ACADEMIC DIVISION OF CHILD HEALTH
SCHOOL OF GRADUATE ENTRY MEDICINE
AND HEALTH
UNIVERSITY OF NOTTINGHAM

USING THE CAFFEINE BREATH TEST TO STUDY DRUG METABOLISM IN PROTEIN-ENERGY MALNOURISHED CHILDREN

By

Kazeem Adeola Oshikoya, *M.B; B.S, MSc*

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ABSTRACT

Malnutrition is a global health problem that affects infants and young children. It is frequently associated with infections and commonly affects children in developing countries. Malnutrition is the cellular imbalance between the supply of energy from macronutrients and micronutrients and the demand of the body for them in order to achieve normal growth, maintenance, and specific functions. Underweight (mild to moderate) and marasmus, marasmic-kwashiorkor, and kwashiorkor (severe) are the spectrum of malnutrition.

Various pathophysiological changes, including fatty changes, abnormal rough endoplasmic reticula and mitochondria, decreased peroxisomes, and decreased quantity and quality of metabolising enzymes, are associated with malnutrition which may significantly influence hepatic drug metabolism. However, the effect of different categories of malnutrition on drug metabolism has not been extensively investigated. This research, therefore, aimed to determine the effect of malnutrition on drug metabolism. The specific objectives are (i) to perform a systematic review of the studies of drug pharmacokinetics in malnourished children, and (ii) to use the caffeine breath test to determine the effects of different types of malnutrition on the metabolising activity of hepatic CYP1A2 enzymes.

The systematic review involved literature searches in the MEDLINE and EMBASE databases covering publications between January 1960 and December 2009. Articles describing drug pharmacodynamics and pharmacokinetic parameters in the four categories of malnutrition, limited to children from 0 to 17 years, were sought using both databases and by reference tracking.

Altogether, 42 publications evaluated the disposition of 34 drugs in malnourished children. The drug absorption rate (Ka) was reported for eight drugs, of which gentamicin, metronidazole, phenytoin, chloramphenicol, paracetamol, and sulphamethoxazole showed no difference in the values of Ka for malnourished children and the control groups. The AUC of seven drugs did not differ for malnourished children when compared to their control groups but significantly decreased for carbamazepine ($p < 0.05$) and chloroquine ($p < 0.001$). By contrast, there was a statistically significant increase in the AUC of six drugs: metronidazole ($p < 0.05$), caffeine ($p < 0.05$), paracetamol ($p < 0.05$), phenobarbitone ($p < 0.05$), sulphadiazine ($p < 0.01$), and sulphamethoxazole ($p < 0.001$). The plasma protein binding of 19 drugs was evaluated in seven *in vitro* and two *in vivo* studies. There was a statistically significant decrease in the protein binding of 17 drugs in kwashiorkor when compared to healthy adults (*p*-values ranged from <0.0005 to <0.05). Nineteen studies evaluated the effects of malnutrition on the volume of distribution (VD) for 14 drugs. For most drugs, malnutrition had no statistically significant effect on VD. However, four drugs: gentamicin, quinine, streptomycin, and theophylline demonstrated contrasting results. The effect of malnutrition on the total clearance (CL) and elimination half-life ($t_{½}$) of nine drugs that are primarily metabolised in the liver was evaluated in 15 studies. There was a statistically significant decrease in the CL of six drugs: acetanilide ($p < 0.025$), antipyrine ($p < 0.05$, $p < 0.0025$, $p < 0.05$), caffeine ($p < 0.01$), sulphamethoxazole, isoniazid ($p < 0.01$), and metronidazole ($p < 0.01$). There was a corresponding statistically significant increase in their plasma half-lives. For six drugs that are primarily eliminated by the kidneys, malnutrition has a varying effect on their total CLs. The total CL was significantly increased for penicillin in children with marasmus ($p < 0.001$),

marasmic-kwashiorkor ($p < 0.01$), and kwashiorkor ($p < 0.01$), as well as increased for streptomycin in children with kwashiorkor ($p < 0.01$). By contrast, the total CL was significantly decreased for penicillin in underweight children ($p < 0.01$). It was also significantly decreased for cefoxitin in children with kwashiorkor ($p < 0.025$). The significantly decreased total CL of most of the drugs primarily metabolised by the liver may reflect decreased activity of the intrinsic hepatic metabolising enzymes. This would suggest a need to reduce drug dosage in malnourished children. More studies are therefore required to assess the activities of the hepatic metabolising enzymes in malnourished children.

Following the systematic review, the caffeine breath test (CBT) identified as a non-invasive approach to study the effects of the four categories of malnutrition on caffeine metabolism. Caffeine is a 1, 3, 7 trimethylxanthine compound that is metabolised in the liver by 1-*N*, 2-*N* and 7-*N* demethylation, and C-8 hydroxylation to 1, 3, 7 trimethyluric acid. CYP1A2 is responsible for the 3-*N* demethylation of caffeine. The CBT involves oral administration of a non-radioactive stable isotope of caffeine (^{13}C on the 3-methyl group). The caffeine undergoes 3-*N* demethylation in the liver which is a CYP1A2 dependent reaction. After *N*-demethylation, the ^{13}C methyl group enters the carbon pool as it is converted to formaldehyde, formate and bicarbonate. The bicarbonate is exhaled as carbon-dioxide. The exhaled labelled $^{13}CO_2$ is known to correlate with CY1A2 activity.

Fifteen children each who were underweight or experiencing marasmus, marasmic-kwashiorkor or kwashiorkor were recruited from Lagos and Kano States in Nigeria. They were studied before and after nutritional rehabilitation. After ingesting labelled caffeine (3mg/kg) at 0900 hours, breath samples were

collected in duplicate at -20, -10, -1 minute and every 15 minutes over 2 hours. The cumulative mean percent ^{13}C-caffeine dose exhaled as $^{13}CO_2$ was measured over 2 hours. Student's *t*-test was used to compare the results for each category of malnutrition, before and after nutritional rehabilitation, at 5% level of significance. The mean cumulative percent ^{13}C-caffeine dose recovered (CPDR) in underweight children was 7.56 ± 4.01% and 7.95 ± 3.68% before and after nutritional rehabilitation, respectively, and there was no significant difference in the mean values (p = 0.603). The CPDR significantly increased after nutritional rehabilitation in children with marasmus (from 6.80 ± 3.00% to 7.67 ± 2.81%, p < 0.001), marasmic-kwashiorkor (from 6.61 ± 2.26% to 7.56 ± 2.46%, p < 0.041), and kwashiorkor (from 6.29 ± 1.06% to 7.20 ± 1.80%, p =0.002). It is concluded that the present study may not have been adequately powered to detect a statistically significant difference in the results for underweight children. Such a difference would have been the basis for validating the results in a larger population of underweight children. However, doses of drugs that are metabolised by CYP1A2 enzyme may require modification in severely malnourished children.

