmonary mechanics and mechanical power of breathing but no improvement in VO_2. The authors contested the role of work of breathing as the cause of increased VO_2 and suggested that factors such as inflammation and repair processes in the lungs may be responsible for the elevated VO_2.

Gamarra (1992) recently compared total energy expenditure (EE) in five premature newborns with BPD to that of six infants who had recovered from respiratory distress syndrome. The postnatal ages of the BPD and control group were 105 ± 45 and 31 ± 6 days respectively. The BPD group had significantly higher mean VO_2 (10.15 vs 8.04 ml/Kg/min $p< 0.01$), mean VCO_2 ($p<0.02$) and mean EE (76 vs 61 Kcal/kg/ day $p<0.02$) compared to the non BPD group. The highest values were encountered in the most severely ill infants. Interestingly, the EE reported in this study is identical to that reported by Yeh et al. (1989).

When comparing resting energy expenditure (REE) between

BPD infants and controls, authors have expressed REE relative to body size. While it is necessary to normalize the data to an index of body size, REE should ideally be expressed in terms of fat free mass. This is because whole body rates of REE depend on the metabolically active body mass, specifically fat free mass. In the clinical setting, fat free mass may be measured by bioelectric impedence in adults; however it has not been widely used nor validated in infants. While normalizing REE data to body weight may overestimate REE or VO_2 in infants with BPD and growth failure, when REE in BPD infants with growth failure was compared to controls matched for body weight in Kurzner's study, REE was still significantly increased in BPD.

In light of the above, growth failure in infants with BPD does appear to be related to an increase in resting energy expenditure; however, the cause of the latter is still unclear. If work of breathing cannot fully account for the increased REE, then to what can this increase be attributed ?

C. Oxidative Stress

a) Free Radicals and Oxidant Damage:

Toxic oxygen free radicals have been implicated as important pathogenic mediators in many clinical disorders (Cross, 1987).

A free radical is any species that contains one or more unpaired electrons (Machilin and Bendich, 1987). Free radicals can originate endogenously from normal metabolic reactions or exogenously as components of tobacco smoke, air pollutants, or from exposure to radiation and hyperoxic environments (Machilin and Bendich, 1987). Because of their unpaired electrons, free radicals are short-lived, very reactive and capable of reversibly or irreversibly damaging organic compounds of all biochemical classes (Kneepkens et al, 1992). These include nucleic acids, proteins and free amino acids, lipids and lipoproteins, carbohydrates and connective tissue macromolecules (Cross, 1987). When free radicals interact with cellular constituents, they may lead to cell damage or cell death (Frank and Sosenko, 1987).

The formation of highly reactive oxygen containing molecules is a normal consequence of a variety of essential biochemical reactions in vivo and is occurring all the time (Wispe and Roberts, 1987). Under hyperoxic conditions, some of these reactive oxygen metabolites--superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical(OH), singlet oxygen ($^1O_2$), and peroxide radical (ROO) are known to be produced intracellularly in excess amounts (Frank and Sosenko, 1987). Oxidant free radical damage can also develop from the release of free radicals at the tissue level by invading polymorphonuclear leukocytes and macrophages involved in the

inflammatory response and tissue repair (Sinkin and Phelps, 1987).

b) Antioxidant Defense System:

Most cells are equipped with a variety of antioxidant systems to protect them against the adverse effects of reactive oxygen species. The diversity of the defense mechanisms, ranging from low molecular weight compounds to complex enzyme networks, allows an efficient protection at the intercellular and subcellular level (Akerboom and Sies, 1989). Catalase, superoxide dismutase and the enzymes of the glutathione (GSH) redox cycle are the primary intracellular antioxidant enzyme defense mechanisms. Non enzyme antioxidant molecules include lipid soluble antioxidants such as vitamin E and beta-carotene and water soluble antioxidants such as vitamin C, uric acid, and glutathione. Some molecules have a wide distribution (vitamin C, uric acid) while others are present in the lipid membranes of tissues (vitamin E and beta carotene) (Heffner and Repine, 1989).

Although catalase and the GSH redox cycle have overlapping capacities for the reduction and elimination of intracellular H_2O_2, experimental and clinical evidence reveal that the GSH redox cycle is the most important antioxidant peroxidase system in mammalian species. Whereas catalase is

largely confined to peroxisomes, enzymes of the GSH redox are distributed throughout the cytosol, increasing their availability and contact with oxidants. While catalase functions maximally at high concentrations of hydrogen peroxide, glutathione peroxidase is an effective scavenger at low concentrations. In addition, glutathione peroxidase utilizes both lipid hydroperoxides and hydrogen peroxide as substrates (Heffner and Repine, 1989).

Emphasis will be placed upon glutathione and it's role in the GSH redox cycle as the latter is a central mechanism for the reduction of intracellular hydroperoxides. Glutathione (GSH) is an antioxidant present intracellularly (Heffner and Repine, 1989). It is also present in large amounts in the epithelial lining fluid in the lung mainly in its reduced form in concentration 100 times that of plasma (Cantin, 1987). The alveolar space recruits glutathione from the epithelial lining fluid to provide antioxidant protection to lung parenchymal cells (Cantin, 1985). As an alveolar antioxidant, glutathione supplements the intracellular antioxidant system in preventing air-way borne oxidant injury. This is of importance because the lungs have a large epithelial surface area suceptible to oxidant-mediated attack (Heffner and Repine, 1989). With catalase and superoxide dismutase, glutathione prevents free radical chain reactions by decreasing available concentrations of free radicals to initiate the process (Heffner and Repine,

1989). Acting as a hydrogen donor, GSH contributes to the reduction of hydrogen peroxide and lipid peroxides to non reactive molecules (Akerboom and Sies, 1990). Reactions (1) and (2) are catalyzed by GSH peroxidase, a selenium-dependent enzyme that has an absolute requirement for glutathione as a substrate (Heffner and Repine, 1989):

$$(1)\quad 2GSH + H_2O_2 \xrightarrow[\text{GSH peroxidase}]{} GSSG + H_2O$$

$$(2)\quad 2GSH + ROOH \xrightarrow[\text{GSH peroxidase}]{} GSSG + ROH + H_2O$$

Thus GSH plays a critical role in interrupting the propagation of free radical reactions leading to lipid peroxidation (Akerboom and Sies, 1990).

During the course of these redox reactions, GSH is continuously oxidized to glutathione disulphide (GSSG) and rereduced by the enzymes GSH-peroxidase and GSH reductase respectively. GSH reductase which catalyzes the regeneration of reduced glutathione from oxidised glutathione uses nicotinamide adenine dinucleotide phosphate (NADPH) formed by the pentose phosphate pathway (Heffner and Repine, 1989; Akerboom and Sies, 1990). Non stressed cells maintain a high intracellular reduced/oxidised glutathione ratio to ensure the availability of reduced glutathione necessary for these antioxidant processes (Heffner and Repine, 1989). GSH redox

activity is highest in erythrocytes and the liver with intermediate levels in the lung and heart (Heffner and Repine, 1989). In isolated liver and lungs, a variety of oxidant stresses have been shown to activate the glutathione peroxidase system in vivo resulting in increased oxidized glutathione, release of oxidised glutathione (GSSG) into the effluent and under conditions of severe oxidant stress, depletion of reduced glutathione (GSH) (White, 1988).

A number of studies have demonstrated lower selenium levels in premature than in term infants (Amin et al, 1980; Sluis et al, 1992). Selenium is an important dietary component of lung glutathione peroxidase. Amin and coworkers (1980) associated poor growth and severe BPD with selenium deficiency in a small infant receiving prolonged parenteral nutrition. Lockitch et al. (1980) reported that baseline selenium concentration and glutathione peroxidase activity in plasma were significantly lower in low birth weight infants than in full term babies. Parenteral nutrition without supplemental selenium provided the majority of calories for all 16 infants during the first two weeks of life and for seven during the subsequent three weeks. Plasma selenium levels were found to decrease from baseline levels over the first 50 postnatal days in 16 sick low birth weight infants who were followed up to 6 weeks because of lung disease. Similar findings were observed by Sluis et al, (1992) who reported dramatic decreases in

plasma selenium and GSH peroxidase with age in premature infants remaining in the neonatal unit. It was observed that of 12 infants with plasma selenium levels less than 0.19 μmol per litre, eight were still requiring oxygen at 28 days and six had BPD. Falcigilia et al. (1988) also found severe selenium deficiency in eight patients who recovered from respiratory distress and 11 patients in whom BPD developed during the first month of life. Plasma selenium did not differ between the two groups; however, vitamin E levels were significantly lower in the BPD group compared to control at 3 days of life (0.58 vs 1.29 mg/dl, $p<0.05$) and oral vitamin E administration failed to increase plasma vitamin E at 3 days in BPD group. These authors suggested that selenium deficiency whether due to prematurity and low stores and/or dietary deficiency postnatally in sick infants impairs selenium dependent antioxidants and may lead to the increased requirement for the antioxidant, vitamin E.

These studies point to the possible role of a deficiency in the antioxidant, selenium, in relation to GSH peroxidase deficiency in the development, enhancement of severity, or prolongation of respiratory problems such as BPD.

c) Oxidative Stress in Premature Infants

The lungs exist in an oxygen rich environment delicately

balanced between the toxicity of oxidants and the protective activities of several intracellular and extracellular antioxidant defense systems. When the critical balance between free radical generation and antioxidant defense is upset, oxidative stress occurs (Machilin and Bendich, 1987). Premature infants might be susceptible to oxidative stress for a number of reasons. The following discussion will attempt to briefly elucidate the mechanisms that might predispose premature infants to oxidative stress.

A number of the cell's overall antioxidant defense systems, such as vitamin A, E, C, beta carotene, sulfur containing amino acids and trace metals such as selenium, iron, copper, zinc are in low concentrations in the premature infant as the placental maternal to fetal passage of these factors is very limited before the third trimester (Frank, 1992).

In addition, experimental studies from 4 different animal species have shown that antioxidant enzyme (AOE) activities were quite low until the final 15-20% of gestation (Frank and Sosenko, 1987). Marked increases in AOE occured up to 500% just prior to birth (Frank, 1992). It was also demonstrated that unlike full-term rabbits who showed consistent elevations in AOE activities when exposed to > 90% O_2, premature rabbits failed to respond to hyperoxia by increased AOE levels (Frank

and Sosenko, 1991). Based on these findings, it was suggested that the pulmonary AOE defense system is not fully developed nor responsive to oxidative stress in the premature human infant, compromising its ability to handle the relative hyperoxia of birth (Frank and Sosenko, 1987). Recent evidence has shown that unlike the animal models studied, the developmental pattern of the antioxidant enzyme catalase in the human lung increases throughout gestation (McElroy et al, 1992). In addition, the expression of copper-zinc and manganese superoxide dismutase in the human lung was demonstrated to be a continuous process throughout development and not a late gestational process as has been shown in animal studies (Strange et al, 1990). These findings are based on postmortem material and require cautious interpretation. In contrast, Ripalda and coworkers (1989) described the developmental pattern of antioxidant defense mechanisms in erythrocytes from neonates with birth weight between 520 and 4210gm and 12 healthy adults. Erythrocyte catalase activity and GSH-peroxidase activity were strongly correlated with increased birth weight and gestational age. Results indicated that most of the changes in the antioxidant activity were detectable from about 31 to 34 weeks gestation equivalent to a fetal weight of approximately 1500-2000g.

Hence, the premature infant's preparedness for independent respiratory function, already affected by the

limited or absent secretion of surfactant, may be further compromised by the underdeveloped antioxidant enzyme system in the immature lung and erythrocyte, and by limited antioxidant vitamins and trace metals (Frank and Sosenko, 1987).

At birth, the lungs are suddenly exposed to substantially higher oxygen tensions than in utero and the infant must rapidly adapt to this sudden increase in pulmonary oxygen tension (Wispe and Roberts, 1987). This relative hyperoxia of birth is frequently compounded by the exposure to higher oxygen concentrations as part of the required treatment of respiratory distress syndrome of prematurity. The more premature the newborn, the more immature the antioxidant enzyme catalase, and yet the more likely the infant will receive high O_2 supportive therapy (Frank and Sosenko, 1988).

Summing up, premature infants are highly suceptible to oxidative stress as a result of the insufficient stores of nutrients needed for effective antioxidant lung defenses, the undeveloped antioxidant enzymes, and the simultaneous increase in reactive O_2 metabolites resulting from high oxygen therapy. Ongoing damage, inflammation, and repair may further contribute to oxidant injury, depletion of antioxidant defenses and perpetuate oxidative stress.

d) Evidence of Oxidative Stress in Premature Infants and Infants with BPD:

Pitkanen and coworkers (1990) measured oxidative stress in 19 very low birth weight infants with respiratory distress syndrome by quantifying ethane and pentane in expired air during the first 5 days postnatally. Ethane and pentane are degradation products of lipid peroxidation, the result of oxygen free radical damage to the phospholipids of cellular and subcellular membranes. They found that infants who later developed BPD or who subsequently died, exhaled significantly more lipid peroxidation products in the first 5 days postnatally, than those who subsequently had no serious neonatal complications and who developed normally during the first year of life. Kuivalein et al. (1991) obtained similar findings in 24 premature infants; expired ethane and pentane increased significantly 5 and 3 fold, respectively, to a maximum at 5-6 days of age ($p<0.001$).

In vivo, lipid peroxidation may be studied through the resulting degradation products - conjugated dienes, lipid hydroperoxides, aldehydes and volatile hydrocarbons (Kneepkens et al, 1992). Considerable attention has been given to aldehydes because of their relatively greater lifetime than the highly reactive radicals. As a result, reactive aldehydes can diffuse over larger distances in the cell and can attack

targets which are not directly in the proximity of the original site of free radical production. Moreover, aldehydes are detected in biological samples more easily than the initially formed radicals offering a more suitable index of lipid peroxidation (Hageman et al, 1992).

Of the aldehydes formed as secondary products of lipid peroxidation, malondialdehyde (MDA) is the single most abundant peroxidation product of polyunsaturated fatty acids (PUFA), notably of PUFA of more than 2 double bonds (Esterbaub and Cheeseman, 1990). Because of its reactivity, MDA rarely remains free in biological samples, but rather, is often found in covalently bound forms. It has been widely used as an index of lipid peroxidation and has been measured in both urine and blood (Draper and Hadley, 1990; Bird and Draper, 1984).

In order to determine the relationship of urinary MDA in preterm neonates with different modalities of oxygen supply and related clinical problems, Schlenzig and coworkers (1993) measured urinary MDA in 45 preterm neonates (25-35 weeks' gestation) during the first month of life. A significantly higher MDA concentration was found in infants with BPD compared to those without ($p< 0.005$). In addition, MDA concentration correlated slightly but significantly with the fraction of inspired oxygen ($r=0.22$, $p<0.02$).

Several precautions must be taken into account when measuring urinary MDA. First, dietary effects should be precluded as rats fed with different fatty acids showed remarkable changes in urinary MDA. Second, only a minor fraction of MDA formed in vivo is excreted in urine due to hepatic metabolism or to a poor capacity of the kidneys to excrete extrarenally produced MDA. Third, MDA is highly reactive and is not very stable in biological samples. Fourth, MDA in urine is mainly bound to other compounds making it hard to estimate the precise origin of MDA when acidic derivatization methods are used (Hageman et al,1992).

Recently, Grigg et al. (1993) found lower concentration of glutathione in bronchoalveolar lavage fluid in the first day of life in seven infants who subsequently developed chronic lung disease compared with 27 infants who did not require supplemental oxygen at 36 weeks postconceptional ($p=0.003$). All infants in this study had a gestational age less than 35 weeks. It was suggested that a deficiency of glutathione in the lung epithelial fluid may predispose premature infants to lung injury.

These results indicate that, in premature infants, free radical activity and it's consequences increase during the first postnatal days and may be associated with injury to tissues and the development of BPD.

While these data are suggestive of an early role for oxidative stress in the pathogenesis of BPD, no data on oxidative stress in infants beyond the first weeks/months of life are available. Little work has addressed oxidant defense and ongoing oxidative stress in the older infant with BPD and no data have examined the possible role of oxidative stress in growth failure and it's relation to energy expenditure in these infants with BPD.

Cellular and molecular damage caused by oxidative stress must be repaired. In addition, ongoing lung injury, cell damage and inflammation may impair energy utilization. Thus, the cost of the repair process plus inefficient utilization may suggest a role for oxidative stress in the increase in energy expenditure seen in BPD. The preliminary data of Bounos and colleagues (1993) seem to support this contention. After supplementing the diet of 3 HIV seropositive individuals with whey protein rich in glutamylcysteine dipeptide groups promoting glutathione activity, an increase in glutathione content of the blood mononuclear cells was observed. In addition, an increase in body weight was documented which could not be explained by increased energy or protein intake. Although energy expenditure (EE) was not measured in this study, it has been shown that resting EE is elevated in HIV seropositive individuals compared to controls (Hommes et al,

1991). If weight gain was achieved by augmenting glutathione without increasing energy intake, then energy expenditure must have been decreased. It is possible that a decrease in EE was associated with a decrease in oxidative stress as glutathione levels improved and this, therefore led to an improvement in weight gain.

SECTION THREE HYPOTHESIS and OBJECTIVES

The following hypothesis is proposed:

Ongoing oxidative stress in some BPD infants contributes to their increased energy expenditure and this leads to growth failure.

The objectives of this study are threefold:

1. To determine the prevalence of growth failure in BPD infants followed at the Montreal Children's Hospital
2. To establish whether growth failure is due to increased energy expenditure and/or decreased energy intake.
3. To determine whether ongoing oxidative stress exists in BPD infants and whether there is a difference between normally growing BPD infants and BPD infants with growth failure.

SECTION FOUR RESEARCH METHODS

A. Study Design

The study consisted of two parts: the first is a clinic census of all BPD infants who were followed in the out patient clinics at the Montreal Children's Hospital during the summer of 1993. Criteria for enrolment in the clinic at the time of discharge from the nospital included a requirement for home oxygen at 40 weeks post conceptual age. Hence, these infants had severe BPD at the time of referral to clinic. However, these criteria were not necessarily present at the time of study. In the second part, patients in a sample of this population were enrolled between August 1993 and January 1994 for the evaluation of their nutritional and oxidative stress status.

B. Study Population

a) Selection Criteria and Recruitment

For the clinic census, all charts of children with BPD less than four years of age were reviewed. For the sample, the study population consisted of BPD infants of willing parents between the ages of 3 and 38 months corrected age

followed in the Home Care/BPD clinic, Neonatal clinic, and Respiratory clinic. Criteria for enrolment included a diagnosis of BPD, regular medical care in one of the clinics at MCH and signed parental consent. Infants were excluded from this portion of the study if they were known to have uncorrected congenital heart disease or any other medical diagnosis in addition to BPD that would influence their growth, peripheral edema, jaundice or intercurrent illness at the time of study.