DEDICATION

This work is dedicated to my future students and those who will join me later to teach rational medicine use for children in the developing countries; to my family, this is another way you have encouraged me to contribute to science and humanity; and to the malnourished children around the world who, over many decades, have been excluded from drug safety studies, I join many others to make a case for you.

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PUBLICATIONS RELATED TO THIS THESIS

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2. Effect of underweight malnutrition on ^{13}C-caffeine metabolism. Oshikoya, K.A., Sammons, H.M., Smith, K., Choonara, I. *(Manuscript in preparation)*.

3. Decreased metabolism of ^{13}C-caffeine via hepatic CYP1A2 in marasmus and kwashiorkor based on breath test. Oshikoya, K.A., Smith, K. (2013). *J Basic Clin Physiol Pharmacol* (DOI 10.1515/jbcpp-2013-0081). Decreased metabolism of ^{13}C-caffeine via hepatic CYP1A2 in marasmus and kwashiorkor based on breath test. Oshikoya, K.A., Smith, K., Sammons, H.M., Choonara, I. (2013). *J Basic Clin Physiol Pharmacol* (Erratum).

CONFERENCE PRESENTATIONS

1. A systematic review of pharmacokinetic studies in children with protein-energy.

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   Chamonix, France.

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ABBREVIATIONS

ABCA	Automated Breath Carbon Analyser
ACT	Artemesinin Combination Therapy
ADME	Absorption, Distribution, Metabolism and Excretion
ADH	Alcohol dehydrogenase
AGP	alpha-1acid glycoprotein
AIDS	Acquired Immunodeficiency Syndrome
AKTH	Aminu Kano University Teaching Hospital
ANOVA	Analysis of Variance
AP	Atom Per cent
APE	Atom Per cent Excess
ART	Antiretroviral therapy
AUC	Area Under the Curve
BBC	British Broadcasting Corporation
BNF	British National Formulary
BSA	Body Surface Area
C	Concentration
C_{in}	Concentration of dug in in-flowing blood

C_{out}	Concentration of dug in out-flowing blood
^{12}C	Carbon 12 Isotope
^{13}C	Carbon 13 Isotope
CBT	Caffeine Breath Test
CL	Clearance
Cmax	Maximum concentration
CO_2	Carbon dioxide
CPDR	Cumulative Per cent ^{13}C-dose Recovered
C_{ss}	Steady state concentration
CYP	Cytochrome
DBS	Dried Blood Spot
DHA	Dihydroartemisinin
ECF	Extracellular Fluid
E	Extraction ratio
E/U/Cr	Electrolyte, Urea, Creatinine
F	Extent of Drug Absorption
FAO	Food and Agriculture Organization
FBC	Full Blood Count
f_u	Unbound free drug

FMO	Flavin Monooxygenase
FTI	Free Thyroxine Index
GC	Chromatography Column
GFR	Glomerular Filtration Rate
GH	Growth Hormone
H	Healthy subject
h	Hour
hGH	Human Growth Hormone
HIV	Human Immunodeficiency Virus
ID	Identity
IRMS	Isotope ratio mass spectrometery
i.m	Intramuscular
i.v	Intravenous
K	Kwashiorkor
K_a	Rate of Drug Absorption
LASUTH	Lagos State University Teaching Hospital
L/Kg	Litre per kilogram
L/h	Litre per hour
L-R	Lactose-rhamose

LUTH	Lagos University Teaching Hospital
M	Marasmus subject
MeSH	Medical Subject Heading
mg	Milligram
mg/h	Milligram per hour
mg/kg	Milligram per kilogram
mg/kg/hr	Milligram per kilogram per hour
mg/L	Milligram per Litre
min	Minute
ml/h	Millilitre per hour
mmol	Millimole
M-K	Marasmic-kwashiorkor subject
MUAC	Mid Upper Arm Circumference
m/z	mass/charge ratio
M3G	Morphine-3-glucuronide
M5G	Morphine-5-glucuronide
N_2	Nitrogen
Na^+-K^+	Sodium-Potassium Pump
NAT	N-Acetyl Transferase

NCHS	National Centre for Health and Statistics
NG	Nasogastric tube
NH	Normal Healthy Subject
O_2	Oxygen
PEM	Protein Energy Malnutrition
PCM	Protein Calorie Malnutrition
PD	Pharmacodynamic
PK	Pharmacokinetic
PPH	Para-hydroxy-phenytoin
PR	Pressure Regular
r	Correlation coefficient
RDA	Recommended Daily Allowance
S.D/s.d	Standard Deviation
SGT	S-glutathione Transferase
SULT	Sulphotransferase
TB	Tuberculosis
TBW	Total Body Water
Tmax	Time to attain maximum concentration
$t_{½}$	Half Life

T_3	Triodothyronine
T_4	Thyroxine
U	Underweight
UDP	Uridyl-diphosphate
UDPGT/UGT	Uridyl-diphosphate glucuronyl transferase
UNICEF	United Nations Children Fund
VD	Volume of Distribution
Vmax	Maximal rate of metabolism
WHO	World Health Organization
WTWP	Wellcome Trust Working Party
XO	Xanthine oxidase
ln 2	Natural logarithm constant

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CHAPTER ONE:

INTRODUCTION

1.1. OVERVIEW OF MALNUTRITION

Malnutrition is a major disease that affects children worldwide. It is sometimes referred to as protein-energy malnutrition (PEM) and, at other times, as protein-calorie malnutrition (PCM). PEM is a common health problem, especially for children in developing countries whose inadequate nutritional intake derives from socioeconomic status (Abidoye & Sikabofori, 2000; WHO*,* 2004). The nutritional deficiency may be macronutrient (i.e., for proteins, carbohydrates, and fats), micronutrient (i.e., for electrolytes, minerals, and vitamins), or both (Müller & Krawinkel, 2005). Macronutrient deficiency rarely occurs alone; it is usually associated with micronutrient deficiency (Thanangkul *et al*., 1980). Since macronutrient deficiency is predominantly responsible for PEM (Müller & Krawinkel, 2005), PEM is thus typically accompanied by both deficiencies.