The recruitment of participants was carried out by the research nutritionist (J.C) by one of two approaches. The first involved direct contact with the parents of eligible participants in the out-patient clinics where an explanation of the purpose of research and the extent of their involvement was provided. The second approach involved mailing parents an invitation letter as well as a consent form which included the details of the study. A week later, this was followed by a telephone call to clarify any questions pertaining to the research and to inquire about their decision concerning entry into the study.

The study was approved by the Montreal Children's Hospital institutional review board. Signed informed consent was obtained from the parent of each patient.

b) Sample Size

Data on plasma malondialdehyde and red cell glutathione in this population (measures of oxidative stress) are not available, making an estimate of the sample size required for study based on these parameters impossible. However, if our hypothesis is correct that oxidative stress results in an increased energy expenditure, resulting in failure to thrive, an estimate of the sample size required can be derived from data on energy expenditure in BPD infants with and without failure to thrive (Kurzner et al, 1988b; Browner et al, 1988). Oxygen consumption, a marker of energy expenditure, was increased by 25% to 43% in infants with BPD compared to controls (Weinstein, 1981; Kao, 1988; Yeh, 1989; Kurzner, 1988b; Gamarra, 1992) with a standard deviation of 1.79 ml/Kg/min (Kurzner, 1988b). The most conservative estimate, therefore, would assume a 25% difference in VO_2 between BPD infants with FTT and thriving BPD infants with a 1.79 standard deviation, a one tailed alpha of 0.05 and a beta error of 0.20. This would require 12 infants (ie 6 infants per group) for this study. Fifteen subjects were recruited for analysis of oxidative stress.

C. Data Collection

For the clinic census, data was collected by review of

hospital records and consisted of birth weight, gestational age, chronological and corrected age, weight, length/height in order to describe the BPD population. Subjects enrolled in the sample had weight, height/length, triceps skinfold, mid arm circumference and mid arm muscle circumference measured by J.C. at the time of the study. Blood samples were measured for plasma malondialdehyde, red blood cell glutathione and hemoglobin concentration. Food intake was recorded by the parents on two separate 3 day food records.

D. Methods of Measurement

a) Anthropometric Measurements

Weight values were obtained in infants less than 24 months without clothing using an electronic pediatric scale (Detecto Scales, Webb city, MO). Values were recorded to the nearest 100 gms. Recumbent length was measured for infants whose age was less than 24 months with the aid of a wooden length board. Measurement was recorded to the nearest 0.5 cm. For children whose age was equal to or exceeded 24 months, weight and height were recorded without shoes and with subjects wearing a minimum of clothing on a beam balance scale (Detecto Scales Inc Brooklyn, NY, USA). Weights were recorded to the nearest 100g and height to the nearest 0.5 cm. The nutritional status of infants was assessed using the National

Center for Health Statistics (NCHS) growth charts (33) where the infant's weight for age, length for age and weight for length was plotted after correcting for gestational age. To correct for gestational age, the number of weeks of prematurity (based on 40 weeks gestation) was subtracted from the child's chronological age. The difference between growth percentiles for corrected and uncorrected postnatal age is statistically significant depending on the growth parameter (Peterson et al, 1984). Age at measurement should be corrected for prematurity up to 24 months for weight, and 3.5 years for height (Peterson et al, 1984). Data were expressed as corrected weight for age, weight for height and height for age z score. Z scores were calculated based on corrected age using software for calculating pediatric anthropometry (ANTHRO version 1.01)

The triceps skinfold (TSF) measurement was made at the midpoint between the acromion and olecranon on the left arm while the arm was bent at a 90 degree angle using a Lange skinfold caliper with the subject in a standing position (age $\geq$ 24 months) or held in mother's lap (age < 24 months). The skinfold was pinched with the fingers approximately 1 to 2 cm above the midpoint and the jaw of the caliper placed at the midpoint (Walker et al, 1985). Readings were taken three times and the average of these measurements was recorded to the nearest 0.5 millimeter.

The measurement of the mid arm circumference was made at the TSF site with the left arm hanging relaxed. A measuring tape was wrapped gently but firmly around the arm and the circumference was recorded to the nearest mm (Gibson, 1990). The mid arm muscle circumference (MAMC) was calculated from the triceps skinfold and midarm circumference (MAC) using the standard equation: MAMC(cm) = MAC (cm) - 3.14 TSF(cm). TSF, MAC and MAMC measurements were compared to the standards by Frisancho (1981) and expressed as percentiles.

b) Dietary Assessment

Energy intakes were assessed by the use of 2 independent 3-day food records with a 1-2 week interval between the food records. One of the food records always included a weekend day. Food records, including instructions on how to record food, were given to mothers. Two forms were prepared: one for infants less than one year and the other for children older than 1 year. Mothers were asked to record the time, quantity of food consumed and method of preparation during the six days. These records were brought in on the next clinic day or mailed in to the research nutritionist. The average of these records was calculated to reflect the daily nutritional intake of these infants/children. The adequacy of the six day food records was assessed by comparing the within-subject variability to between-subject variability and calculating the

number of days necessary to estimate energy intake to within 20% of the true mean intake 90% of the time according to the following formula (Willett et al, 1990):

$$n = (Z\ CVw/Do)^2$$

where n= the number of days needed per person

Z = the normal deviate for the percentage of times the measured value should be within a specified limit
CVw = the within-person coefficient of variation
Do = the specified limit (as a percentage of long-term true intake)

Nutritional data collected from the dietary records were coded and analyzed using the database Nutritional Assessment System (NUTS Quilchenia Consulting Ltd) .

c) Assessment of Oxidative Stress

Because the direct measurement of oxygen free radicals is very difficult in biologic systems, the presence of oxidative stress in vivo is often assessed by demonstrating degradation products of free radical injury in tissues and body fluids and/or by identifying a depletion of antioxidant defences. We assessed oxidative stress by measuring malondialdehyde in plasma, a degradation product of lipid peroxidation and red blood cell glutathione, a measure of one antioxidant defense. All subjects were asked to fast for a minimum of 3 hours to avoid any dietary effects and blood was withdrawn between 10:00am and 1:00pm to avoid diurnal variations.

(i) Determination of Plasma Malondialdehyde (MDA)

Samples for MDA were collected into EDTA tubes on ice, centrifuged within an hour and separated into plasma and packed cells. Plasma was stored at -80°C for a maximum of 6 weeks until assayed.

MDA was measured in plasma by a new high performance liquid chromatography (HPLC) technique which is more specific, reliable, and reproducible than previously available (Lepage et al, 1991). To 500 microliters (μl) of plasma, 2 ml of water and 500μl of 0.5% butylated hydroxytoluene in methanol were added to prevent further formation of MDA. This was followed by the addition of 200μl of 0.66N H_2SO_4 and 150 μl of 10% Na_2WO_4 in order to precipitate proteins and allow complete recovery of MDA. The mixture was thereafter centrifuged at 1000 x g for 10 minutes (min). Maximum formation of the MDA-TBA complex was obtained by adjusting the pH between 2.5 and 4.5. The MDA-TBA complex was heated at 100°C for 60 min, then completely extracted with n-butanol at pH < 0.75 which was then evaporated at 37°C under nitrogen, 20 μl of the sample were injected into the HPLC, and the peak of the MDA-TBA complex determined. MDA was expressed as nmol/liter.

(ii) Blood Glutathione

Samples for red blood cell glutathione were collected in heparinized collection tubes. Erythrocyte pellets were obtained by centrifuging at 500 x g for 15 min at room temperature. The plasma and buffy coat were then removed and erythrocytes were washed twice in 1.25 ml of sterile 0.9 g/L NaCl solution. Erythrocyte lysates were prepared according to the method of Picot et al (1992) with modifications. Erythrocyte pellets were frozen and thawed two times, followed by the addition of three volumes of ice-cold distilled water. Cell membranes were removed by centrifugation (6000 x g for 10 min at 4°C) and the supernate was frozen at -80°C until the determination of GSH was performed. The latter was carried out by the DTNB-GSSG reductase recycling assay for total glutathione (Anderson, 1985). The method combines the usefulness of the colorimetric reaction of DTNB (5,5'-dithiobis-2-nitrobenzoic acid) with the specificity of GSH-reductase and offers a high sensitivity for total glutathione. One hundred microliters of sample were added to 700 μl of the working buffer containing 0.3mM NADPH (pH 7.4) after diluting in stock buffer [0.143M sodium phosphate containing 6.3mM EDTA (pH 7.5)]. One hundred microliters of DTNB solution (6mM in stock buffer) were added with mixing and the assay was initiated by the addition of 10μl of GSSG reductase (120U/mg) diluted in stock buffer. The rate of TNB formation was then

followed spectrophotometrically at 412nm for 2 minutes and compared to a standard curve of known quantities of glutathione. Values were calculated as nanomoles of glutathione and expressed in μmol/g of hemoglobin.

E. Data Analysis

Data were collected by the research nutritionist and filed into software program Lotus 123. The data were then imported into statistical software program SPSS for Windows (version 6.0). SAS was used for repeated measures analysis of variance for determining the variability in energy intake. The following model was used to determine the within-subject variability and between-subject variability (Willett, 1990) : Nutrient Y = μ + Subject + ϵ ; where μ is the mean energy intake, subject represents between-subject variability and ϵ represents within-subject variability.

Data were expressed as group means and standard deviation for all variables. Student's unpaired t test was used to compare the means between the two groups. Relationships between two variables were expressed as Pearson correlation coefficients. P values were considered significant if less than 0.05. Significance levels for testing correlations are reported as one tailed while significance levels comparing means between two groups are reported as two tailed.

SECTION FIVE RESULTS

I. Descriptive Study

A. Characteristics of the BPD Population

The medical records of thirty eight BPD patients less than four years of age followed at the Montreal Children's Hospital were reviewed between June 1993 and August 1994. The population consisted of twenty nine males and nine females. Table 1 displays the characteristics of this population. The corrected age of the population varied from 3 to 40 months with a mean of 18.3 ± 11.5. The mean gestational age was 27.3 ± 2.7 weeks with a range of 22.7 to 30 weeks. The mean birth weight was 981.5 ± 440.9 with a range of 450 to 2850g. Comparing the characteristics by sex, there were no significant differences in gestational or corrected age between females and males. However, females followed in the clinic had a significantly lower birth weight than the males who were being seen. Four patients (2 males, 2 females) were small for gestational age at birth, defined as a birth weight less than the 10^{th} percentile for gestational age; the remainder had birth weights appropriate for gestational age, defined as birth weight between the 10th and 90th percentile for gestational age.

DESCRIPTIVE STUDY
CHARACTERISTICS OF THE BPD POPULATION

	FEMALES (N = 9) MEAN ± SD	MALES (N = 29) MEAN ± SD	TOTAL (N = 38) MEAN ± SD
CHRONOLOGICAL AGE (month)	25.39 ± 14.22	20.28 ± 10.52	21.49 ± 11.50
CORRECTED AGE (month)	22.08 ± 14.63	17.16 ± 10.34	18.33 ± 11.48
GESTATIONAL AGE (week)	26.41 ± 2.28	27.53 ± 2.83	27.26 ± 2.72
BIRTHWEIGHT (gm)	716.67 ± 274.72	1063.62 ± 453.62*	981.45 ± 440.89

* $P < 0.05$

TABLE 1

DISTRIBUTION OF WEIGHT FOR AGE Z SCORE(WAZ) IN BPD POPULATION

(N=38)

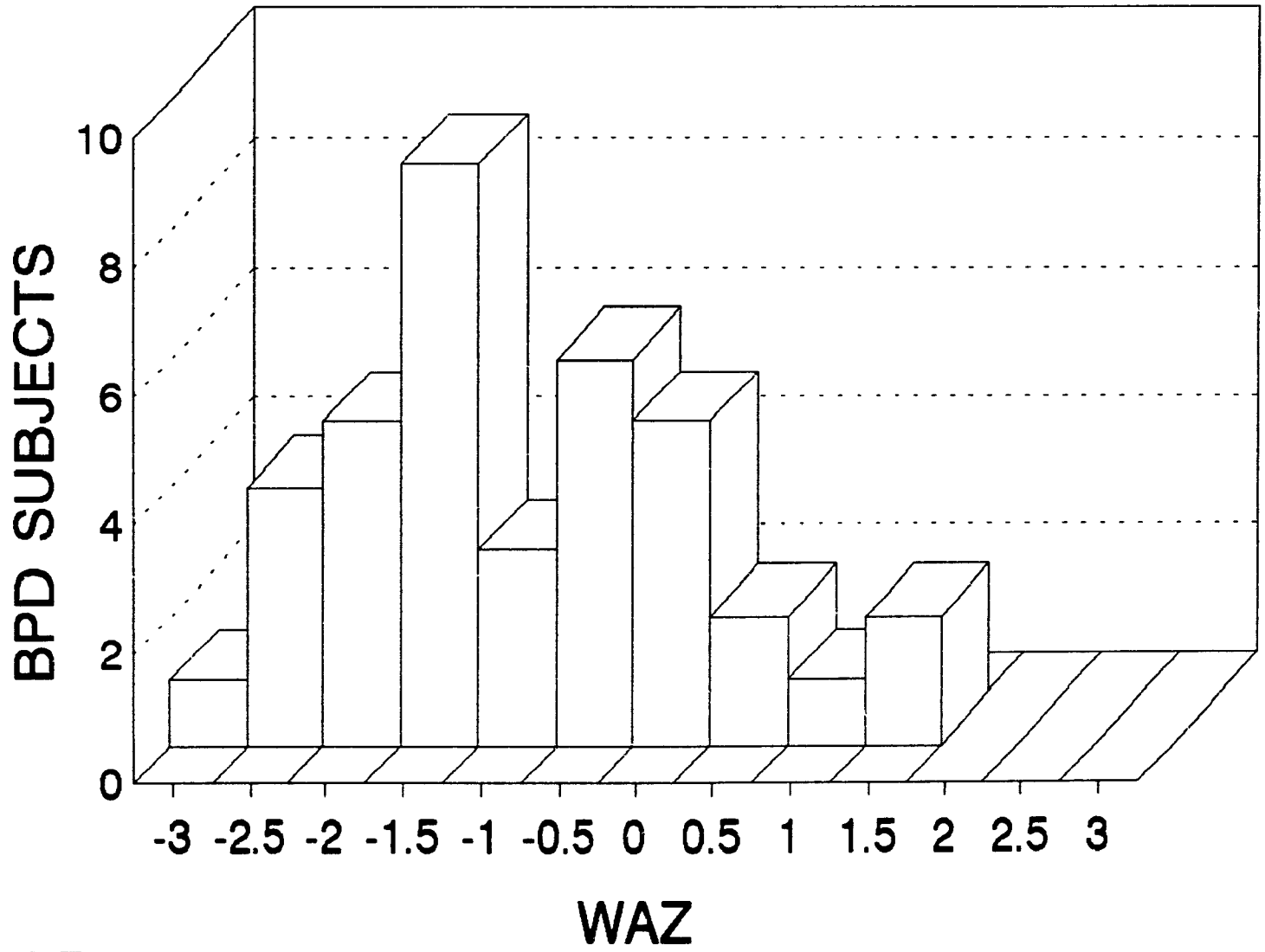

FIGURE 1

B. Anthropometric Measurements of the BPD population:

The weight for age z score (WAZ), weight for height z score (WHZ) and height for age z score (HAZ) distributions of the BPD population are depicted graphically in figures 1, 2, and 3 respectively. Figure 1 illustrates the following: 77.68% (28/38) of the population fell below the median for weight for age, 13.15% (5/38) were at the median while only 13.15% (5/38) were above the median. Similiarly, the distribution of WHZ (fig.2) revealed that 76% (29/38) were below the median, 8% (3/38) were at the median while 16% (6/38) fell above. The least affected parameter was the height for age, where 57.8% (22/38) of patients were below the median, 10.52% were at the 50th percentile and 32% (12/38) were above. The means and standard deviations (SD) of the three indices (WAZ, WHZ, and HAZ) are reported for the population as well as for each sex in Table 2. The mean weight for age and weight for height z scores for the population were approximately 1 SD below the median and 0.5 SD below the median for height for age. There were no significant differences between males and females in any of the above indices although females tended to be lighter, shorter, and thinner than their male counterparts. Excluding the SGA infants from the analysis, the mean WAZ, WHZ and HAZ improved by 12.75%, 41% and 26% respectively.