According to the World Health Organization (WHO), malnutrition is a spectrum of pathological conditions associated with protein and calorie deficiency in infants and young children (WHO, 2000). It is frequently associated with infections and commonly affects children of developing countries (de Onís *et al.,* 1993). Using the WHO's global database on child growth, de Onís *et al.* (1993) have more broadly defined malnutrition as 'the cellular imbalance between the supply of energy from macronutrients and micronutrients and the demand of the body for them in order to achieve normal growth, maintenance, and specific functions'. PEM ranges from underweight malnutrition (mild and moderate) to marasmus, marasmic-kwashiorkor, and kwashiorkor (severe) (Jahoor *et al.,* 2008).

1.2. EPIDEMIOLOGY

PEM is a prevailing global public health problem that affects children in African, Asian, Latin American, and Caribbean regions (de Onís *et al.,* 2000; Khor, 2003; Pelletier, 1994). Worldwide, approximately 800 million people were undernourished from 2000 to 2002; the majority of these people were living in developing countries (FAO, 2004). The prevalence of PEM is highest in South Asia and sub-Saharan Africa (FAO, 2004; Schofield & Ashworth, 1996).

According to WHO, 11 million children under the age of 5 years die each year, and over 90% of these deaths occur in developing countries (WHO, 2002). As a result, malnutrition is responsible for about half of all childhood deaths in developing countries (Murray & Lopez, 1997). It is responsible for the direct mortality of 300,000 deaths per year and the indirect mortality of at least half of all deaths of young children (Figure 1.1) (Fernandez *et al.,* 2002; Müller & Krawinkel, 2005).

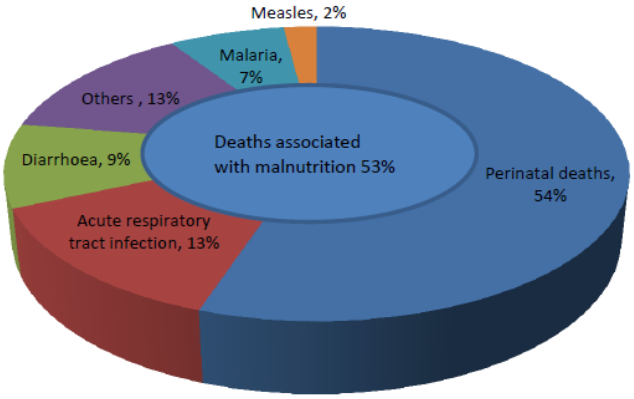


Figure 1.1: Causes of death among children under 5 years of age, 2000–2003, worldwide (Adapted from Muller & Krawinkel (2005) *CMAJ.* 173: 279-286)

PEM usually manifests in early childhood between the age of 6 months and 2 years. It is frequently associated with early weaning, delayed introduction

of complementary foods, intake of a low-protein diet, and recurrent severe infections (Kleinman *et al.,* 2003; Müller *et al.,* 2003).

Poverty is the primary cause of malnutrition. Factors associated with poverty are many and include poor socioeconomic status, high risk of infections, food insecurity, and inadequate household food (Müller & Krawinkel, 2005). These factors are also important determinants of severe malnutrition (Figure 1.2). The types of PEM and their frequency in a given population depend on factors such as political and economic situations, the level of education of the parents, environmental sanitation, seasonal and climatic conditions, food production, cultural and religious food habits, breastfeeding habits, the prevalence of infectious diseases, the existence and effectiveness of nutrition programmes, and the availability and quality of health services (FAO, 2004; Young *et al*., 2004).

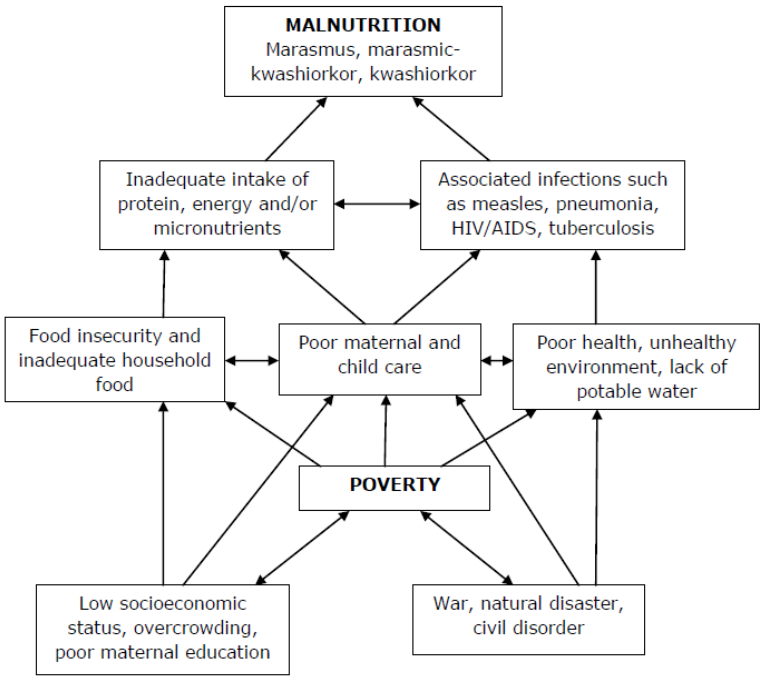


Figure 1. 2: Direct and indirect causes of malnutrition (Adapted from Muller & Krawinkel (2005) *CMAJ.* 173: 279-286).

1.3. CLASSIFICATION OF PROTEIN-ENERGY MALNUTRITION

Before WHO developed a unified method of classification for malnutrition, many methods had been used to describe a child's nutritional status. The various classifications were based on weight and height (Gómez *et al.,* 1956a; Jelliffe, 1966; McLaren & Read, 1975; Waterlow, 1972; Wellcome Trust Working Party, 1970; WHO, 1999), as well as the presence or absence of clinical signs of malnutrition.

There is no specific classification recommended for studies of drug disposition of children with PEM. However, in previous studies of drug disposition in children with PEM, the Gómez (1956a), Waterlow (1972), and Wellcome Trust Working Party (1970) classifications have been used more extensively than the WHO (1999) classification.

1.3.1. Gómez classification

The Gómez classification system is one of the first methods used to classify PEM. It is based on aged-related body deficit and compares the weight of a patient to that of a healthy child of the same age (Table 1.1).

Table 1.1: Gomez classification of malnutrition

Percentage of expected weight for age	**Interpretation**
> 90%	Normal
75- 90%	Grade 1 (mild malnutrition)
60- 75%	Grade 2 (moderate malnutrition)
< 60%	Grade 3 (severe malnutrition)

The method is useful for population screening and public health evaluations of malnutrition. Mathematically, the percentage of the expected weight for age = [(patient's observed weight) / (weight of a healthy child of the same age)] ×

100. A major limitation of the Gómez classification is its assumption that all children of a certain age are of the same weight. Also included in the classification are actively malnourished children and underweight children who had previously suffered malnutrition. The Gómez classification may, therefore, overestimate the prevalence of PEM.

1.3.2. Waterlow classification

Waterlow (1972) reported that the weight-for-height anthropometry could measure the current status of malnutrition in a child better than the weight for age. This classification compares the weight of a patient to that of a healthy child of similar height to determine the extent of wasting. It also compares the height of the patient to that of a healthy child of similar age to determine the extent of stunting (Table 1.2). The relationship is presented mathematically as a percentage of weight-for-height for the patient = [(weight of the patient) / (weight of a healthy child of similar height)] × 100, as well as the percentage of height-for-age for the patient = [(height of the patient) / (height of a healthy child of similar age)] × 100.

Table 1.2: Waterlow classification of malnutrition

Percentage of weight for height (wasting)	**Percentage of height for age (stunting)**	**Interpretation**
> 90%	> 95%	Normal (Grade 0)
80- 90%	90- 95%	Mild (Grade 1)
70- 80%	85- 90%	Moderate (Grade 2)
<70%	85%	Severe (Grade 3)

Stunting is a consequence of chronic malnutrition, while wasting is the effect of malnutrition on a large proportion of the body. The Waterlow classification is a very useful method of assessing the nutritional status of children in population-based studies.

1.3.3. Wellcome Trust Working Party classification

This Wellcome Trust Working Party (WTWP) classification is based on both the anthropometric measurement of the patient (using the Gómez classification) and a single clinical sign from physical examination. The percentage of body weight deficit in the presence or absence of oedema serves as the criterion for classification (Table 1.3).

Table 1.3: Wellcome Trust Working Party classification of malnutrition

Percentage expected weight for age (Gomez)	**Presence of oedema**	**Absence of oedema**
60- 80%	Kwashiorkor	Underweight
< 60%	Marasmic- kwashiorkor	Marasmus

According to the WTWP classification, the major diseases along the spectrum of severe PEM are marasmus, marasmic-kwashiorkor, and kwashiorkor (Jahoor *et al.,* 2008). Each disease in the spectrum presents with different clinical features. Thanangkul *et al.* (1980) have reported an experience with underestimating the diagnosis of kwashiorkor among Thai children, since many health workers wrongly classified kwashiorkor as marasmic-kwashiorkor because average Thai children were smaller than international reference standards. Thus, the WTWP classification tended to wrongly classify stunted but not wasted children as sufferers of severe PEM.

1.3.3.1. Marasmus

Marasmus is derived from the Greek word *marasmos,* which means 'wasting' (Scheinfeld *et al.,* 2010). Marasmus refers to a state of chronic nutritional deficiency resulting from inadequate intake of macronutrients (proteins, calories, and fats) and is characterised by wasting (Haider & Haider, 1984). It is an adaptive response to starvation from both protein and calorie deficiency (Scheinfeld *et al.,* 2010). During adaptation, all of the proteins and calories reserved in the body are depleted, which results in wasting of the skeletal muscle and the depletion of body fat. The major clinical features of marasmus are listed in Table 1.4.

Table 1.4: Clinical features of marasmus (Haider & Haider, 1984)

Marked weight loss resulting in prominent ribs, thinness of the body, marked loss of subcutaneous fat and muscle wasting
Slowing of linear growth
Impaired vital muscle functions resulting in behavioural changes such as irritability, apathy, decreased physical activity and social responsiveness
Dried, wrinkled and loose skin
The face looks older than the age due to a loss of buccal fat pads
Inconsistent cutaneous findings include: • *Fine, brittle hair* • *Alopecia* • *Fissuring of the nails.* • *Hairs are in the telogen (resting phase)rather than in the anagen (active phase): a reverse of normal* • *Occasionally, marked growth of lanugo hair may be present*
Decreased basal energy metabolism
Diarrhoea and vomiting may be present

1.3.3.2. Kwashiorkor

Cecily Williams, a British nurse, was the first to introduce the word *kwashiorkor* to medicine in 1933 (Williams, 1933). She took the word from the Ga language in Ghana, where it describes the illness resulting from improper weaning. Kwashiorkor now refers to the 'disease a first child gets when the second one is on the way' (Williams, 1933). Williams noted that kwashiorkor was dietary in origin but that infection, psychosocial problems, and cultural habits also played a significant role. Kwashiorkor is a consequence of physiological maladaptation to unbalanced deficiency of calories and proteins. Oedema is a major feature of kwashiorkor. It and other clinical features of kwashiorkor are presented in Table 1.5.

Table 1.5: Clinical features of kwashiorkor (Mukherjee & Jelliffe, 1955)

Constant or typical features • *Oedema* • *Failure to gain weight or growth retardation* • *Lethargy and apathy* • *Decreased muscle mass*
Usual presenting signs • *Moon face* • *Anaemia* • *Hypoproteinaemia* • *Changes in skin pigmentation* • *Hair changes in both texture and colour: depigmentation of hair causes it to be reddish yellow to white; curly hair becomes straightened; if periods of poor nutrition are interspersed with good nutrition, alternating bands of pale and dark hair, respectively, called the flag sign may occur; hairs may also become dry, lustreless, sparse and brittle, and can be pulled out easily.*
Occasional presenting signs • *Enlarged liver* • *Protruding abdomen* • *Cardiomyopathy and cardiac failure* • *Dehydration and shock from vomiting and diarrhoea* • *Features of avitaminosis: xerophthalmia from vitamin A deficiency, keratomalacia, glossitis, angular stomatitis, and cheilosis* • *Signs of infection due to impaired immunity* • *Signs of hypoglycaemia* • *Thin and soft, fissured or ridged nail plates* • *Skin changes and rashes ("mosaic skin" and "flaky paint")*

1.3.3.3. Marasmic-kwashiorkor

A mixed form of marasmus and kwashiorkor may exist where kwashiorkor is superimposed upon some degree of marasmus. Under these circumstances, the term *marasmic-kwashiorkor* is considered to be more appropriate. The condition is characterised by features of both marasmus and kwashiorkor and occurs when oedema is present in a chronically ill and starved patient due to superimposed stress. As a result, body fat stores, as well as somatic and visceral protein stores, become depleted (Haider & Haider, 1984). This condition is marked by impaired immunity, risk of infections, and a high mortality rate. Clinical features usually consist of the typical features of kwashiorkor and some features of marasmus.