DISTRIBUTION OF WEIGHT FOR HEIGHT Z SCORE(WHZ) IN BPD POPULATION

(N=38)

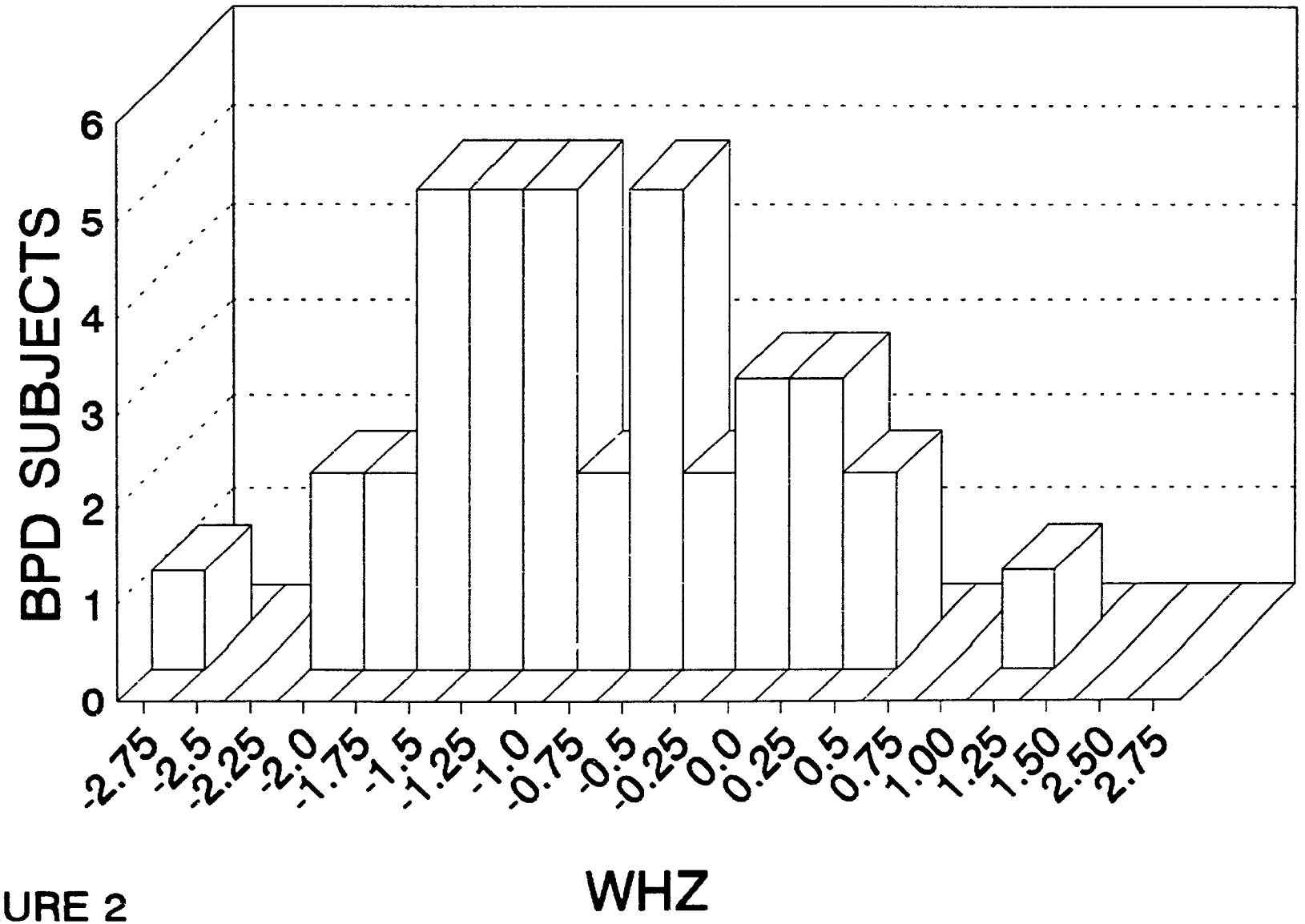

FIGURE 2

DISTRIBUTION OF HEIGHT FOR AGE Z SCORE(HAZ) IN BPD POPULATION
(N=38)

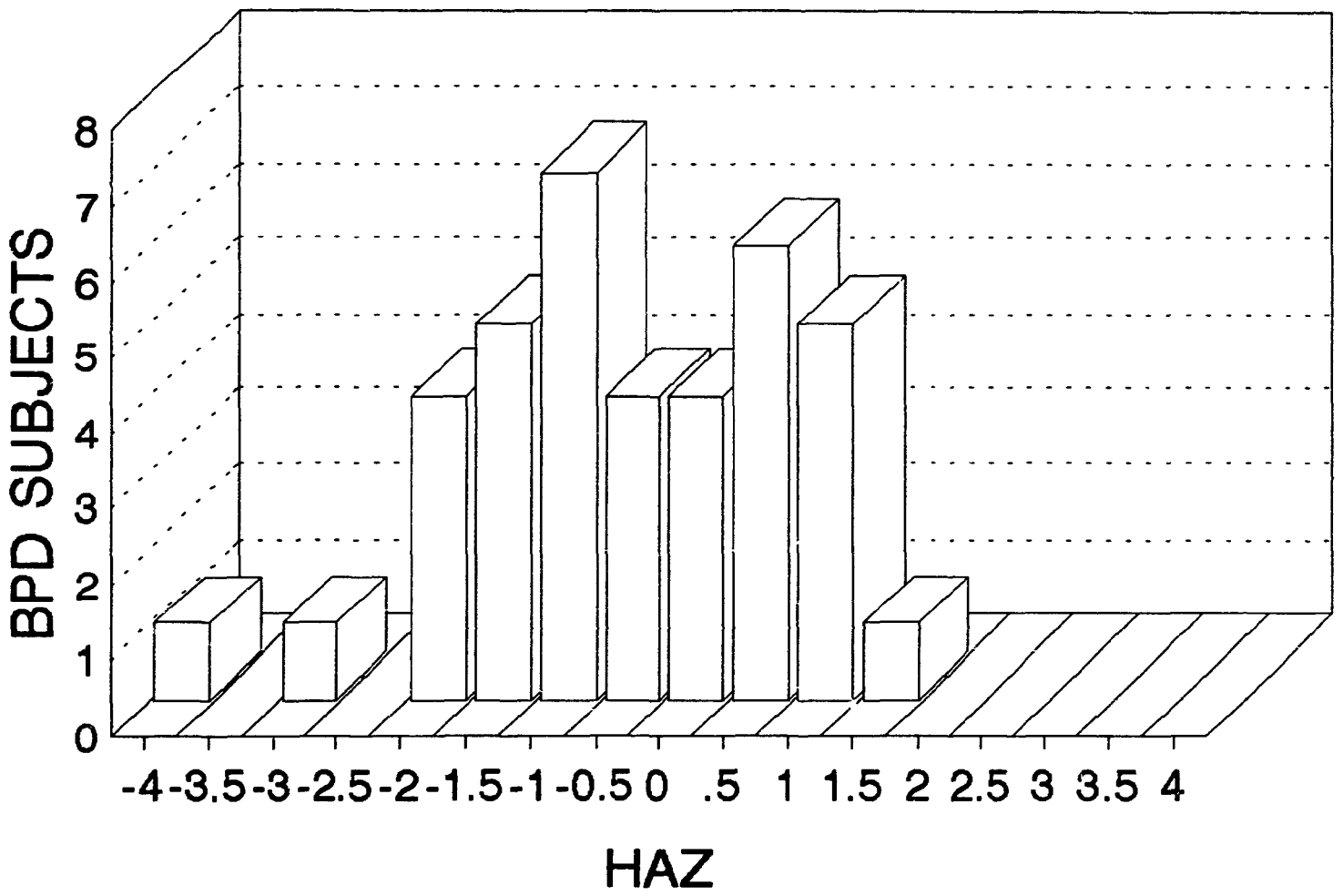

FIGURE 3

GROWTH STATUS OF BPD INFANTS

	FEMALES (N = 9) MEAN ± SD	MALES (N = 29) MEAN ± SD	TOTAL (N = 38) MEAN ± SD	TOTAL* (N = 34) MEAN ± SD
WAZ	-1.56 ± 0.88	-0.86 ± 1.17	-1.02 ± 1.14	-0.89 ± 1.08
HAZ	-1.12 ± 1.37	-0.37 ± 1.13	-0.54 ± 1.22	-0.40 ± 1.12
WHZ	-1.02 ± 0.44	-0.73 ± 0.93	-0.80 ± 0.85	-0.73 ± 0.79

* Small for gestational age infants excluded

TABLE 2

The prevalence of growth failure in this population was determined based on two indices, weight for age and weight for height. Growth failure was defined as weight for age and weight for height less than one standard deviation below the median. This corresponds to weight for age and weight for height less than the tenth percentile and is similiar to criteria previously used in the literature reporting growth failure in BPD (Kurzner et al, 1988a; Meisels et al, 1986). The prevalence of growth failure based on a cutoff point of 1 SD below the median for weight for age was 55%. Based on a cutoff point of 1 SD below the median for weight for height, the prevalence of growth failure was 45%.

C. Relationship of Neonatal Characteristics to Growth Indices:

In order to determine whether gestational age accounted for growth failure, we examined the relationship of gestational age to WAZ, WHZ and HAZ (table 3). Gestational age did not correlate with any of the above z scores and thus, did not account for our findings. In contrast, birth weight was strongly correlated to WAZ ($r=0.45$; $p=0.002$) (Fig 4) and HAZ ($r=0.39$; $p= 0.008$) (Fig 5) but to a lesser extent with WHZ ($r=0.26$;$p=0.055$) (Fig 6). Because two outlying points on the graphs (shown with stars) seemed to strengthen the correlations, analyses was redone excluding them. The correlations between birth weight and WAZ ($r=0.39$; $p=0.012$)

RELATIONSHIP OF GESTATIONAL AGE TO GROWTH PARAMETERS

	Pearson's Correlation Coefficient	P value
Gestational age and		
• WAZ	**0.1975**	**0.117**
• WHZ	**0.0180**	**0.475**
• HAZ	**0.1991**	**0.115**

TABLE 3

RELATIONSHIP OF BIRTH WEIGHT TO WEIGHT FOR AGE Z SCORE (WAZ) (N=38)

(r=0.45; p=0.002)

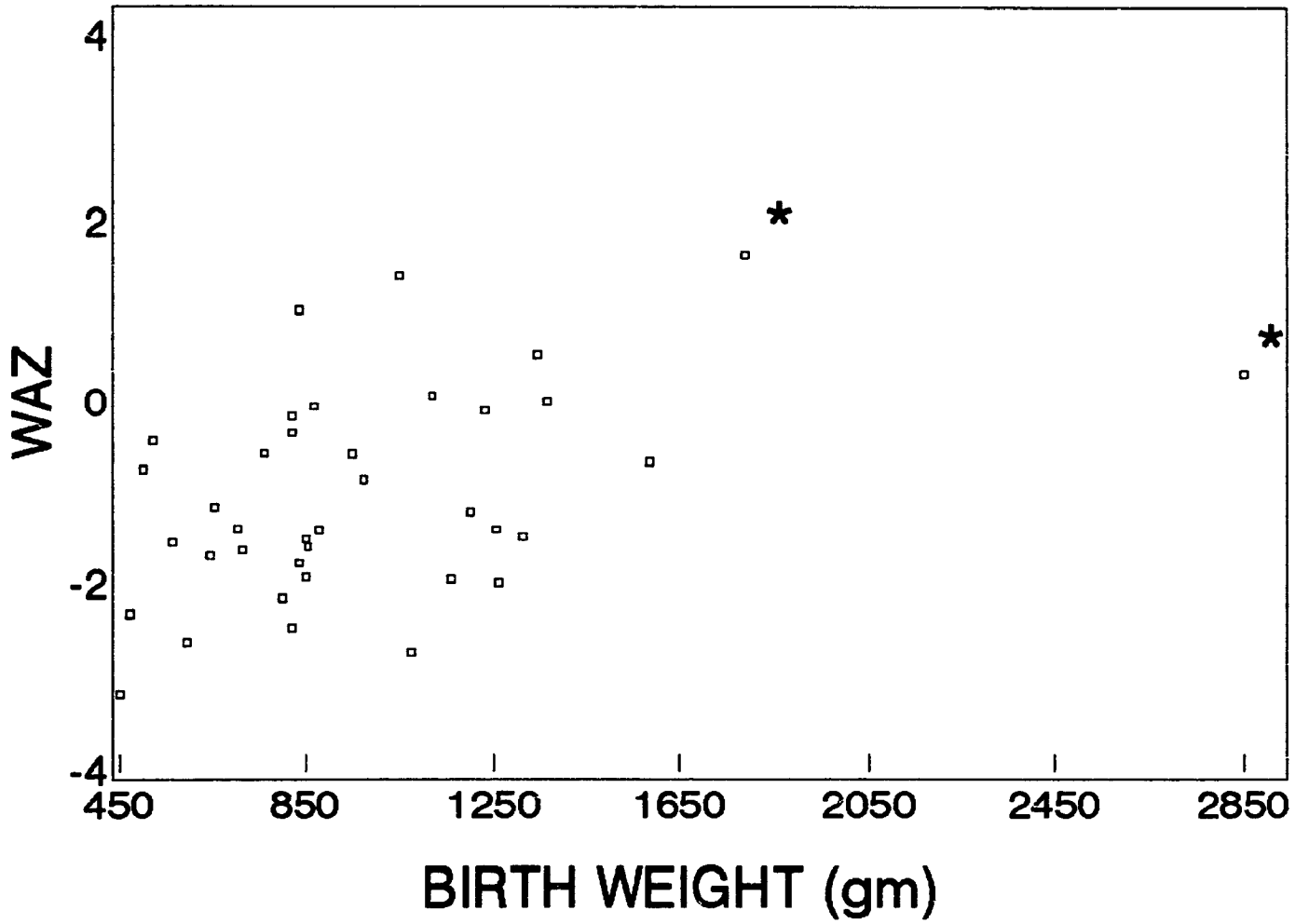

FIGURE 4

RELATIONSHIP OF BIRTH WEIGHT TO HEIGHT FOR AGE Z SCORE(HAZ) (N=38)

(r=0.39; p=0.008)

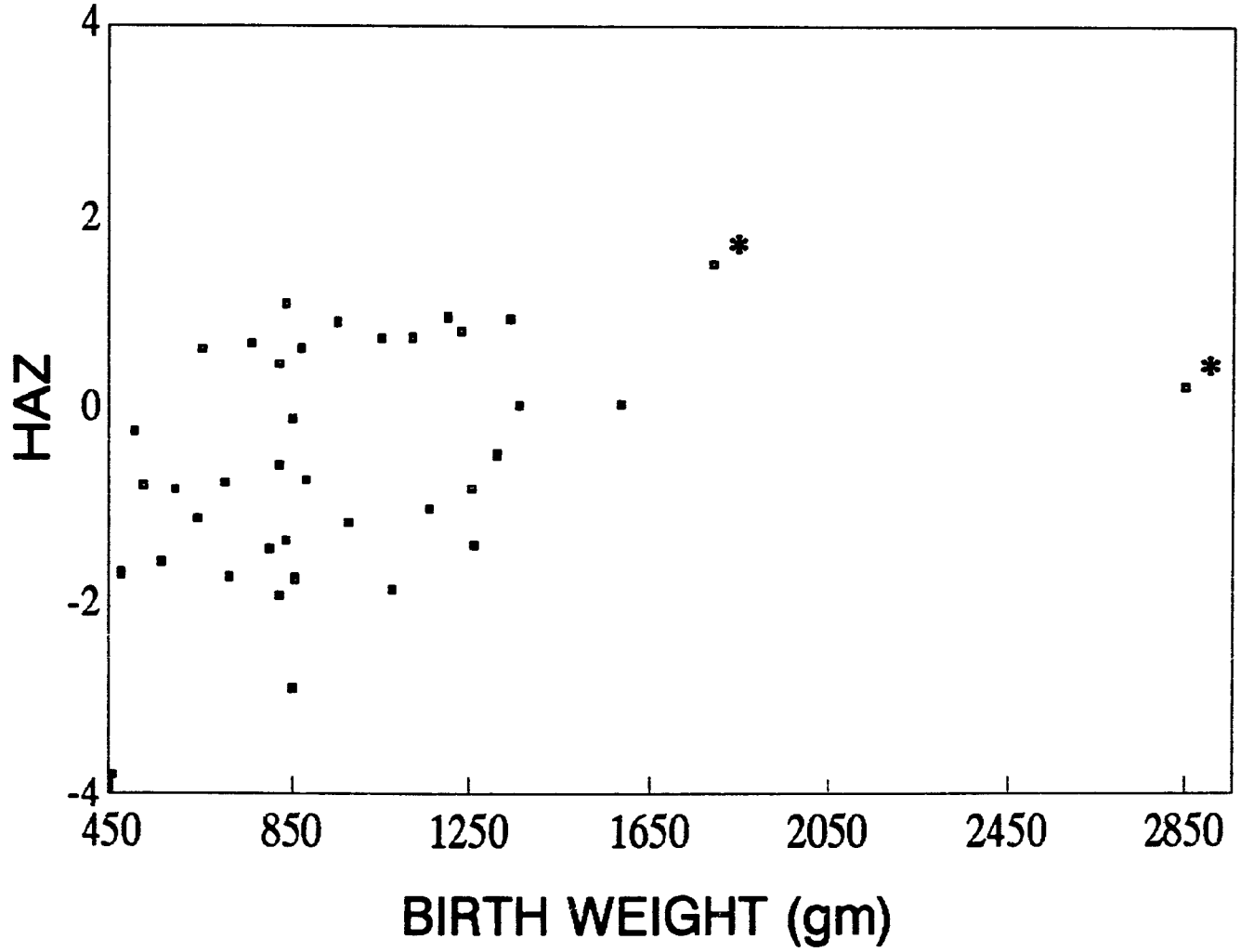

FIGURE 5

RELATIONSHIP OF BIRTH WEIGHT TO WEIGHT FOR HEIGHT Z SCORE (WHZ) (N=38)
(r=0.26,p=0.055)

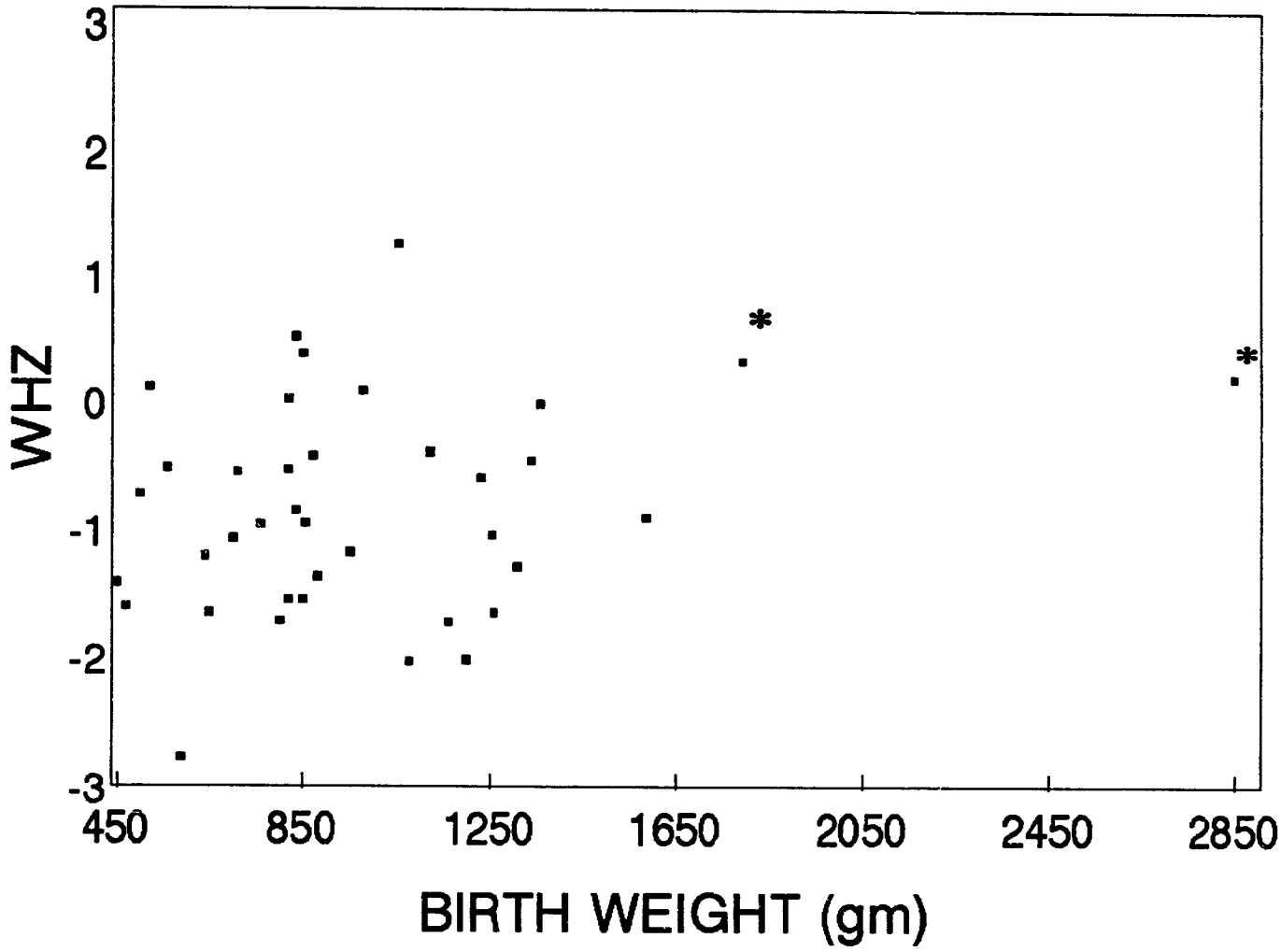

FIGURE 6

and HAZ (r=0.30;p=0.041) remained significant; however, the relationship between birth weight and WHZ disappeared (r=0.22;p=0.106). These findings suggest that birth weight is a predictor of subsequent weight and length but not weight for height.