1.3.4. Classification of malnutrition according to WHO

After recommending the use of a universal method to assess the nutritional status and growth of children, WHO's classification method harmonised all other classification systems of malnutrition. WHO's classification method uses a growth reference based on the *Z*-scores of the anthropometric measures of children (WHO, 1999). In defining normal child anthropometry, WHO considered global ethnic diversities in regards to diet, growth, and environmental and genetic differences, as well as exclusive breastfeeding for the first 6 months of life (Manary & Sandige, 2008).

The WHO classification of malnutrition is presented in Table 1.6. Assessment of the nutritional status of a patient is based on the weight-for-height and height-for-age *Z*-scores. This method compares the patient's weight to that of a healthy reference population of children of similar height. It also compares the patient's height to that of a healthy reference population of children of a similar age. The weight or height is usually expressed in a unit of

standard deviation (*SD*) and compared to the reference values for a paediatric population (WHO, 1999).

Table 1.6: Classification of malnutrition according to the World Health Organization

Index	**Classification**	
	Moderate malnutrition	**Severe malnutrition (type)**
Symmetrical oedema	No	Yes (oedematous malnutrition)**
Weight-for-height	-3 ≤ SD-score <- 2	SD-score < - 3 (severe wasting)
Height-for-age	-3 ≤ SD-score <- 2	SD-score < - 3 (severe stunting)

** Includes kwashiorkor and marasmic-kwashiorkor in older classifications. SD= standard deviation with reference to the median NCHS/WHO reference values for weight-for-height or height-for-age respectively (WHO Multicentre Growth Reference Study Group, 2006).

The calculated *Z*-scores are compared to the mean and *SD* of a reference population, thereby reflecting the reference distribution. As standardised quantities, *Z*-scores are comparable across ages, sexes, and anthropometric measures. *Z*-scores can be analysed as a continuous variable in studies, as well as quantify both under- and over-nutrition at both ends of the distribution. However, *Z*-scores are more useful in epidemiological studies than in clinical settings (Wang & Chen, 2012).

In some health facilities it may be difficult to measure the height of a severely malnourished child. Therefore, the mid-upper-arm circumference (MUAC) can be used in place of the weight-for-height *Z*-score. MUAC measures lean body mass and its value rarely changes between the ages of six months and five years. A MUAC of <110 mm has been used to predict the risk of death from malnutrition (Briend & Zimicki, 1986). The value also has been used to define severe malnutrition in a study of the Bangladeshi context (Briend *et al.,* 1986).

The WHO classification differentiates acute malnutrition into moderate or severe. Moderate malnutrition is defined as a weight-for-height *Z*-score between 2 and 3 standard deviations (*SD*) below the mean (WHO, 1999). Severe malnutrition occurs when the weight-for-height *Z*-score is below the mean for the general population by more than 3 *SD*, with a MUAC of <110 mm, or in the presence of nutritional oedema. Absence of nutritional oedema in moderate or severe malnutrition defines a child as marasmic, while when oedema is present, the child is said to have kwashiorkor.

1.4. PATHOPHYSIOLOGY OF MALNUTRITION

The pathophysiology of PEM is complex; its development is attributed to inadequate dietary intake, increased metabolic demands, or increased nutrient losses (Corish & Kennedy, 2000). The clinical features of PEM usually result from pathophysiological changes. An understanding of the pathophysiology is necessary to identify changes that may affect drug disposition in children with malnutrition.

1.4.1. Infection and immunity

The primary aetiology of malnutrition is the inadequate intake of macronutrients. However, the associated infections, particularly diarrhoeal disease and intestinal parasitosis, may play contributory roles (Müller & Krawinkel, 2005). An inadequate intake of macronutrients predisposes a patient to anorexia. It is also associated with intestinal mucosal damage, as well as decreased nutrient absorption, increased metabolic requirements, and direct nutrient losses (Brewster *et al.,* 1997a; Pinstrup–Andersen *et al.,* 1993). In such a case, the immune system is stimulated by infection that increases both catabolism and the demand for metabolically derived anabolic energy and associated substrates.

These increases may adversely affect the nutritional status of a patient and further increase susceptibility to infection. An inflammatory process is induced by sepsis and associated with a release of mediators, which further increases body catabolism, as demonstrated by an increased use of arginine. Arginine depletion is responsible for T-cell immune response impairment (Bronte & Zanovello, 2005). A negative nitrogen balance occurs after excessive endogenous arginine production (Kurpad, 2006).

Due to protein deficiency, immunity (lymphocyte functions) and innate host defence mechanisms (macrophages and granulocytes) are impaired in severe malnutrition. Reduced immune functions predispose malnourished children to infections such as bronchopneumonia (Chisti *et al*., 2009), tuberculosis (Vijayakumar *et al*., 1990), sepsis (Sunguya *et al*., 2006), and HIV/AIDS (Scrimshaw & SanGiovanni, 1997). Following nutritional rehabilitation, there is a delay in the recovery of immunological functions compared to an early recovery of anthropometric and biochemical parameters (Chevalier *et al.,* 1998).

Intestinal parasitic infestation and shigellosis are common occurrences in PEM (Bastow, 1982; Schaible & Kaufmann, 2007; Van den Broek *et al*., 2005). The effects of intestinal parasitosis on drug absorption have not yet been studied in severely malnourished children. In addition to malaria, bronchopneumonia and measles that frequently complicate PEM in African children may occur, as well as urinary tract infection, gastrointestinal tract infection, and septicaemia (Brewster, 2006; Nnakwe, 1995; Phillips & Wharton, 1968). Most bacterial infections in children are caused by Gram-positive and Gram-negative organisms and are usually implicated by infections in severe PEM (Noorani *et al.,* 2005; Purtilo & Connor, 1975; Shimeles & Lulseged, 1994).

1.4.2. Oedema and other body fluid distribution

Despite many years of research, the aetiology of kwashiorkor is still uncertain. The pathophysiology of kwashiorkor was initially based on the hypotheses of prolonged breastfeeding, inadequate protein intake, severe anaemia, presence of aflatoxins (Hendrickse, 1991), and reduced dietary antioxidants (Jahoor *et al.,* 2008). These hypotheses are no longer tenable, since the diets of marasmic children are known to have similar deficiencies (Lin *et al.,* 2007). Further research by Ciliberto *et al.* (2005) has shown that supplementing the diet of children at risk of kwashiorkor with proteins and antioxidants did not prevent the development of kwashiorkor. Golden (1982) has shown that a protein-restricted diet can lead to early oedema resolution in kwashiorkor. The presence of kwashiorkor in a paediatric population without evidence of aflatoxin ingestion and with evidence of aflatoxin in its member's post-mortem tissues has invalidated the hypothesis that implicated aflatoxin intoxication in the aetiology of kwashiorkor (Golden, 1998).

New potential mechanisms have been proposed for the formation of oedema in kwashiorkor. One mechanism is related to the release of water normally bound to glycosaminoglycans. Glycosaminoglycans are long-chain polysaccharides with repeated sulphated carbohydrate units that are bound to the short-protein core in all connective tissues and basement membranes throughout the body (Manary *et al.,* 2009). They contain oxidisable and reducible moieties that strongly bind water by cohesive forces. Abnormal renal architecture is responsible for the loss of glycosaminoglycans in children with kwashiorkor (Golden *et al.,* 1990). Glycosaminoglycans, in the form of heparan sulphate proteoglycans, are also lost in the intestines of children with kwashiorkor (Amadi *et al.,* 2008). The inability to retain the gel-like shape of

water, due to decreased glycosaminoglycans levels, has been attributed to be the cause of oedema in kwashiorkor (Manary *et al.,* 2009).

There are reports suggesting that synergistic or antagonistic effects of the gut microflora on human hosts occur as the microflora metabolically consume and process nutrients (Karasov & Carey, 2009). Swann *et al*. (2009) have reported the ability of gut microbiota to render some dietary toxins harmless. However, the bacteria may also produce toxic metabolites capable of damaging the vital organs such as the brain, liver, or kidneys (Michalke *et al.,* 2008). Manary *et al.* (2009) have postulated that kwashiorkor may be a consequence of either changes to the gut microbiota, which favours the production of metabolites causing insult and loss of integrity to the human cell membrane in malnourished hosts, or the disruption of the protective function of gut microbiota by environmental toxins.

Another hypothesis derives from a change in vanadium metabolism. Vanadium, like other micronutrients, may be deficient in the diet of children with kwashiorkor, thus leading to sodium retention (Anonymous, 1981; Golden & Golden, 1981). At physiological concentrations, vanadium is a powerful sodium pump inhibitor. The sodium pump is responsible for transportation across cell membranes and the reabsorption of sodium from the kidneys. An inability to inhibit the sodium reabsorption, either due to vanadium deficiency or the ionisation of its molecules to the inactive vanadyl ion, could result in salt and water retention. This retention will eventually lead to oedema formation. Burger and Hogewind (1974) have reported a low serum level of vanadium in children suffering from kwashiorkor.

The pathophysiology of kwashiorkor is broad and complex, for it affects most major organs and systems. The basic pathology in kwashiorkor is the

extensive damage to cell membranes throughout the body and an associated electrolyte imbalance. The damage results in a loss of potassium and water from all cell types, thus leading to dysfunction in all organ systems (Manary *et al.,* 2009). In such a case, an extreme reduction in whole-body intracellular ions (potassium, magnesium, and phosphorus) occurs (Golden, 1998). Life-threatening hypokalaemia and hypophosphataemia are more common in severe kwashiorkor. Despite hyponatraemia, total body sodium increases. Cardiac output becomes reduced to about 30% in kwashiorkor, while adrenal fractional sodium excretion is diminished by up to 70%.

Severe PEM may be complicated by dehydration and chronic hypovolaemia, as well as secondary hyperaldosteronism, all of which further aggravate fluid and electrolyte imbalance (Hansen *et al.,* 1965). Muscular dystrophy in PEM triggers the mobilisation of much of the body's potassium, which is then lost through urinary excretion. Therefore, affected children rarely show signs of hyperkalaemia (Brewster *et al.,* 1997b; Manary *et al.,* 2009).

1.4.3. Plasma proteins

Dietary protein is essential in providing amino acids for the synthesis of body proteins and other useful compounds necessary for various functions. Protein deficiency is a major problem of severe malnutrition. It is, however, more pronounced in kwashiorkor and marasmic-kwashiorkor than in marasmus (Thanangkul *et al.*, 1980). Nevertheless, the role of hypoproteinaemia in oedema formation in kwashiorkor and marasmic-kwashiorkor has not been completely ruled out (Heikens & Manary, 2009). Hypoproteinaemic oedema is primarily caused by both a decrease in the amount of proteins, especially albumins, in the plasma and by a drop in the colloidal osmotic, or oncotic,

pressure of plasma, the latter of which is accompanied by a release of fluid from the capillary bed into the tissues (Zweifach, 1972).

1.4.4. Changes in the gastrointestinal system

Diarrhoea and vomiting are two important gastrointestinal features of severe PEM. Various degrees of intestinal malabsorption related to food, sugar, and milk have been reported in severely malnourished children (Arroyave *et al.,* 1959; Gómez *et al.,* 1956b). Generally, the malabsorptive state disappears after recovery from nutritional rehabilitation (Shakir & Morley, 1974), but some of the morphological changes in the gastrointestinal system persist despite improved nutritional status (Schneider & Viteri, 1972).

Despite hepatomegaly in PEM, liver function tests remain normal (Porta & Hartroft, 1970; Tandon *et al.,* 1974). However, fatty changes, abnormal rough endoplasmic reticula and mitochondria, and decreased peroxisomes have been major findings in liver biopsies of severely malnourished children (Brooks *et al.,* 1992; Brooks *et al.,* 1994). The hepatic changes were completely reversed after nutritional rehabilitation.

1.4.5. Changes in renal function

Only a few studies have described the renal function of children with severe malnutrition. One study measured the serial clearance of inulin as a determinant of renal function and observed both a diminished renal blood flow and glomerular filtration rate (GFR), the latter of which was worsened by dehydration (Alleyne, 1967). However, both conditions reverted to normal after nutritional rehabilitation (Alleyne, 1967). Unfortunately, the time taken for the GFR to revert to normal was not determined in the cited study. Other studies used less precise measurements of renal function, such as creatinine clearance or serum creatinine, to study the effects of PEM on renal function. Results were similar to

those that used inulin (Klahr & Tripathy, 1966). Normal blood urea nitrogen and creatinine, an absence of glycosuria and proteinuria, and a lack of formed elements, such as casts, red blood cells, and epithelial cells in the urine of children with PEM, have also been documented (Klahr & Alleyne, 1973).

Despite the lack of evidence of established renal damage in severely malnourished children, the oedema observed in marasmic-kwashiorkor and kwashiorkor has been attributed to the inability of the kidneys to adequately excrete excess fluid and sodium (Alleyne, 1967). Other studies have attributed the oedema to the presence of hypoproteinaemia and aflatoxins (Hendrickse, 1991).