We further examined the relationship between corrected age and growth parameters. There was a strong positive correlation between corrected age and each of WAZ (r= -0.38; p=0.01) (figure 7) and WHZ (r=-0.45; p= 0.002) (figure 8) suggesting a deterioration in the growth outcome with progression of age. Corrected age; however, did not correlate with HAZ (r=0.26; p=0.164) (figure 9) suggesting that linear growth was not more affected in the older children.

RELATIONSHIP OF CORRECTED AGE TO WEIGHT FOR AGE Z SCORE(WAZ)

(r=-0.33, p=0.01)

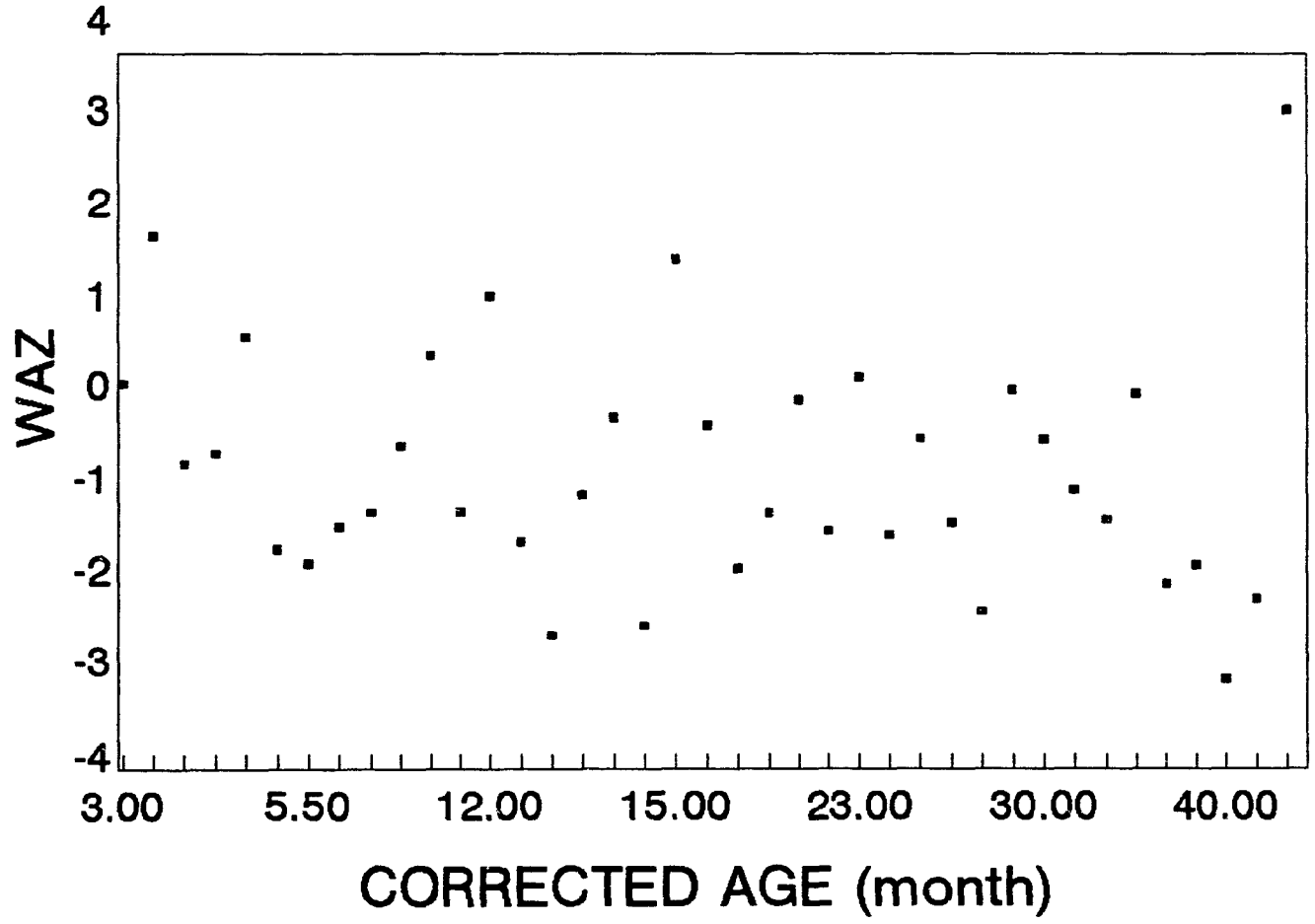

FIGURE 7

RELATIONSHIP OF CORRECTED AGE TO WEIGHT FOR HEIGHT Z SCORE (WHZ)(N=38)

(r=-0.45, p=0.002)

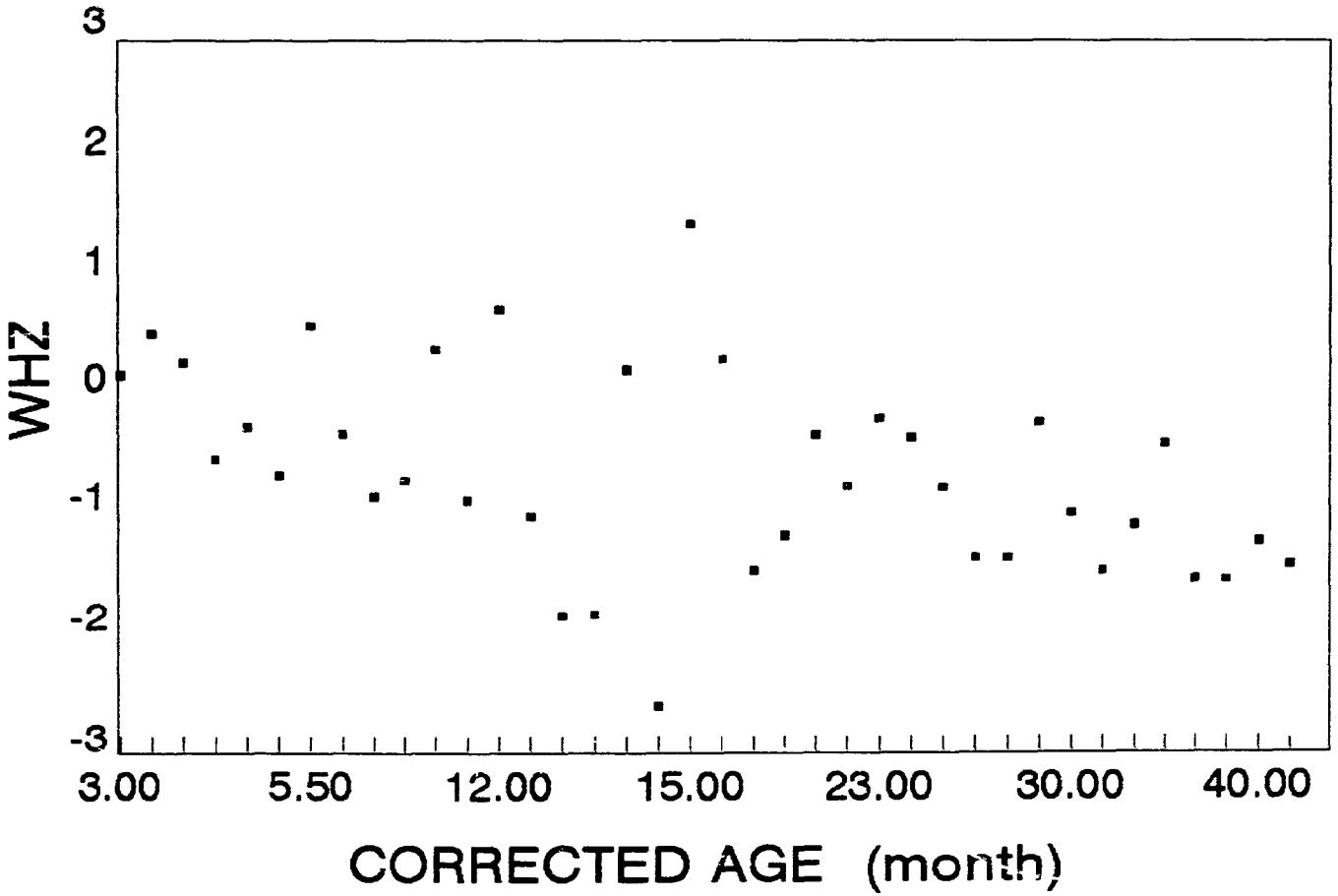

FIGURE 8

RELATIONSHIP OF CORRECTED AGE TO HEIGHT FOR AGE Z SCORE(HAZ)(N=38)

(r=0.26, p=0.164)

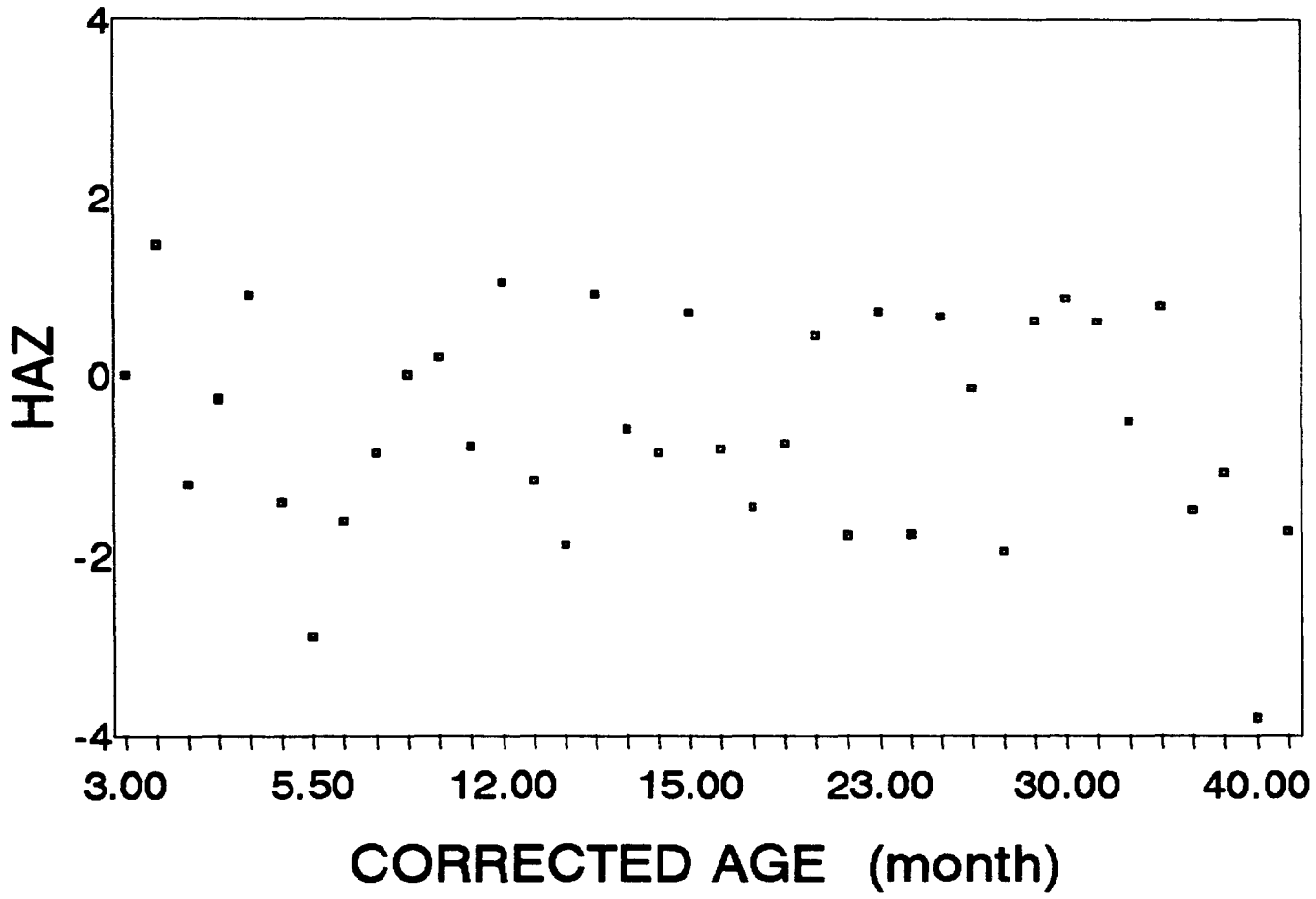

FIGURE 9

II. Sample Study

A. Characteristics of the Sample

Parents of fifteen patients consented to participate in the study of the evaluation of nutritional status, energy intake and oxidative stress status. Two parents refused participation due to lack of interest while nineteen parents objected to the blood test required. The research nutritionist was unable to locate two parents. Thus, the participants represented approximately 40% of the total BPD clinic population. Ten males and five females were included in the sample. The mean (± SD) chronological and corrected age of the fifteen patients studied were 29.23 ± 11.3 and 26.33 ± 11.17 months respectively (table 4). The sample was significantly ($p<0.005$) older than the total population. However, both gestational age and birth weight were comparable to the total population. Two infants (2 females) had birth weights small-for-gestational-age. No significant differences were apparent between females and males on any of the measures although females tended to have had lower birth weights than males.

B. Anthropometric Measurements:

The anthropometric measurements of the BPD sample are summarized in table 5. The means for WAZ, WHZ and HAZ

CHARACTERISTICS OF A SAMPLE OF BPD PATIENTS (N=15)

	FEMALES (N=5) MEAN ± SD (RANGE)	MALES (N=10) MEAN ± SD (RANGE)	TOTAL (N=15) MEAN ± SD (RANGE)
CHRONOLOGICAL AGE (month)	33.80 ± 9.13 (24.50 - 43.00)	26.95 ± 12.06 (7.00 - 41.50)	29.23 ± 11.3* (7.00 - 43.00)
CORRECTED AGE (month)	30.90 ± 9.13 (21.50 - 40.00)	24.05 ± 11.82 (4.00 - 38.50)	26.33 ± 11.17* (4.00 - 40.00)
GESTATIONAL AGE (week)	27.97 ± 1.19 (27.00 - 30.00)	28.64 ± 3.24 (24.71 - 36.00)	28.42 ± 2.69 (24.71 - 36.00)
BIRTHWEIGHT (gm)	781.00 ± 351.11 (450.00 - 1310.00)	1211.50 ± 605.63 (800.00 - 2850.00)	1068.00 ± 561.38 (450.00 - 2850.00)

* $P < 0.005$ Significantly different from BPD population

TABLE 4

GROWTH STATUS OF BPD SAMPLE (N=15)

	FEMALES MEAN ± SD (N = 5)	MALES MEAN ± SD (N = 10)	TOTAL MEAN ± SD (N = 15)
WAZ	-1.76 ± 1.12	-1.01 ± 1.27	-1.26 ± 1.24
WHZ	-1.12 ± 0.42	-0.98 ± 0.77	-1.03 ± 0.66
HAZ	-1.47 ± 1.60	-0.32 ± 1.16	-0.71 ± 1.39

TABLE 5

distribution fell at approximately 1 SD below the median for weight for age, weight for height and height for age. The three growth indices of the sample were comparable to the indices of the total population. Significant differences for the z scores were not noted between females and males. However, there was a trend for females to have poorer growth outcomes than males because of the two small for gestational age (SGA) infants in the female group. Upon exclusion of these SGA infants, the growth indices were comparable between males and females.

The distribution of triceps skinfold (TSF), midarm circumference (MAC) and midarm muscle circumference (MAMC) according to the standards of Frisancho (1981) is illustrated in figure 10. Fifty four percent of the sample had triceps skinfold below the 5th percentile, 31% were distributed between the 5th and 25th while only 15% were between the 25th and 50th percentile. The midarm circumference distribution revealed that 20% of the sample had their MAC below the 5th percentile, 38% fell between the 5th and 25th, 23% between the 25th and 50th while only 15% had their MAC above the 50th percentile. As to the MAMC, 31% had their MAMC distributed between the 5th and 25th, 15% were between 25th and 50th percentile while 54% had their MAMC above the 50th percentile. These findings suggest depletion of fat stores for the majority of infants while lean tissue mass or protein reserves

ANTHROPOMETRIC PERCENTILES

(N=13)

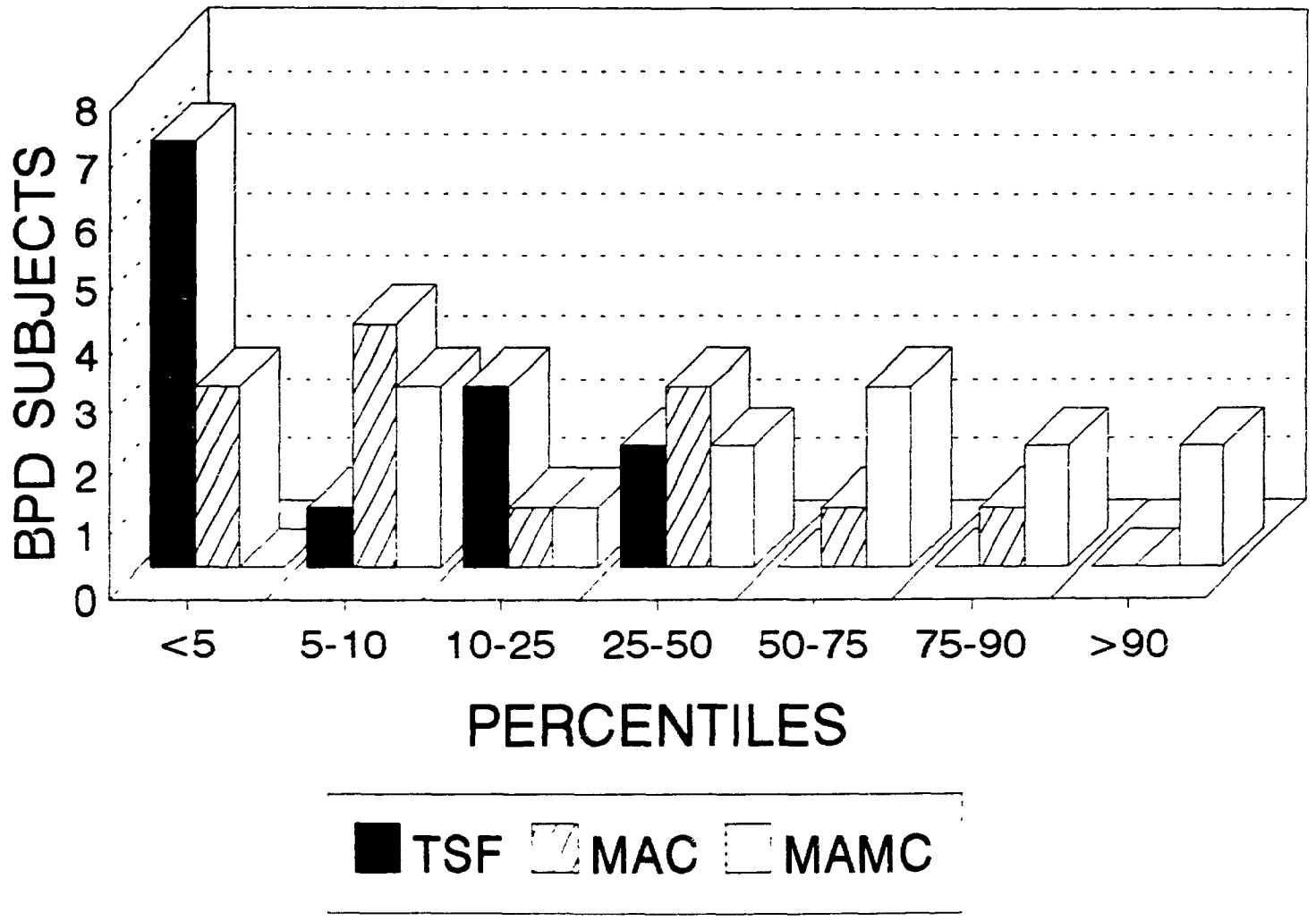

FIGURE 10

were spared.

The mean values of triceps skinfolds, midarm circumference and midarm muscle circumference for females were at the 10th, 50th and 75th percentile respectively. The mean values of triceps skinfold, midarm circumference and midarm muscle circumference for males were below the 10th percentile, between the 10th and 25th, and at the 50th respectively. This suggests that males had relatively lower fat stores as well as protein stores than females.

C. Energy Intake :

Thirteen of the fifteen mothers of BPD patients returned a completed six day food record. Calculated energy intake based on analysis of the records, varied from 84.49 to 166.17 kcal/kg/day for females and from 63.51 to 178.30 kcal/kg/day for males. The average energy intake expressed as Kcal per Kg of body weight per day for females and males was 116.64 ± 34.08 and 121.11 ± 44.48 respectively (table 6). There was no significant difference in energy intake between females and males. Energy intake was expressed as a percent of the recommended nutrient intake (RNI) (Health and Welfare Canada, 1990). The mean (± SD) energy intake as a percent of RNI for females and males was 120 ± 34 and 124 ± 46 respectively.

NUTRIENT CONSUMPTION OF BPD PATIENTS (N=13): ENERGY AND MACRONUTRIENT INTAKE

	FEMALES (N = 5) MEAN ± SD (RANGE)	MALES (N = 8) MEAN ± SD (RANGE)
ENERGY (Kcal/Kg/day)	116.64 ± 34.08 (84.49 - 166.17)	121.11 ± 44.48 (63.51 - 178.30)
CARBOHYDRATE (gm/kg/day)	16.06 ± 6.81 (11.14 - 25.74)	16.23 ± 7.20 (7.52 - 27.15)
FAT (gm/kg/day)	3.93 ± 0.79 (2.67 - 4.84)	4.68 ± 1.64 (2.48 - 7.07)
PROTEIN (gm/kg/day)	4.50 ± 1.50 (2.84 - 6.85)	4.84 ± 2.66 (1.59 - 9.28)

TABLE 6

Table 7 reports the macronutrients as a percent of total energy intake. Carbohydrate contributed 54 and 55 percent of the total energy intake for females and males respectively; fat constituted 30% and 35% of total calories for females and males respectively, while 15% and 16% of energy were derived from protein for females and males respectively. The recommended amount of carbohydrate, fat and protein as a percent of total energy is 50-60, 30, and 10-20 respectively (Health and Welfare Canada, 1990). The diet of these subjects in this study conformed well with these recommendations.

In order to determine whether a six day food record would be sufficient to assess energy intake of subjects, we examined within subject to between subject variability (Willett, 1990) and determined the number of days needed per person to estimate an individual's energy intake. Using repeated measures analysis of variance, results revealed that 68% of the variation in energy intake was explained by between-subject variability while only 22% of the variation was explained by the within-subject variability. The ratio of within-subject variability to between-subject variability was less than one (0.52). The number of days/daily records needed per subject to estimate the individual's dietary energy intake to within 20 percent of their true mean 90% of the time was 4 days or daily records. Our dietary intake was assessed on 6 daily records and therefore represents a reasonably good

NUTRIENT CONSUMPTION OF BPD PATIENTS (N=13)
NUTRIENT AS A PERCENT OF TOTAL ENERGY

	FEMALES (N = 5)	MALES (N = 8)
CARBOHYDRATE (% OF TOTAL ENERGY)	55.00	54.00
RANGE	38.20 - 88.30	24.84 - 89.70
FAT (% OF TOTAL ENERGY)	30.00	35.00
RANGE	20.61 - 37.35	18.43 - 52.50
PROTEIN (% OF TOTAL ENERGY)	15.00	16.00
RANGE	9.70 - 23.50	5.30 - 30.60

TABLE 7

assessment of dietary intake for individuals.

D. Oxidative Stress Status

The mean values of plasma MDA and red cell glutathione (GSH) per gram of hemoglobin for the sample were 123.99 ± 49.99 nmol/l and 5.22 ± 1.70 umol/g respectively. Normal values of plasma MDA and red cell GSH for this age group are not available. However, Lepage et al, (1991) using the same methodology as in this study, measured plasma MDA in 17 controls whose mean age was 14 years and obtained mean MDA levels of 69.1 ± 2.6 nmol/l. In a subsequent study, Lepage et al (1993) found a mean value of 55 ± 3 nmol/l for MDA in controls whose age ranged between 19 and 22 years. In the present study, fourteen of fifteen subjects (93%) exhibited levels of MDA at least double the amount reported in older controls, suggesting increased lipid peroxidation. Only one subject (7%) had a mean plasma MDA level comparable to the control values.

The mean value of normal red cell GSH per hemoglobin for adults is 6.57 ± 1.04 umol/gm. The mean glutathione level of our subjects was comparable to mean adult control levels. However, six patients (40%) had decreased levels of red cell GSH compared to control levels suggesting depletion of this antioxidant. Two subjects (13%) showed elevated levels while

seven infants (47%) had normal GSH levels compared to adult controls. We further examined whether oxidative markers were related to age. There were no correlations between corrected age and plasma MDA ($r= 0.11$; $p=0.352$) or red cell glutathione ($r=0.18$; $p= 0.265$).

E. Comparison between BPD Infants with Growth Failure and BPD Infants with Normal Growth:

a. Defining the Groups

We defined FTT on the basis of weight for age and weight for height z scores. The WAZ, WHZ and HAZ distribution for the sample are presented in figures 11, 12, and 13 respectively. From the WAZ and WHZ distribution, it appeared that the sample was comprised of two distinct populations at a cutoff point of 1 SD below the median for both weight for age and weight for height. Infants were classified in the same manner by both of these measures. There was no clear separation when plotting the HAZ distribution. Therefore, a cutoff point of 1 SD below the median for weight for age was selected and subjects were classified accordingly.

Based on a cutoff point of 1 SD below the median for weight for age and weight for height, nine infants were failing to thrive compared to six infants who were thriving.

DISTRIBUTION OF WEIGHT FOR AGE Z SCORE IN BPD SAMPLE
(N=15)

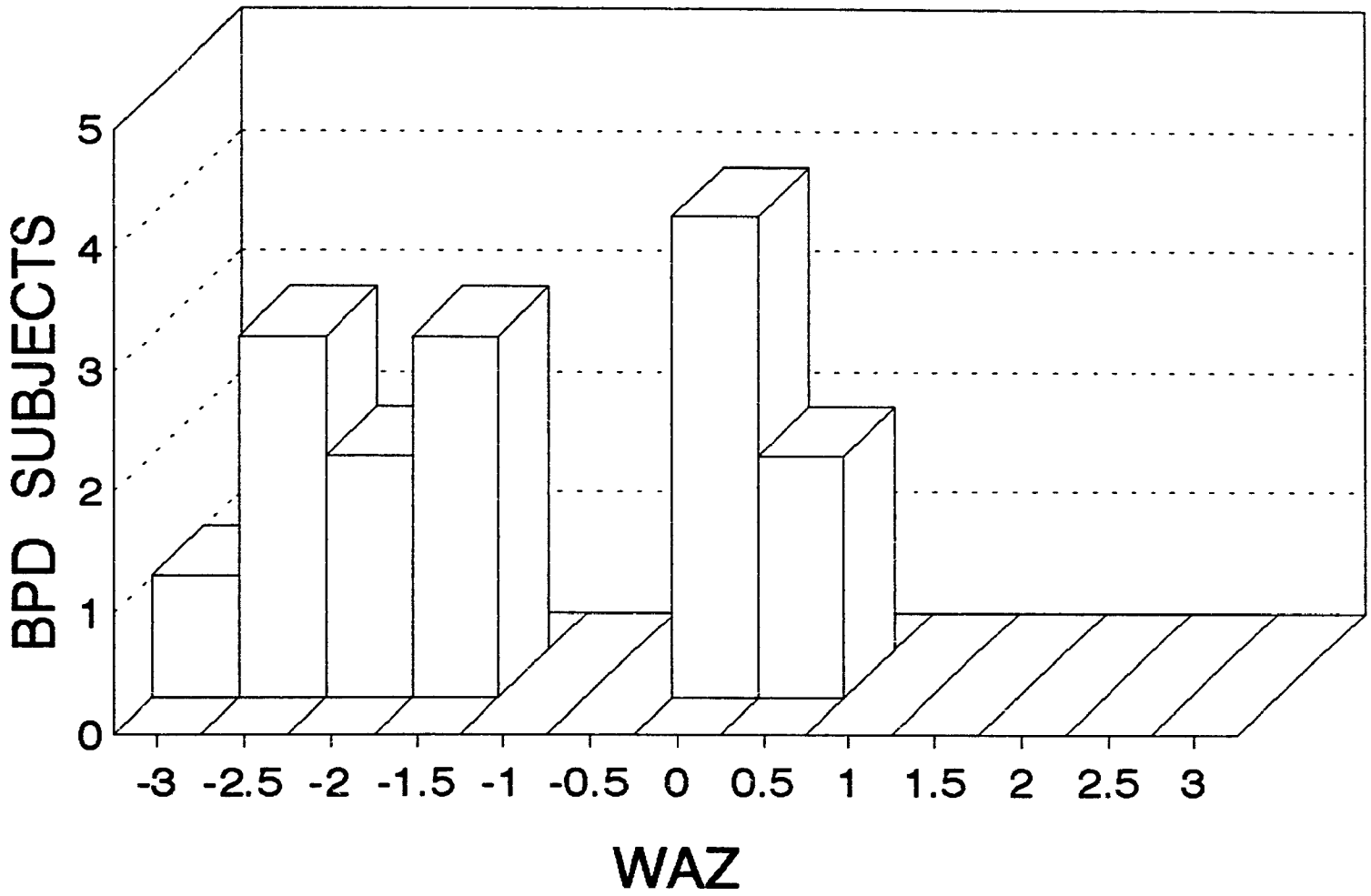

FIGURE 11

DISTRIBUTION OF WEIGHT FOR HEIGHT Z SCORE(WHZ) IN BPD SAMPLE
(N=15)

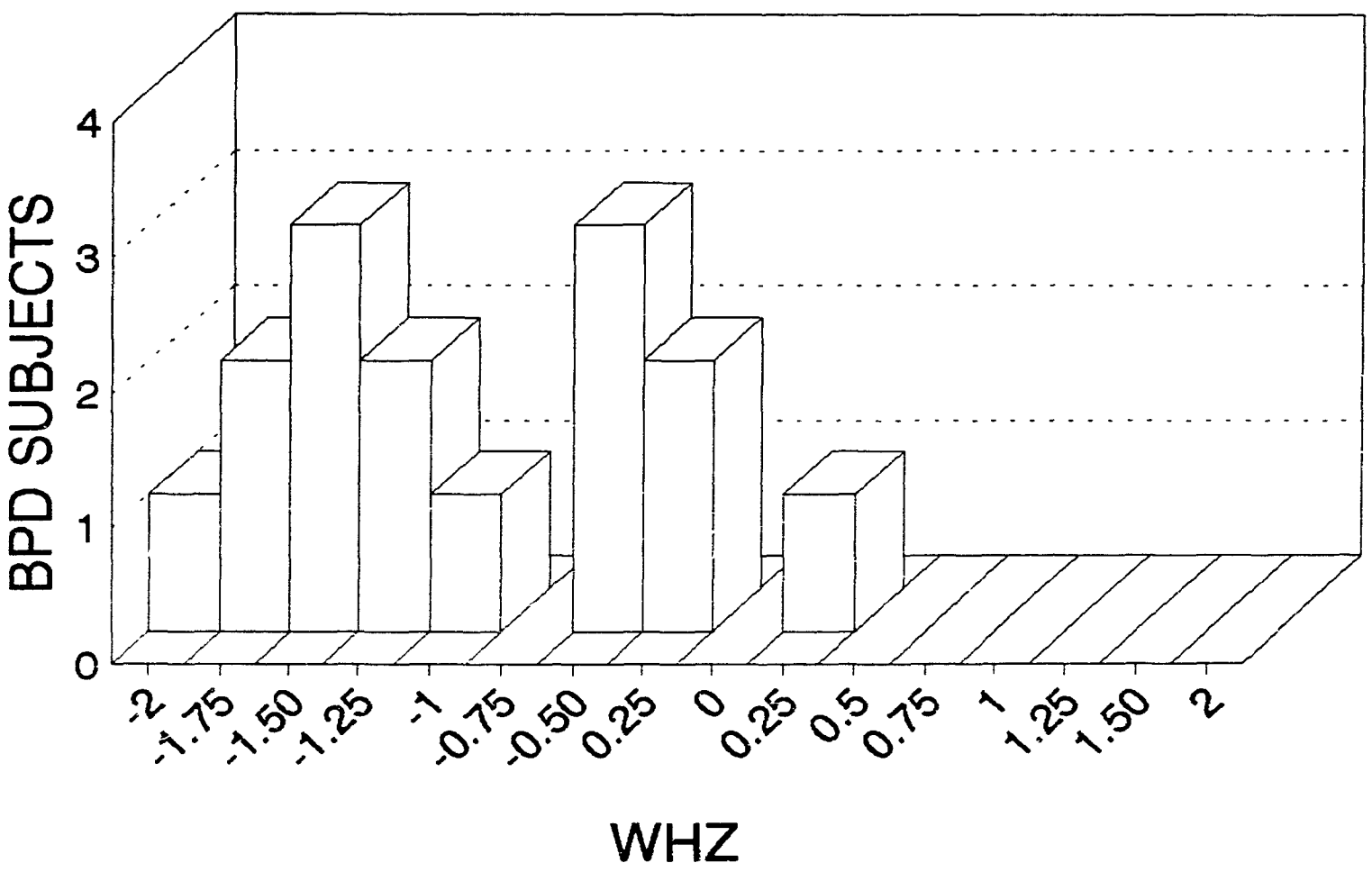

FIGURE 12

DISTRIBUTION OF HEIGHT FOR AGE Z SCORE(HAZ) IN BPD SAMPLE
(N=15)

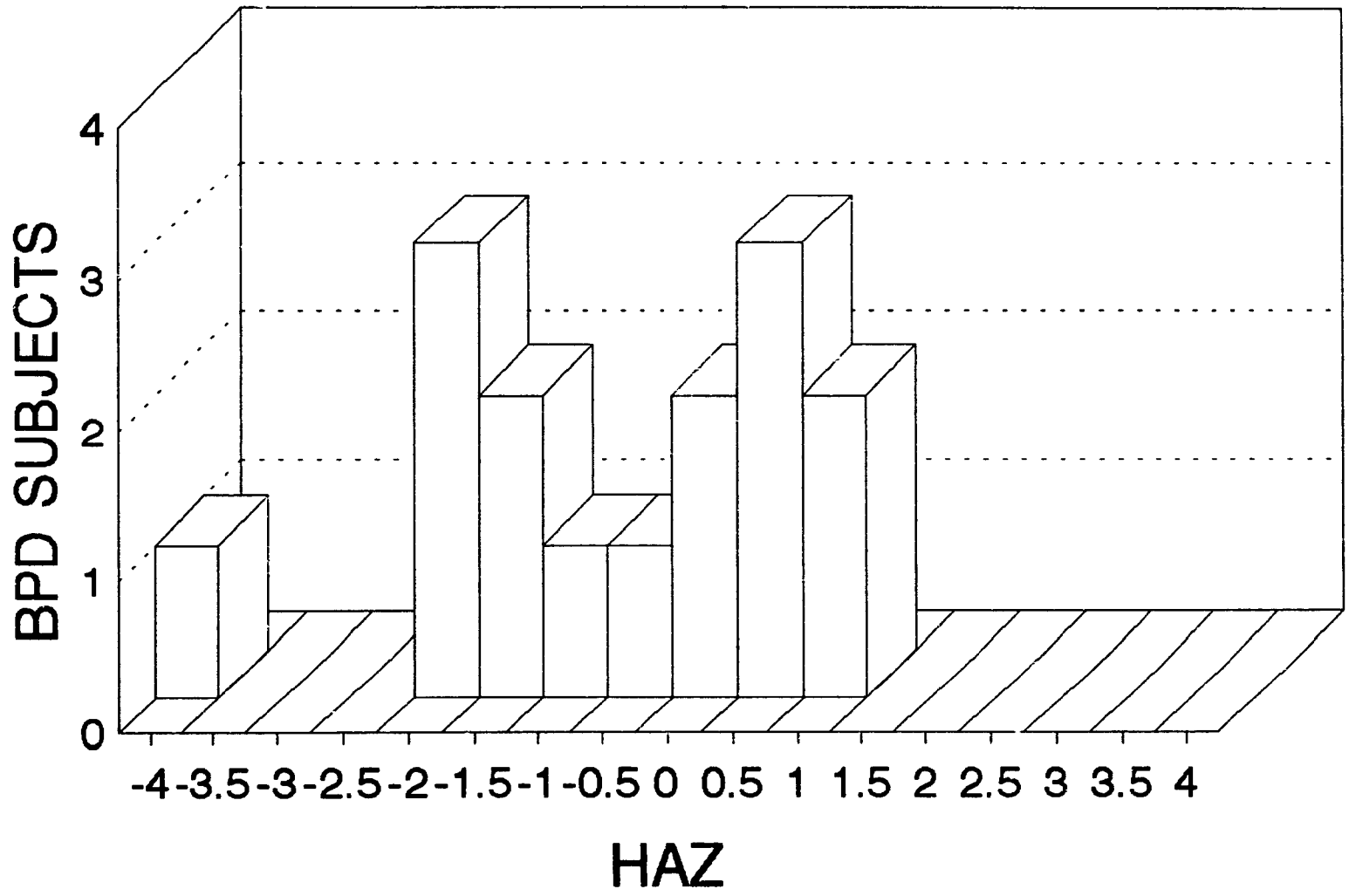

FIGURE 13

Failing to thrive BPD infants were significantly older than thriving BPD infants. However, birth weight and gestational age did not differ between the two groups (Table 8).

b. Energy Intake

We assessed energy intake to determine whether growth failure in BPD infants is associated with increased or decreased intakes for age. Energy intakes expressed as percent of RNI (mean ± SD) were 133 ± 35% and 104 ± 46% for infants with growth failure and normally growing BPD infants respectively (Table 9). These figures indicate a mean energy intake that is 33% in excess of requirements for age in infants with growth failure and only a 4% mean increase for thriving infants.