1.4.6. Changes in the cardiovascular system

Severely malnourished children have smaller and thinner hearts, which reduces stroke volume (Gillespie, 1999). The inability of the kidneys to adequately excrete excess fluid and sodium in children with nutritional oedema may also adversely affect the heart. Therefore, circulatory overload tends to occur more easily in children with marasmic-kwashiorkor and kwashiorkor. Oxidative stress is a potential cause of damage and leakage to the cell membrane of the heart (Gillespie, 1999). The Na-K pumps in the cell membrane are drastically reduced in malnutrition as a bodily adaptation affected to conserve energy. Consequently, intracellular sodium tends to accumulate, while leakages of potassium are enhanced, the latter of which leads to fluid and electrolyte imbalance (Gillespie, 1999).

Inappropriate fluid therapy may contribute to cardiac failure in acute severe malnutrition (Ashworth, 2001; Rabinowitz *et al.,* 2009). Circulatory insufficiency may be associated with a prolonged circulation time, inadequate absorption, and insufficient distribution of drugs and nutrients. Oedematous

malnutrition is characterised by sodium and water retention (Klahr & Alleyne, 1973) and may contribute to heart failure (Rabinowitz *et al.,* 2009). Fluid retention expands the extracellular fluid volume and increases the apparent volume of distribution of water-soluble drugs.

1.4.7. Changes in endocrine function

In acute, severe malnutrition, serum insulin levels were suppressed, while growth hormone (GH) was elevated among children suffering from kwashiorkor (Laditan, 1983). The values of growth hormones were also elevated in marasmic children, though the levels were much lower than those reported in children suffering from kwashiorkor. Studies have shown a steady fall in GH and a steady rise in insulin of severely malnourished children, especially those with kwashiorkor, after nutritional rehabilitation (Laditan, 1983). Brooks *et al*. (1992) have noted atrophic changes in the adrenal glands of children with malnutrition; however, these children exhibited elevated plasma cortisol concentrations that were unaltered by corticotrophin challenge. It was postulated that, since cortisol binds to serum proteins, hypoalbuminaemia would increase the free plasma cortisol level, thus contributing to the abnormal glucose tolerance and oedema seen in kwashiorkor and marasmic-kwashiorkor. The contrasting features of marasmus and kwashiorkor have been attributed to the variable response of the adrenal cortex in adapting to the diverse dietary stress that characterises marasmus and kwashiorkor (Rao, 1974).

Hypopigmentation of the hair and skin is a common feature of PEM and results from protein substrate (tyrosine) and coenzyme deficiency, the two major substances required for pigment synthesis (Collins *et al.,* 2006).

1.5. DIAGNOSIS OF PEM

The diagnosis of PEM is based on diet history, a thorough physical examination, and anthropometric measurements (Haider & Haider, 1984). Dietary history includes a 24 h food recall and a food frequency history (Joyce *et al.,* 1998). The physical examination should focus on the clinical features of the three types of severe malnutrition (marasmus, marasmic-kwashiorkor, and kwashiorkor) listed in Tables 1.4 and 1.5.

Anthropometric measurement is a key feature of nutritional assessment of children with malnutrition. The anthropometric measures most relevant to the classification of PEM are weight (kg), height (m), and MUAC, the last of which can be used instead of the weight-for-height *Z*-score to identify malnutrition when measuring height proves difficult (Briend & Zimicki, 1986).

Other causes of secondary malnutrition, such as HIV/AIDS, tuberculosis, cancer, liver disease, chronic renal failure, diabetes, pancreatic disease, and digestive or absorptive diseases, should be excluded during the process of diagnosing primary malnutrition (Haider & Haider, 1984).

Laboratory investigations of plasma urea, electrolytes, and creatinine; glucose, proteins, and lipids; full blood count and differentials; and blood film for malaria parasites are necessary, as well as urinalysis, urine microscopy, and examinations of blood culture sensitivity and of the stool for ova and parasites. However, most laboratory results for these tests prove normal (Rabinowitz *et al.,* 2009). WHO has also recommended laboratory investigations for associated diseases, such as HIV, pneumonia, and tuberculosis (WHO, 1999).

1.6. MANAGEMENT

Children with severe PEM are exclusively managed as inpatients (Collins *et al.,* 2006). While underweight children and children with moderate marasmus can be managed as outpatients, children with severe marasmus and kwashiorkor require inpatient treatment (Rabinowitz *et al.,* 2009).

Fluid and electrolyte imbalances are major problems of PEM. Hypokalaemia, hypocalcaemia, hypomagnesaemia, and hypophosphataemia are the most common electrolyte imbalances and should therefore be monitored and corrected (Whitehead & Alleyne, 1972). Hypoglycaemia should be corrected with intravenous or oral glucose administration. Hypothermia should be prevented by being covered with warm clothes, by avoiding cold baths, and by being nursed in a warm environment.

Broad-spectrum antibiotics (co-trimoxazole, chloramphenicol or ampicillin, and gentamicin) are used for the empirical treatment of infections stemming from malnutrition, even in the absence of typical symptoms since the inflammatory response to infection may be suppressed by PEM (WHO, 2000). However, specific infections should be treated with appropriate antibiotics based on the results of blood culture sensitivity pattern. Antimalarial (i.e., artemisinin-based combination) therapies are recommended for use in malaria endemic regions (WHO, 2006). Tuberculosis and HIV infection may coexist with PEM and require additional drug treatment.

Oral zinc supplements should be given in the cases of diarrhoea and skin ulcers in patients with malnutrition (Scheinfeld, *et al*., 2012). Multivitamin supplements are given daily to provide other micronutrients, such as iron, folate, and multivitamins.

Dietary therapy is important and requires the use of nutrient rich supplemental foods that will provide the recommended daily dietary allowance of all macronutrients (Manary & Sandige, 2008).

Table 1.7 shows a list of the necessary management steps for malnutrition.

Table 1.7: The WHO 10-step objectives of management of severe malnutrition (Ashworth *et al.,* 2004)

Treat or prevent hypothermia
Treat or prevent hypoglycaemia
Treat or prevent dehydration.
Correct electrolyte imbalance
Treat infection
Correct micronutrient deficiencies
Begin feeding and keep protein and volume load low
Increase feeding to recover lost weight
Stimulate emotional and sensorial development
Discharge when stable and monitor at the follow up clinic.