While there was no statistical difference in mean energy intake between the two groups, infants with growth failure had energy intakes more than sufficient to meet normal requirements for age. A significant negative relationship was found between average energy intake and WAZ (Figure 14). This may reflect the clinic practice of intervening and supplementing dietary intake in patients with growth failure.

COMPARISON BETWEEN BPD INFANTS WITH NORMAL GROWTH TO BPD INFANTS WITH GROWTH FAILURE

CHARACTERISTICS	INFANTS WITH GROWTH FAILURE WAZ $\leq$ -1 (N = 9)	INFANTS WITH NORMAL GROWTH WAZ > -1 (N = 6)
CHRONOLOGICAL AGE (month)	34.00 $\pm$ 8.99	22.08 $\pm$ 11.32*
CORRECTED AGE (month)	30.94 $\pm$ 9.19	19.42 $\pm$ 10.90*
GESTATIONAL AGE (week)	27.87 $\pm$ 2.18	29.25 $\pm$ 3.38
BIRTHWEIGHT (gm)	865.56 $\pm$ 288.09	1371.66 $\pm$ 751.78

*P < 0.05

TABLE 8

COMPARISON OF ENERGY INTAKE BETWEEN BPD INFANTS WITH NORMAL GROWTH AND GROWTH FAILURE

	INFANTS WITH GROWTH FAILURE WAZ $\leq$ -1 (N = 8)	INFANTS WITH NORMAL GROWTH WAZ > -1 (N = 5)
DIETARY ENERGY INTAKE (kcal/kg/day)	131.04 $\pm$ 32.33	100.76 $\pm$ 45.86
% RNI	133.00 $\pm$ 35.00	104.00 $\pm$ 46.00

TABLE 9

RELATIONSHIP OF ENERGY INTAKE TO WAZ (N=13)

(r=-0.46, p=0.055)

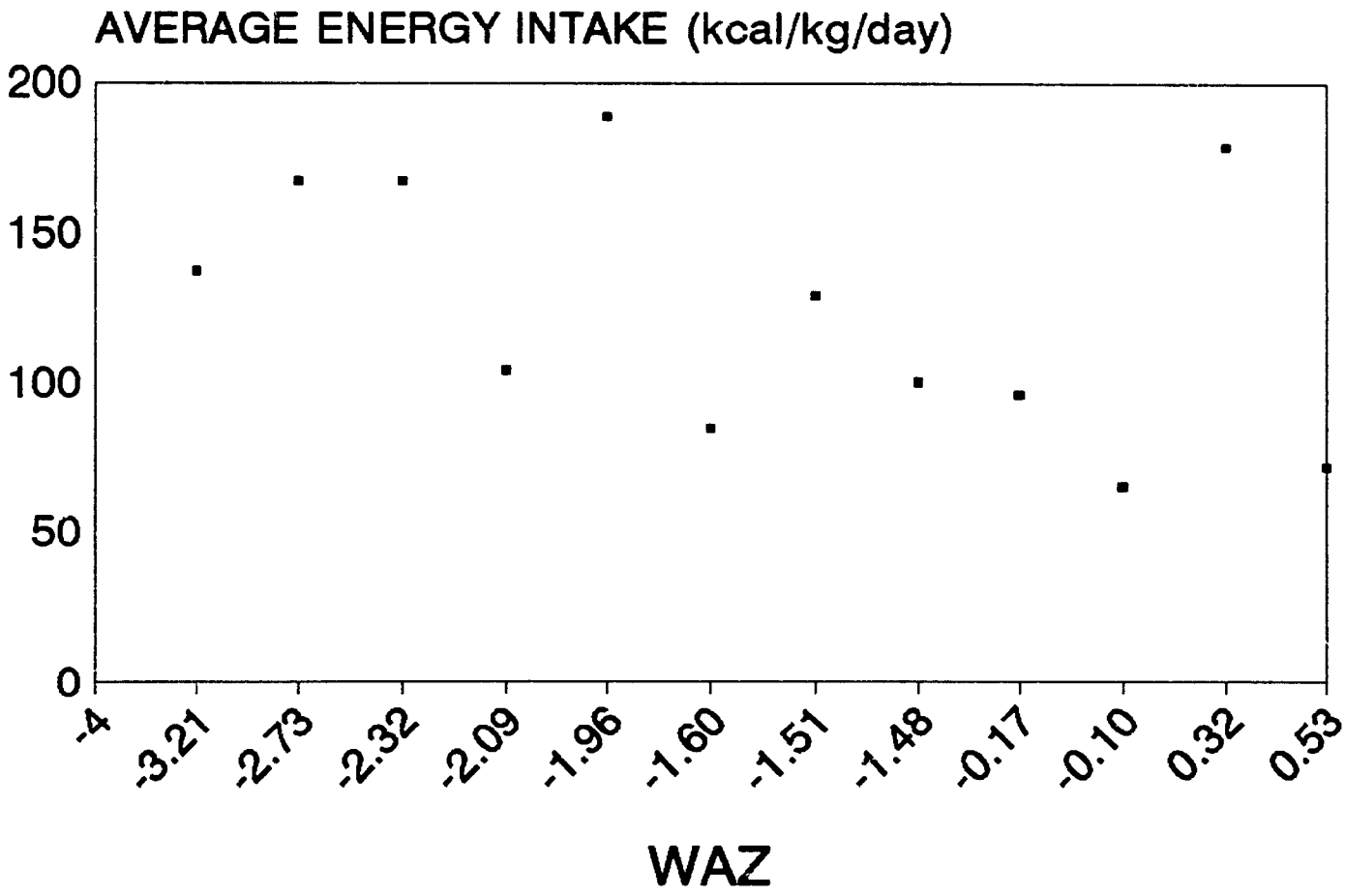

FIGURE 14

c. Oxidative Stress Status

The comparison of oxidative stress markers between the two groups is presented in table 10. There were no significant differences in mean red blood cell (RBC) glutathione per hemoglobin or in mean plasma malondialdehyde levels between the groups. Out of six subjects with low glutathione levels, four were failing to thrive while two were thriving. Similiarly, nine of the fourteen patients with elevated MDA levels were failing to thrive. Because two infants in the sample were receiving supplemental O_2 and the use of the latter might confound the results, analysis was redone excluding these cases. There were no statistical differences between failing to thrive infants and normally growing BPD infants in mean RBC glutathione ($p>0.1$) and mean plasma MDA ($p>0.1$) upon exclusion of the two cases. Furthermore, there was no correlation between either MDA or GSH and WAZ, WHZ and HAZ.

The relationship of energy intake to each of the oxidative stress markers was examined. A significant positive association was demonstrated between energy intake and glutathione levels ($r=0.48$; $p=0.048$) (figure 15). Because glutathione is a tripeptide of glycine, cystine, and glutamic acid, the association between glutathione and protein intake was further tested. A positive association was observed but

COMPARISON OF OXIDATIVE STRESS MARKERS BETWEEN BPD INFANTS WITH NORMAL GROWTH AND GROWTH FAILURE

OXIDATIVE STRESS MARKERS	INFANTS WITH GROWTH FAILURE WAZ $\leq$ -1 (N = 9)	INFANTS WITH NORMAL GROWTH WAZ >-1 (N = 6)	P VALUE
MALONDIALDEHYDE (nmol/L)	133.83 $\pm$ 55.79	109.24 $\pm$ 39.76	0.370
GLUTATHIONE (μmol/gm Hgb)	5.10 $\pm$ 1.56	5.40 $\pm$ 2.04	0.745

TABLE 10

RELATIONSHIP OF ENERGY INTAKE TO GLUTATHIONE (N=13)

(r=0.48, p=0.048)

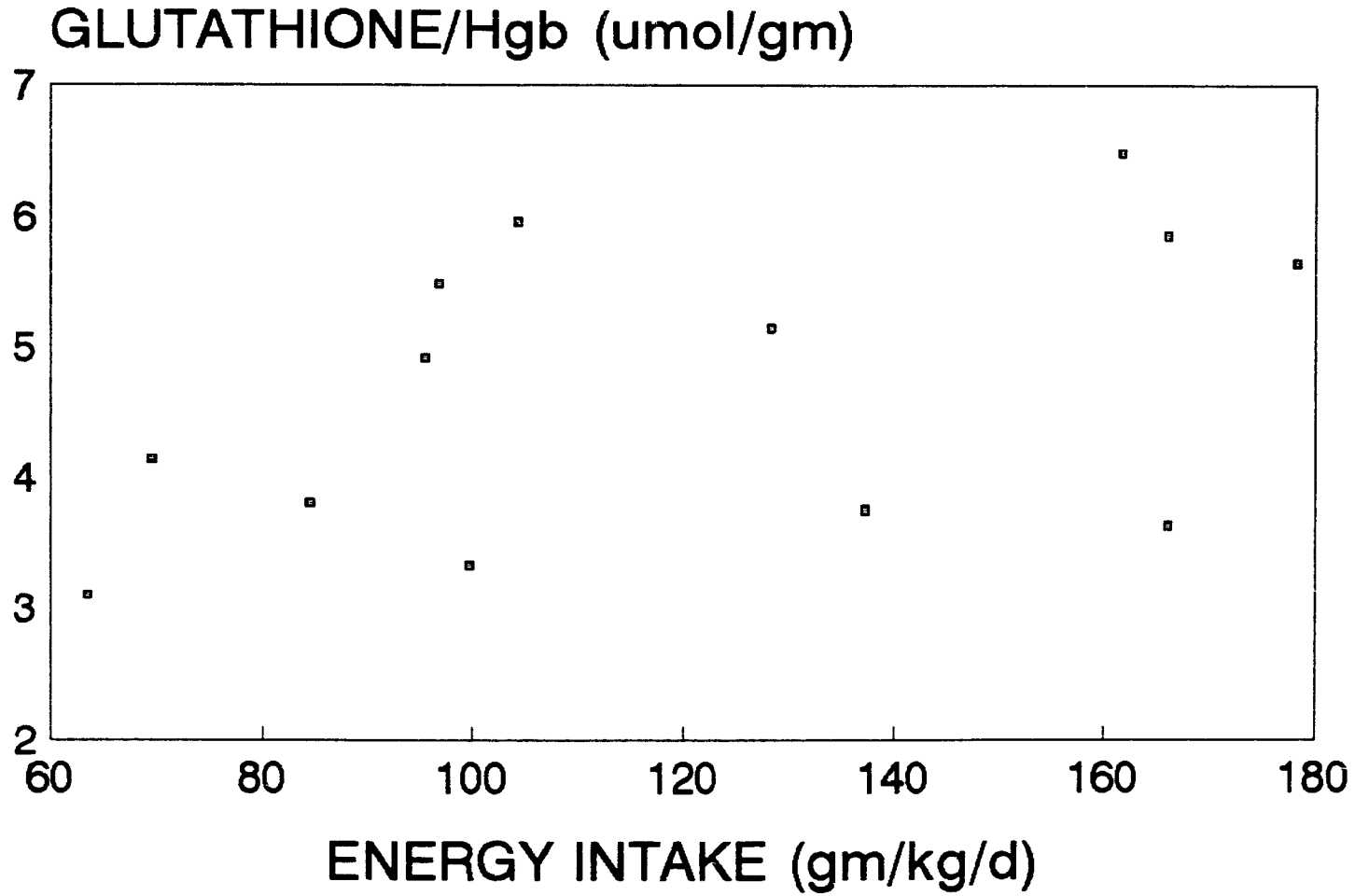

FIGURE 15

the relationship did not reach statistical significance (r= 0.41; p= 0.08). Unlike GSH, MDA did not correlate with energy intake (r= 0.15; p=0.308).

Due to the observed positive association of energy intake and glutathione levels, and because infants failing to thrive had higher energy intakes than thriving infants, energy intake might have negatively confounded the results. We, therefore, reanalyzed the data using multivariate analysis to adjust for energy intake. GSH levels remained comparable between the two groups even after adjusting for energy intake in the analysis.

SECTION SIX DISCUSSION

I. DESCRIPTIVE STUDY

The current study is a census of BPD infants followed at the outpatient clinics at the MCH between June 1993 and August 1994. It differs from studies in the literature in a number of important respects. Many studies in the literature reporting on the growth outcome of BPD infants have followed all BPD survivors longitudinally up to 2 years of age (Markstead and Fitzhardinge, 1981; Vohr et al, 1982; Yu et al, 1983; Davidson et al, 1990) rather than a select population requiring medical care at tertiary clinics at a single point in time. Compared to cross-sectional studies reporting on BPD infants (Gamarra, 1992; Kurzner et al, 1988; Weinstein and Oh, 1981; Yeh et al, 1989), the mean age of our population is relatively old. This is attributed to the fact that our subjects were studied while they were being followed at outpatient clinics as opposed to infants still in intensive care units who require mechanical ventilation. In addition, in this institution, only BPD infants requiring home oxygen at 40 weeks post conceptual are followed as opposed to all BPD infants. The mean gestational age and birth weight were comparable to other BPD populations.

The reported high ratio of males to females (3:1) in our study is consistent with the literature. Markstead and

Fitzhardinge (1981) reported a ratio of males to females of 4:1 in 20 BPD survivors. The incidence of BPD has been shown to be higher in the male sex and the latter constitutes a risk factor for BPD (Hazinski, 1990). Recent findings indicate that males have lower glutathione levels at birth which may contribute to their greater suceptibility to this disease. While the finding of lower birth weight in females than males is comparable to that of the normal population, on average, the birth weight of males exceeds that of females by only 80g. In our population, however, the difference between males and females was more pronounced. Because females are more protected from developing BPD, it is possible that females had to have a significantly lower birth weight in order to develop BPD. This is supported by the finding of an increased incidence of BPD with a decrease in birth weight (Bancalari, 1988).

We have examined the growth status of these infants with respect to the three indices: weight for age, weight for height and height for age. Weight for age reflects whether a patient is underweight or overweight. The index is used as an indicator of protein-energy malnutrition in children from six months to seven years (Gibson, 1990). This index, which fails to account for height differences, may overestimate the prevalence of malnutrition in constitutinally small children if used alone (Gibson, 1990). Consequently, Waterlow et al,

(1977) recommended the use of a combination of weight for age and weight for height indices. Peterson et al, (1984) noted that the definition of malnutrition based solely on weight for age may include 3-5% of the population who are genetically small but within normal limits of growth. Furthermore, this definition may exclude severely malnourished infants suffering from edema. The authors recommended that the description of malnutrition be based on weight for age, weight for height and height for age. Weight for height is a sensitive index of current nutritional status (Gibson, 1990). In contrast to weight for age, this index detects wasting, when weight is inappropriately low for height. The height for age index, on the other hand, reflects past and possibly ongoing nutritional status (Gibson, 1990). In our population, the distribution of weight for age was comparable to that of weight for height. Both distributions revealed that more than three quarters of the clinic patients were below the 50th percentile while only one quarter were at or above the median. Therefore, the above findings do not conform with the distributions of the reference population. The height for age distribution did not show the shift from the reference distribution observed for weight for age and weight for height distribution. Because weight for height was as affected as weight for age, while height for age was spared, it is clear that these children are not simply small constitutionally, but rather demonstrate a nutritional depletion. Given that the height for age was not

affected, we conclude that this population has suffered a relatively recent nutritional deficit of relatively short duration.

One of the objectives of the study was to determine the prevalence of growth failure in this population. Growth failure was defined as weight for age and weight for height less than the tenth percentile (z score $\leq$ -1). Because weight for age does not account for height differences and may overestimate the prevalence of malnutrition, the use of weight for height index was essential to identify those infants who were wasted. This was evident when we calculated the prevalence of growth failure in BPD infants based on weight for age and weight for height. Using weight for age, the prevalence was 55%. This was a slight overestimate as the prevalence based on weight for height was 45%. The rational for the use of the cutoff point less than the 10^{th} percentile (z score $\leq$ -1) is for the purpose of comparison with previous studies reporting on the growth outcome of infants with BPD, which have defined growth failure as weight for age and height for age less than the tenth percentile (kurzner et al, 1988a; Meisels et al, 1986). Because our population included BPD infants requiring continued follow up in the clinics, the reported prevalence may be an overestimate of the problem in the general BPD population. However, Meisels et al (1986) obtained a prevalence of growth failure as high as 67% in 17

infants with BPD in their second year of life based on weight for age below the tenth percentile.

Older subjects in our study had poorer growth outcomes than younger ones. This is in contrast to the findings of Markstead and Fitzhardinge (1981) and Vohr et al, (1982) who reported improvement in growth outcomes for both sexes at two years post-term. In both studies, improvement in weight and length was associated with improvement in respiratory symptoms. There are two possibilities for the inconsistency between our study and the aforementioned studies. One explanation may be ascribed to the different populations under study. Whereas the populations in these studies included all BPD survivors who were followed prospectively, our population was comprised of the sickest BPD infants who continued to require follow up in our center. The other possibility may be attributed to the change in the management of the BPD patient in the neonatal unit at the hospital. During the past two years, steroids have been used more aggressively during the neonatal period accounting for the the improved overall outcome of infants. Most of the infants receiving steroids during that time were discharged and are therefore, not followed in the outpatient clinics. This is reflected in the few young infants followed at the outpatient clinics, as opposed to a large number of older infants who would not have received such a treatment at that time and continue to have

poor nutritional outcomes.

We did not find any correlation between gestational age and any of the growth indices suggesting that the degree of prematurity does not explain the growth outcome. On the other hand, the strong positive correlation between birth weight and WAZ and HAZ suggests that in our patients, birth weight did affect subsequent weight and height. According to Garn and Shaw (1977), birth weight is the single most important determinant of individual linear growth during the first seven years of life in normal term infants. Moreover, Davidson et al (1990) found that birth weight was the best predictor of 12 and 21 month post-term growth achievements in infants with and without BPD. The lack of an association between birth weight and WHZ in our data, upon exclusion of SGA infants, suggests that this indicator in our subjects was not an artifact of small body size at birth, but clearly reflects nutritional status.

II. SAMPLE STUDY

A. Characteristics

Fifteen patients were further evaluated for their nutritional and oxidative stress status. The finding that subjects in the sample were older than the clinic population may have resulted from the bias introduced because of the need for parental consent. Parents who agreed to participate may have been self-selected as they were interested in the nutritional problems which were prevalent in the older infants. This may have led to the increased participation of parents of older infants versus younger ones in an effort to improve their children's outcome. Parents of younger children were less likely to participate because of the overprotective nature of parents towards their younger ones and the lower prevalence of nutritional problems in this group.

In the sample, nine infants were defined as failing to thrive compared to six infants who were thriving. Because older infants had poorer nutritional outcomes than younger ones in the clinic population, it was not surprising that failing to thrive infants were significantly older than normally growing BPD infants. To date, only one study has compared failing to thrive infants to thriving BPD infants. In

the study conducted by Kurzner et al, (1988a) failing to thrive BPD infants had significant lower birth weight and shorter gestation than thriving BPD infants. Although significant differences in the present study were not noted in birth weight or gestational age between the two groups, infants with growth failure tended to have lower birth weight than infants with normal growth. However, we have already shown that the nutritional problem was not an artifact of small body size at birth rendering the difference in birth weight between the two groups of minor importance.

B. Anthropometric Measurements

The distribution of TSF, an index of fat and energy stores, revealed that more than half of the sample had depleted fat stores (below the 5th percentile). The midarm circumference revealed that approximatly one quarter of the sample were below the 5th pecentile suggesting either a reduction in muscle mass, a reduction in subcutaneous fat or both. However, midarm muscle circumference (MAMC), an index of the muscle mass and therefore protein reserves of the body, was least affected. More than half of the sample had adequate protein stores. Therefore, the decreased MAC reflected decreased fat stores but not a reduction in muscle mass. There are two possible explanations for the finding of depleted fat stores but adequate protein reserves. One is that protein

stores were depleted at one time but were replenished by the time of the study due to the nutritional intervention received in the clinic. Therefore, we could not detect a depletion in protein reserves at the time of assessment. Another possibility is that malnutrition was not severe enough to deplete the protein stores. This is supported by the finding of few infants who had weight for height z score less than two standard deviations below the median.

Errors may occur in nutritional anthropometry affecting the precision, accuracy and validity of the measurements. Two major errors are: measurement errors and use of invalid assumptions in the derivation of body composition from anthropometric measurements.

Measurement errors arise from examiner error due to inadequate training, instrument error, and measurement difficulties (eg. skinfold thickness). These errors were minimized in our study by ensuring that the examiner (JC) received thorough training in the use of standardized procedures, and precise and correctly calibrated instruments. Furthermore, to improve the precision of the triceps skinfold, measurements were performed in triplicate and the mean reported. It has been reported that the intra-examiner measurement errors for triceps skinfolds and midarm cirumference measurements are small provided that training in

standardized procedures is given (Gibson, 1990).

Invalid assumptions may lead to erroneous estimates of body composition when derived from anthropometric measurements, especially in protein energy malnutrition and obesity. The use of skinfold thickness to estimate body fat assumes that a) the thickness of the subcutaneous adipose tissue reflects a constant proportion of the total body fat and b) the site selected represents the average thickness of subcutaneous tissue. Siervogel et al. (1982) concluded that the triceps skinfold was the most representative of the total subcutaneous layer for infants. The relationship between subcutaneous fat, total body fat, and internal fat is nonlinear and varies with age and disease state. In malnourished persons there is a shift of fat storage from subcutaneous to deep visceral sites. Measuring skinfold thickness should therefore, be considered a qualitative measure of the amount of total body fat (Gibson, 1990). In our study, we used skinfold thickness to assess the degree of loss of subcutaneous fat, due to malnutrition, in BPD infants compared to age matched controls. These measurements were used in conjunction with other anthropometric data (weight for age, weight for height, mid arm circumference) to describe nutritional status of our population rather than to derive precise determination of body composition.

C. Energy Intake

In order to examine the relationship between growth failure and energy intake, we assessed energy intake using an estimated food record. The use of the food record system has a number of limitations. Food records may cause parents to modify their children's habits to make recording easier. The parents may feed their children and/or report what they presume the investigator would want to see. Nevertheless, food records, unlike 24hr recall data, do not depend on memory. To date, only one study has objectively evaluated the usual food intake in children. Livingstone et al. (1992) evaluated the usual food intake in children and adolescents aged 7 to 18 years by 7 day weighed dietary record in comparison to total energy expenditure by the doubly labelled water method. Results indicated that there was good agreement [assessed by pair-wise comparison showing the relative bias (mean diference) and limits of agreement (mean difference ± 2SD of the difference) between weighed dietary record and total energy expenditure] between the mean energy intake using a weighed 7 day food record and mean total energy expenditure in 7 and 9 year old children. However, there was an increasing divergence between energy intake and total energy expenditure in 12, 15 and 18 year old subjects. The authors concluded that the weighed dietary record is a valid tool to assess dietary intake in 7 and 9 year old children, but individual estimates

of weighed dietary record lacked precision. The validity of this tool in 7 and 9 year old children was attributed mainly to the good compliance of parents in reporting the food intake of these children as opposed to self-reporting in adolescents. Recently, Trumbell-Waddell et al. (1993) examined the reliability and validity of the 3-day estimated food record of 146 preschool children aged 24 to 47 months. Parents and caregivers completed a 3 day estimated food record and then were randomly assigned to on of two groups. One group completed another estimated record (reliability test) while the second group completed a weighed record (validity test). Results showed that the 3-day estimated record was reliable and valid at the group level. However, this did not apply at the individual level since the confidence intervals for differences between individual mean intakes were wide. Because energy intake is to be averaged over a number of subjects, the use of food record in this study is justified on this basis.

Large intraindividual variation in the dietary factor under study (energy intake in our case) may mask significant correlations when interindividual variation is small in relation to intraindividual variation. Moreover, it may also mask significant differences when comparing average nutrient intakes between two groups. We, therefore, assessed intra-subject and inter-subject variability. The ratio of intra-subject variability to inter-subject variability (variance

ratio) reported in our study, did not exceed one. The inter-subject variability explained 68% of the variability in energy intake while 22% was explained by intra-subject variability. Nelson and coworkers (1989) reported a variance ratio of energy intake for both sexes less than one for infants studied at 15 and 18 months of age. When the energy intake of the same infants was assessed at 24 and 36 months, the variance ratio exceeded one. Nevertheless, variance ratios for energy intake and for 28 nutrients were relatively low for toddlers compared to children aged 5-17 years. Trumbell Wadell et al. (1993) found that intra-subject variation in energy and nutrient intake exceeded inter-subject variation (60-90% vs 10-40 % of total variation) in preschool children. Our results are not in agreement with those of Trumbell-Waddell et al. (1993). A possible explanation for the greater variability in the latter study is attributed to the fact that both the mother and the caregiver reported the child's food intake. In the present study, however, the mother was the sole provider of nutritional information on the child's intake.

Nelson et al. (1988) found that 7 dietary records were required to estimate energy intake for toddlers (age range 1-4 years) for both sexes to ensure that the correlation between the observed and true energy intake was greater or equal to 0.9. The number of dietary records calculated by Nelson et al. for toddlers exceeded that of our study. We calculated the

number of days required to estimate energy intake to within 20 percent of the true energy intake, to be four repeated dietary records. The estimated difference in required records is attributed to the higher ratios of within-to between subject variances in the study of Nelson et al. In the present study, the low variance ratio of energy intake may be explained by the higher proportion of males to females. Nelson et al. found that females across all age groups (15 months up to 68 years) generally have higher ratios of within to between-subject variances than males. The authors ascribed this finding to overall lower caloric intakes and smaller between-subject variance in the females. Similiarly Beaton et al. (1979) showed that women have larger ratios of within to between-subject coefficient of variation than men for energy, fat, and cholesterol.

In order to determine whether growth failure was associated with a decrease in energy intake and/or an increase in energy expenditure, we compared the energy intake of thriving and non thriving infants. The finding that energy intake met the recommended intake for age in thriving infants suggests that these infants do not have increased energy requirements. In contrast, infants with growth failure tended to have an increase in mean energy intake compared to the recommended energy intake for age, yet continued to fail to thrive. These findings suggest that the metabolic demands were

increased in these infants to account for their growth failure. Our results are consistent with the work of Kurzner et al. (1988) who observed elevated energy expenditure in BPD infants with growth failure compared to BPD infants with normal growth ($p< 0.005$) and controls ($p< 0.001$). Furthermore, these investigators demonstrated a non significant trend toward increased overall intake in infants with growth failure compared to infants with normal growth. Because growth failure was not associated with decreased energy intake but with increased energy expenditure, we examined whether the increase in metabolic demands demonstrated in infants with growth failure was associated with oxidative stress.

D. Oxidative Stress Status

Oxidative stress in vivo can be assessed by the determination of degradation products of free radical injury or by the identification of depleted antioxidant defences. The former method needs to be controlled for degradation products which may have been generated during or after sampling, as opposed to in vivo. The latter approach may suggest oxidative stress among other possible causes of depletion, but does not by itself confirm the presence of oxidative stress. A combination of the two approaches provides stronger evidence in support of oxidative stress.

(i) oxidative injury: Although all macromolecules may undergo peroxidative damage, recent emphasis has been on lipid peroxidation. This is because polyunsaturated fatty acids (PUFA) which are present in the phospholipids of cellular and subcellular membranes (Kneepkens et al, 1992), are the main cellular targets for free radical attack Lipid peroxidation is a very destructive and self perpetuating chain reaction that can directly damage membranes and indirectly damage other cell components by the production of reactive aldehydes (Cheeseman and Slatter, 1993). In vivo, lipid peroxidation may be studied through the resulting degradation products- conjugated dienes, lipid hydroperoxides, aldehydes and volatile hydrocarbons (Kneepkens et al, 1992). MDA is one of several low-molecular weight end products formed via the decomposition of certain primary and secondary lipid peroxidation products.

The TBA (thio-barbituric acid) test has been widely used to measure MDA in biological samples. The method requires incubating samples containing TBA under acidic conditions at elevated temperatures and finally measuring the MDA-TBA product spectrophotometrically at 532nm. However, this essay is subject to several flaws. TBA can react with other endogenous compounds such as bilirubin and aldehydes other than MDA (Hageman et al, 1992). In addition, biological samples contain only a small amount of free MDA and in tests

where prolonged incubation is applied, the majority of MDA formed is by the decomposition of lipid hydroperoxides and further peroxidation during the heating stage of the assay itself (Holley and Cheeseman, 1993). Peroxide decomposition produces radicals that can start peroxidation of other lipid molecules during the essay, amplifying the response. The greater the lipid content of the biological sample tested, the greater the TBA reactivity due to the amplification during the test. It has been concluded that this assay is not specific to MDA (Hermann and Cheeseman, 1990).

Recently, modifications in the preparation of biologic samples and in the HPLC measurement of MDA have improved the specificity, recovery, reliability and reproducibility of this essay (Lepage et al, 1991).

Although a number of sensitive analytical methods exist for the unambiguous isolation and direct quantification of MDA, several important considerations must be taken into account when considering MDA as an index of lipid peroxidation. First, MDA yield as a result of lipid peroxidation varies with the nature of PUFA peroxidized depending upon its degree of unsaturation. Second, only certain lipid oxidation products decompose to yield MDA. Third, MDA is only one of several aldehydic end products of fatty peroxide formation and decomposition. Fourth, the

peroxidation environment influences both the formation of lipid-derived MDA precursors and their decomposition to MDA. Fifth, MDA is not only formed during the peroxidation of lipids. The deoxyribose of DNA, several other carbohydrates and a number of amino acids release MDA when reacting with oxygen radicals (Janero, 1990).

Therefore, utilizaton of MDA analysis and/or the TBA test and interpretation of sample MDA content and TBA test response in studies of lipid peroxidtion require caution and correlative data from other indices of fatty peroxide formation and decomposition (Janero, 1990).

At present, there are no standards for MDA concentrations in the normal pediatric population. A control group could not be recruited for the purpose of comparison of oxidative stress markers because of the limited access to normal infants for research purposes. Mean (± SEM) plasma MDA levels in adults are 55 ± 3 nM/l (Lepage et al, 1993) and older pediatric patients 69.1 ± 2.6 nM/l (Lepage et al, 1991). Compared to these levels, almost all BPD infants showed increased lipid peroxidation. One explanation for this phenomenon is that the antioxidant system in these premature infants is insufficient to protect against normal free radical activity (Frank and Sosenko, 1987). Another explanation may be that lipid peroxidation occurs due to an ongoing inflammatory process in

the lungs which would generate free oxygen radicals. Alternatively, these levels may be part of the physiological processes which maintain the normal turnover rate of membranes and cells in growing infants.

MDA may be generated by oxidative injury to non lipid molecules (such as protein, DNA) however, it is usually the result of lipid peroxidation of PUFA via one of two mechanisms. It is the end product of nonenzymatic metabolically uncoupled PUFA oxidative degradation. It may also be a side product of enzymatic eicosanoid formation, prostaglandins and thromboxanes via cyclooygenase, and then thromboxane synthase (Janero, 1990). Because the MDA content of a sample per se cannot give qualitative or quantitative information on the precise molecular nature of the MDA precursor or their origin (Janero, 1990), the MDA levels of our subjects may be a consequence of either the non enzymatic lipid peroxidation of PUFA or eicosanoid formation through the enzymatic pathway.

(ii) antioxidant depletion: Low levels of some antioxidants, such as vitamin A, E, selenium, etc, may reflect poor nutritional status and either decreased intake or malabsorption of the substance, rather than depletion from oxidative stress. However, the antioxidant system utilizing glutathione is an interesting model. Nearly all of the

glutathione in red blood cells is present in the reduced form GSH, which does not readily cross the membranes of the erythrocytes (Jackson, 1986). However, during antioxidant defence, GSH is oxidized to GSSG, which must then be rereduced to GSH. During oxidative stress, the production of oxidized glutathione (GSSG) is increased relative to its rate of reduction back to GSH. Unlike GSH, GSSG is actively transported out of the red cell. A low RBC GSH may, therefore, be the result of a relative increase in the red cell content of GSSG and it's active removal from the cell. Hence, low levels of GSH or increased levels of GSSG can serve as markers of oxidative stress.

There are no standards for glutathione in the normal pediatric population. The red cells of normal adults contain 6.57 ± 1.04 umoles of glutathione per gram of hemoglobin (mean ± SD) (Beutler et al, 1963). Based on this, four subjects with failure to thrive had low glutathione levels compared to two thriving infants. One of the two thriving infants with low glutathione levels, was the youngest subject studied (4 months corrected age). Because glutathione levels may be deficient at birth, the decreased level of glutathione in this subject may possibly be attributed to an age effect rather than oxidative injury.

Possible confounding factors were considered. These

included corrected age, gestational age, oxygen supplementation and energy intake. Although significant differences in corrected age were noted between the two groups, the absence of an association between corrected age and oxidative stress markers rules out corrected age as a confounding variable. Gestational age and birth weight are potential confounding variables. The expression of antioxidants, specifically glutathione peroxidase, is dependent on age as has been demonstrated in a number of studies using animal models (Frank and Sosenko, 1987a,1987b). With regard to MDA, Schlenzig et al. (1993) demonstrated small but significant correlations between MDA and gestational age ($r=-0.33$; $p=0.0002$) and birth weight ($r=-0.32$; $p=0.0003$). Significant differences between gestational age and birth weight were not noted between the two groups in this study and therefore, these variables were not expected to affect our results. Because energy intake was positively correlated with glutathione, we thought that the tendency to increased energy intake in failing to thrive infants compared to thriving infants may have masked any differences in red cell GSG levels between the two groups. Therefore, we examined the possibility of energy intake as a negative confounding variable. The comparable values in glutathione levels between the two groups after controlling for energy intake ruled out this possibility. In addition, because it has been demonstrated that oxygen-treated neonates exhibited significantly higher

MDA levels than those without supplementary oxygen (Schlenzig et al, 1993), we controlled for this confounding factor by excluding infants on supplemental oxygen from the analysis. MDA and GSH levels, however, remained comparable.

Oxidative stress, therefore, did not account for the differences in energy requirments between thriving and non thriving infants. Perhaps, these infants have inefficient substrate utilization which may account for their increased energy requirements.

Several cautions/limitations must be taken into consideration when interpreting the results of our study. A selection bias is observed in the relatively older age of our subjects compared to the clinic population and the nutritional intervention received by the infants in the clinic. An important limitation to consider when interpreting the results of our study on oxidative stress is the sample size. The sample size for oxidative stress could not be calculated prior to the study. While most infants had elevated MDA, the sample size may not have been adequate to detect differences in the oxidative stress marker glutathione. The sample size of this study was based on detecting a 25% difference in energy expenditure and not on a difference in oxidative stress because there were no data on which to make comparisons. The absence of an age matched control group renders comparison

between BPD infants and controls with respect to oxidative stress measurements difficult. Hence, normal values of plasma MDA and red cell glutathione in the pediatric population must be determined. Given the above limitations, extrapolation of our findings to the general BPD population must be applied with caution but this area of investigation clearly warrants further study.

SECTION SEVEN CONCLUSION

The present study which took place between August 1993 and January 1994 was undertaken to examine three objectives. First, to determine the prevalence of growth failure in BPD patients followed at the out-patient clinics at the MCH. The descriptive study revealed that the prevalence of growth failure ranged between 45% and 55%. Second, we set out to determine whether growth failure is associated with either poor nutritional intake, increased energy expenditure or both. Results from the sample study indicated that infants with growth failure had energy intakes in excess of normal requirements while thriving infants had normal intakes. Therefore, growth failure in BPD infants was not associated with decreased energy intake but with increased energy expenditure. Third, we wished to examine whether ongoing oxidative stress exists in the BPD population and whether significant differences in oxidative stress markers exist between failing to thrive and thriving infants to explain the difference in energy requirements. Almost all infants in both groups exhibited elevated MDA levels compared to adult controls suggesting increased lipid peroxidation. When we compared glutathione levels to adult controls, four out of nine failing to thrive subjects showed decreased glutathione levels compared to two out of six thriving infants indicating

a depletion of this antioxidant. Larger numbers of subjects would need to be studied to determine if this finding is important. Significant differences in plasma MDA and red cell glutathione were not detected between the two groups. These are the first values of plasma MDA and red cell glutathione to be reported for the infant pediatric population. Therefore, we could not rule out whether this generation of MDA (lipid peroxidation) was due to BPD or simply to the age of our subjects.

Future studies must address whether oxidative stress exists in the relatively older BPD population (beyond 4 months of age) and to determine the cause of the elevated VO_2 in BPD infants.

BIBLIOGRAPHY

Akerboom T, Sies H. Glutathione transport and its significance in oxidative stress. In: Glutathione: Metabolism and Physiological Functions. CRC Press, Boca Raton 1990.

Amin S, Chen SY, Collipp PJ, Castro-Magna M, Maddaiah VT, Klein SW. Selenium in premature infants. NUTR METAB 1980; 24:331-340.

Anderson ME. Tissue glutathione. In CRC Handbook of Methods for Oxygen Radical Research, Greenwald RA. CRC Press, Boca Raton, FL 1985.

Bancalari E, Gerhardt T. Bronchopulmonary dysplasia. Pediatric Clinics of North America 1986;33(1): 1-23.

Bancalari E. Pathogenesis of bronchopulmonary dysplasia: An overview. In Bancalari E, Stocker JT. Bronchopulmonary Dysplasia. Hemisphere Publishing, Cambridge (Mass.); 1988:3-15.

Beaton GH, Milner J, Corey P. Sources of variance in 24- hour dietary recall data: implications for nutrition study design and interpretation. Am J Clin Nutr 1979; 32:2546-59.

Bergman J, Farrell EE. Neurodevelopmental outcome in infants with bronchopulmonary dysplasia. Clinics in Perinatology 1992;19 (3):673-695.

Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963;61:882-890.

Bilibrey JG, Wooldridge NH, Lyrene R. Growth and nutritional intake in infants with mild, moderate, and severe bronchopulmonary dysplasia. The American Dietetic Association 1993; supplement 93 (9): A-106.

Bird RP, Draper HH: Comparative studies on different methods of malondialdehyde determination. Meth Enzymol 1984;105:299-305.

Birwick DM. Nonorganic failure to thrive. Pediatrics in Review 1989; 1:265-270.

Bithoney WG, Rathburn JM. Failure to thrive. In: Levine MD, Carey WB, Crocker AC, Gross RT. Developmental Behavioral Pediatrics. Philadelphia, W.B. Saunders Company; 1983: 557-572.

Bounous G, Baruchel S, Falutz J, Gold P. Whey proteins as a

food supplement in HIV-seropositive individuals. Clin Invest Med 1993;16(3):204-209.

Brown ER. Bronchopulmonary dysplasia. In: Rubin IL, Crocker AC. Developmental Disabilities. Delivery of Medical Care for Children and Adults. Philadelphia, Lea and Febiger, 1989; 223-229.

Browner WS, Black D, Newman TB, Hulley SR. Estimating Sample size and power. In: Hulley SB, Cummings SR. Designing Clinical Research: An Epidemiological Approach. Williams and Wilkins; 1988: 139-150.

Burchfield D, Neu J. Neonatal parenchymal diseases. In: Koff PB, Eitzman D, Neu J. Neonatal and Pediatric Respiratory Care. 2nd New York, Mosby-Year Book; 1993: 75-91.

Cantin AM, North SL, Hubbard RC, Crystal RG. Normal alveolar epithelial lining fluid contains high levels of glutathione. J Appl Physiol 1987; 63:152-7.

Cantin AM, North SL, Crystal RG. Glutathione is present in normal alveolar epithelial lining fluid in sufficient concentration to provide antioxidant protection to lung parenchymal cells (abstract). Am Rev Respir Dis 1985;131: A372.

Cheeseman KH, Slater TF. An introduction to free radical biochemistry. British Medical Bulletin 1993;49(3):481-493.

Cross CE: Oxygen radicals and human disease. Ann Int Med 1987;107:526-45.

Cummings JJ, D'Eugenio DB, Gross SJ. A controlled trial of dexamethasone in preterm infants at high risk for bronchopulmonary dysplasia. N Engl J Med 1989;320:1505-10.

Davidson S, Schrayer A, Wielunsky E, Krikler R, Lilos P, Reisner S. Energy intake, growth, and development in ventilated very-low-birth-weight infants with and without bronchopulmonary dysplasia. AJDC 1990;144:553-559.

Draper HH, Hadley M. Malondialdehyde determination as an index of lipid peroxidation. Meth Enzymol 1990;186:421-431.

Esterbaub H, Cheeseman KH. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Meth Enzymol 1990;186:407-421.

Falcigilia HS, Ginn- Pease M. Vitamin E and selenium levels of premature infants with severe respiratory distress syndrome and bronchopulmonary dysplasia. J Pediatr Perinatal Nutr 1988;

2 (1):35-49.

Foster RLR, Hunsberg MM, Anderson JJT. Family-Centered Nursing Care of Children. Philadelphia, W.B. Saunders Company, 1989.

Frank DA, Zeisel SH. Failure to thrive. The Pediatric Clinics of North America 1988;35 (6):1187-1206.

Frank L, Sosenko IRS. Prenatal development of lung antioxidant enzymes in four species. J Pediatr 1987;110:106-110.

Frank L, Sosenko IRS. Development of lung antioxidant enzyme system in late gestation: Possible implications for the prematurely born infant. J Pediatr 1987;110 (1):9-14

Frank L, Sosenko IRS. Undernutrition as a major contributing factor in the pathogenesis of bronchopulmonary dysplasia. Am Rev Respir Dis 1988;138:725-729.

Frank L. Nutrition:influence on lung growth, injury and repair, and development of bronchopulmonary dysplasia.In Bancalari E, Stocker JT. Bronchopulmonary Dysplasia.Hemisphere Publishing. Cambridge (Mass.), 1988;78-106.

Frank L, SosenKo IRS. Failure of premature rabbits to increase antioxidant enzymes during hyperoxic exposure:increased susceptibility to pulmonary oxygen toxicity compared with term rabbits. Pediatr Res 1991;29:292-296.

Frank L. Antioxidants, nutrition and bronchopulmonary dysplasia. Clinics in Perinatology 1992;19(3):541-562.

Frisancho AR. New norms of upper limb fat and muscle areas for assessment of nutritional status. Am J Clin Nutr 1981;34:2540.

Gamara E. Energy expenditure in premature newborns with bronchopulmonary dysplasia. Biol Neonate 1992;61:337-344.
Gibson RS. Principles of Nutritional Assessment. Oxford University Press, New York, 1990.

Georgieff MK, Mills MM, Lindeke L, Iverson S, Johnson DE, Thompson TR. Changes in nutritional management and outcome of very-low-birth-weight infants. AJDC 1989;143:82-85.

Goetzman BW. Understanding bronchopulmonary dysplasia. Am J Dis Child 1986;140(4):332-334.

Grigg J, Barber A, Silverman M. Bronchoalveolar fluid glutathione in intubated premature infants. Arch Dis Child 1993;69:49-51.

Gross SJ, Oehler JM, Eckerman CO. Head growth and developmental outcome in very low-birth-weight infants. Pediatrics 1983;71:70-75.

Goldbloom RB. Failure to thrive. Pediatric Clinics of North America 1982; 29 (1):151-166.

Hack M, Merkatz IR, Gordon D, et al. The prognostic significance of postnatal growth in very low-birth weight infants. Am J Obstet Gynecol 1982;143:693-699.

Hageman JJ, Bast A, Vermeulen NPE. Monitoring of oxidative free radical damage in vivo: analytical aspects. Chem Biol Interactions 1992;82:243-293.

Hazinski TA. Bronchopulmonary dysplasia. In: Kendig E. Kendig's Disorders of the Respiratory Tract in Children. 5th ed. W.B. Saunders Company 1990;300-320.

Health and Welfare Canada. Nutrition Recommendations. The Report of the Scientific Review Committee. 1990.

Heffner J, Repine J. Pulmonary strategies of antioxidant defense. Am Rev Respir Dis 1989;140:531-554

Holley AE, Cheeseman KH. Measuring free radical reactions in vivo. British Medical Bulletin 1993;49(3):494-505.

Hommes MJT, Romijn JA, Endert E, Sauerwein HP. Resting energy expenditure and substrate oxidation in human immunodeficiency virus (HIV)-infected asymptomatic men: HIV affects host metabolism in the early asymptomatic stage. Am J Clin Nutr 1991;54:311-315.

Jackson A. Blood glutathione in severe malnutrition in childhood. Transactions of The Royal Society of Tropical Medicine and Hygiene 1986;80:911-913.

Janero DR. Malondialdehyde and thiobarbituric acid- reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radical Biology and Medicine 1990;9:515-540.

Kalhan SC, Denne SC. Energy consumption in infants with bronchopulmonary displasia. J PEDIATR 1990;116(4):662-664.

Kao LC, Warburton D, Sargent CW, Platzker ACG, Keens TG. Furosemide acutely decreases airway resistance in chronic bronchopulmonary dysplasia. J PEDIATR 1983; 103:624.

Kao LC, Durand DJ, Nickerson BG. Improving pulmonary function does not decrease oxygen consumption in infants with bronchopulmonary dysplasia. J PEDIATR 1988;112:616-21

Karlberg P. The development of children in a Swedish urban community: A prospective longitudinal study.III. Physical growth during the first three years of life. Acta Pediatr Scan 1968;187:48.

Kelly FJ. Free radical disorders of preterm infants. British Medical Bulletin 1993;49(3):668-678.

Kneepkens C.M., Ferreira C, Lepage G, Roy C. The hydrocarbon breath test in the study of lipid peroxidation: principles and practice. Clin Invest Med 1992;15(2): 163-186.

Kuivalainen E, Pitkanen OM, Hallman M, Anderson SM. Lipid peroxidation in premature infants. Pediatr Res 1991;29:221A

Kurzner SI,Garg M,Bautista DB, Sargent CW, Bowman CM, Keens TG. Growth failure in bronchopulmonary dysplasia: elevated metabolic rates and pulmonary mechanics. Pediatr 1988;112:73-80.

Kurzner SI, Garg M, Bautista DB,et al. Growth failure in infants with bronchopulmonary dysplasia:nutrition and elevated resting metabolic expenditure. Pediatrics 1988;81:379-84.

Lepage G, Munoz G, Champagne J, Roy CC. Preparative steps necessary for the accurate measurement of malondialdehyde by high-performance liquid chromatography. Anal Biochem 1991;197:277-83.

Lepage G, Kneepkens CMM, Smith L, Lacaille F, Ronco N, Champagne J, Osberg I, Roy CC. Low - Beta carotene may contribute to the accumulation of free radicals in cystic fibrosis. FASEB 1991;5:A1075.

Lepage G, Ronco N, Champagne A, Lamarre A, Roy CC. Beta-carotene inhibits lipid peroxidation and improves peroxisomal function in cystic fibrosis. FASEB J 1993;7:A3[illegible].

Livingstone MBE, Prentice AM, Coward WA, Strain JJ, Black AE, Davies PSW, Stewart CM, McKenna PG, Whitehead RG. Validation of estimates of energy intake by weighed dietary record and diet history in children and adolescents. Am J Clin Nutr 1992;56:29-35.

Lockitch G, Jacobson B, Quigley G, Dison P, Pendray M. Selenium deficiency in low birth weight neonates: An unrecognized problem. J PEDIATR 1980; 114: 865-70

McElroy MC, Postle AD, Kelly FJ. Catalase, superoxide dismutase and glutathione peroxidase activities of lung and liver during human development. Biochimica et biophyisca acta 1992;1117:153-158.

Machilin LJ, Bendich A. Free radical tissue damage: protective role of antioxidant nutrients. FASEB J 1987;1:441-445

Markstead T, Fitzhardinge PM. Growth and development in children recovering from bronchopulmonary dysplasia. J PEDIATR 1981;98:597-602

Meisels SJ, Plunkett JW, Roloff DW, Pasick PL, Stiefel GS. Growth and development of preterm infants with respiratory distress syndrome and bronchopulmonary dysplasia. Pediatrics 1986;77:345-352.

Moison RMW, Palinckx JJS, Roest M, Houdkamp E, Berger HM. Induction of lipid peroxidation of pulmonary surfactant by plasma of preterm babies. Lancet 1993;341:79-82.

Monin P, Vert P. The management of bronchopulmonary dysplasia. Clinics in Perinatology 1987;14 (3):531-49.

Nelson M, Black AE, Morris JA, Cole TJ. Between- and within-subject variation in nutrient intake from infancy to old age: estimating the number of days required to rank dietary intakes with desired precision. Am J Clin Nutr 1989; 50: 155-67.

Niermeyer S. Nutritional and metabolic problems in infants with Bronchopulmonary Dysplasia. Bancalari E, Stocker JT. Bronchopulmonary Dysplasia. Hemisphere Publishing. Cambridge (Mass.), 1988 :313-336.

Northway WH Jr. Bronchopulmonary dysplasia: then and now. Arch Dis Child 1990;65:1076-81.

Nutritional Assessment System (Version 4). Quilchenia Consulting Ltd. 1992.

O'Brodovich HM, Mellins RB. Bronchopulmonary dysplasia: unresolved neonatal acute lung injury. Am Rev Respir Dis 1985;132:694-709.

Oh W. Nutritional management of infants with bronchopulmonary dysplasia: Report of the Ninetieth Ross Conference on Pediatric Research 1986,96-105

Parker RA. Lindstorm DP. Cotton RB. Improved survival accounts for most, but not all, of the increase in bronchopulmonary dysplasia. Pediatrics 1992; 90 (5):663-8.

Peterson KE, Rathbun JM, Herrera G. Growth data analysis in FTT treatment and research. In Drotar D: New Directions in Failure to Thrive: Implications for research and practice. Plenum Press, New York, 1984.

Picot IC, Trivier JM, Nicole A, Sinet PM, Thevenin M. Age-correlated modifications of copper-zinc superoxide dismutase and glutathione- related enzyme activities in human erythrocytes. Clin Chem 1992;38 (1):66-70.

Pitkanen OM, Hallman M, Anderson SM. Correlation of free oxygen radical-induced lipid peroxidation with outcome in very low birth weight infants. J PEDIATR 1990;116:760-4

Ramsey BW, Farrell PM, Pencharz P and the Consensus Committee.Nutritional assessment and management in cystic fibrosis: a consensus report. Am J Clin Nutr 1992;55:108-16

Ripalda MJ, Rudolph N, Wong SL. Developmental patterns of antioxidant defense mechanisms in human erythrocytes. Pediatr Res 1989;26:366-369.

Ross G, Krauss AN, Auld PA. Growth achievement in low-birth-weight premature infants. Relationship to neurobehavioral outcome at one year. J Pediatr 1983;103:105-108.

Schlenzig J-S, Bervoets K, Loewenich V, Bohles H. Urinary malondialdehyde concentration in preterm neonates:is there a relationship to disease entities of neonatal intensive care? Acta Paediatr 1993;82:202-205.

Sinkin AR, Phelps LD. New strategies for the prevention of bronchopulmonary dysplasia. Clinics in Perinatology 1987;14(3) 599-615.

Sluis K, Darlow B, George P, Morgridge N, Dolamore B, Winterbourn C. Selenium and glutathione peroxide levels in premature infants in a low selenium community. PEDIATR RES 1992;32: 189-194.

SPSS for Windows:Base System User's Guide, Release 5.0, 1992.

Strange RC, Cotton W, Fryer AA, Jones p, Hume R. Lipid peroxidation and expression of copper-zinc and manganese superoxide dismutase in lungs of premature infants with hyaline membrane disease and bronchopulmonary dysplasia. J Clin Lab Med 1990;116:666-673.

Sullivan KM, Gorstein J. ANTHRO Software for calculating pediatric anthropometry (Version 1.01).

Thibeault DW, Clutario B, Auld PAM. The oxygen cost of breathing in the premature infant. Pediatrics 1966; 37:954

Trumble-Wadell JE, Campbell ML, Armstrong L, Macpherson B. The reliability and validity of food intake provided by parents and caregivers of preschool children. Journal of the Canadian Dietetic Association 1994 (supplement);55 (1):25.

Vohr BR, Bell EF, Oh W. Infants with bronchopulmonary dysplasia:Growth pattern and neurologic and developmental outcome.Am J Dis Child 1982 ;136:443-447

Walker WA, Hendricks KM. Manual of Pediatric Nutrition. WB Saunders, 1985;1-51.

Waterlow JC, Buzina R, Keller W, Lane JM, Nichaman MZ, Tanner JM. The presentation and use of height and weight data for comparing the nutritional status of groups of children under the age of 10 years. Bulletin of the World Health Organization 1977; 55:489-498.

Weinstein MR, OH W. Oxygen consumption in infants with bronchopulmonary dysplasia. J PEDIATR 1981;99:958-61.

White CW. Pulmonary Oxygen toxicity: cellular mechanisms of oxidant injury and antioxidant defense. In Bancalari E, Stocker JT. Bronchopulmonary Dysplasia. Hemisphere Publishing, Cambridge (Mass.), 1988; 22-41.

WHO (World Health Organization). Measuring Change in Nutritional status. Guidelines for Assessing the Nutritional Impact of Supplementary Feeding Programmes for Vulnerable Groups. World Health Organization, Geneva, 1983.

Willett W. Nutritional Epidemiology. New York: Oxford University Press, 1990.

Wilson DC, McClure G, Halliday HL, Reid MC, Dodge JA. Nutrition and bronchopulmonary dysplasia. Arch Dis Child 1991;66:37-38.

Wispe JR, Roberts RJ. Molecular basis of pulmonary oxygen toxicity. Clinics in Perinatology 1987;14(3): 651-666.

Yeh TF, McClenan DA, Ayahi OA, Pildes RS. Metabolic rate and energy balance in infants with bronchopulmonary dysplasia. J PEDIATR 1989;114:448-51.

Yu VYH, Orgill AA, Lim SB, Bajuk B, Astbury J. Growth and development of very low birthweight infants recovering from bronchopulmonary dysplasia. Arch Dis Child 1983; 58:791-4.