1.7. PRINCIPLES OF PHARMACOKINETICS AND PHARMACODYNAMICS

On the one hand, pharmacokinetics (PK) relates the effects of the body on a drug. PK usually involves the measurement and interpretation of drug concentrations in the systemic circulation (Rang *et al*., 2007). On the other hand, pharmacodynamics (PD) describes the action of a drug on the body. PD relates the events that occur following the binding of a drug to its receptor at the

primary site of action (Baca & Golan, 2012). Both pharmacokinetics and pharmacodynamics help to elucidate the relationship between the dose of and the response to a drug. The pharmacological response elicited by a drug is determined by the drug's binding to its site of action. The concentration of the drug that binds to the target site determines its effect.

This section focuses on the pharmacokinetic parameters: the determinants of the onset of action, the duration of action, and the intensity of the drug's effects. It also examines at the general relationship between a drug and its response, and emphasises how PK in children differs from that in adults.

Several qualitative and quantitative differences exist in the basic anatomy, physiology, and metabolic processes of children and adults, accounting for the variation in their PK (Ginsberg *et al*., 2004). Pharmacokinetic differences are responsible for the age-related variations in the dose and dosing intervals needed to maintain the therapeutic concentrations of many drugs. The paediatric population is divided into several age groups: premature neonates (born before 37 weeks gestation), neonates (less than 1 month old), infants (1to 24 months old), children (2 to 11 years old), and adolescents (12 to 17 years old) (Gennery, 2000).

Irrespective of the route of administration, the pharmacological actions and effects of a drug are influenced by four PK parameters: absorption, distribution, metabolism, and elimination (Thomson, 2000). The PK parameters enable clinicians to design and optimise treatment regimens in regards to route of drug administration, as well as appropriate dosage, dose frequency, and treatment duration.

The relationship between PK parameters and a drug's PD is presented in Figure 1.3. To produce the pharmacological effect at the site of action, a drug

must be absorbed and distributed to its target tissues. Thereafter, the drug is metabolised and excreted.

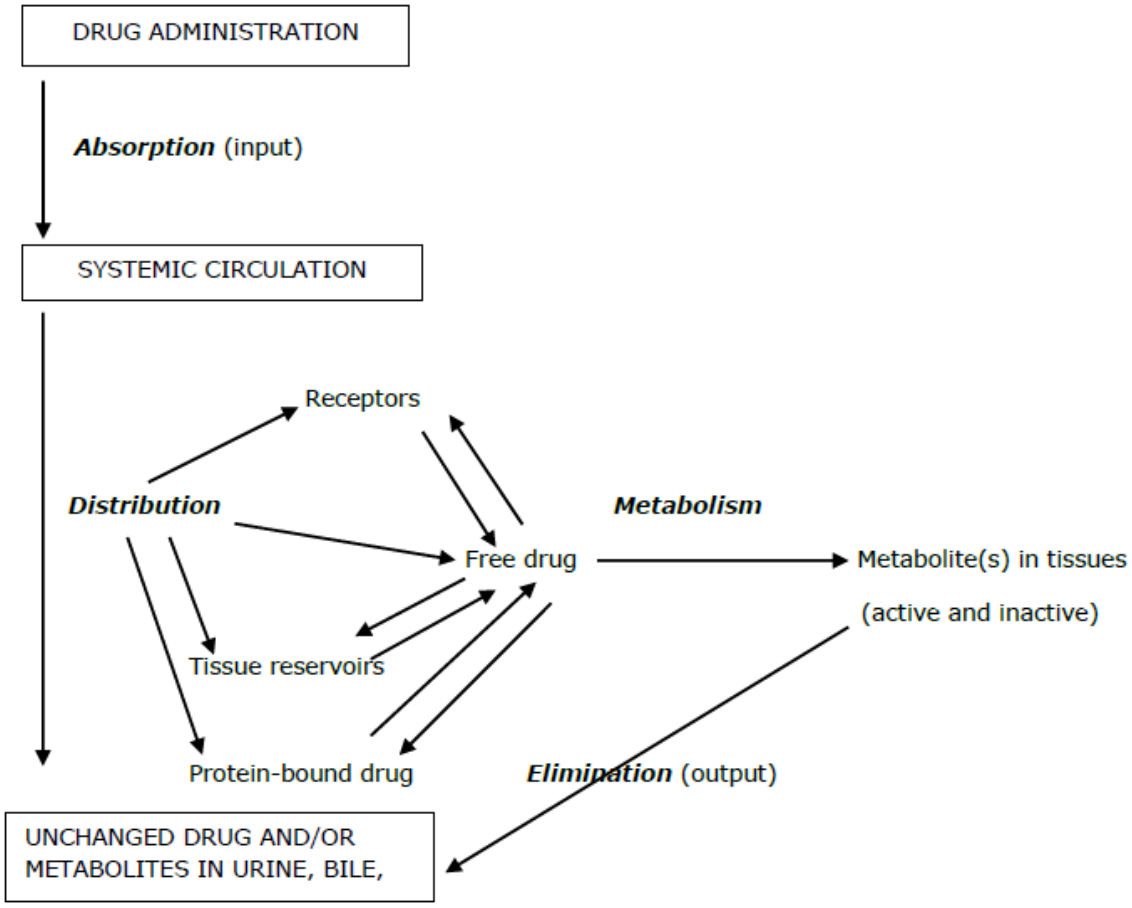


Figure 1.3: Drug absorption, distribution, metabolism, and elimination (Adapted from Baca & Golan (2012) in Principles of Pharmacology: The pathophysiologic Basis of Drug Therapy)

1.7.1. Drug absorption

The bioavailability of a drug (*F*) is one of the parameters that describe absorption. Bioavailability refers to the fraction of the administered drug dose that reaches the systemic circulation (Thomson, 2000). It is usually less than 100% for all routes of drug administration, excepting the intravenous route.

Traditionally, bioavailability is the ratio of the area under curve concentration (AUC) versus the time curve for a particular drug's administration

route to the AUC with intravenous route of administration (Rang *et al.,* 2007). This ratio assumes no changes in drug elimination following the two routes of administration. AUC (mg × hr × L^{-1}) represents the total amount of drug absorbed, defines the extent of absorption of a drug, and depends on a combination of dose, absorption, and clearance or elimination (Urso *et al.,* 2002).

Bioavailability is particularly relevant for orally administered drugs because many physiological processes in the gastrointestinal system tend to affect absorption. For oral drugs, bioavailability is AUC_{oral} / AUC_{iv} (oral = oral route; i.v = intravenous route). First-pass metabolism contributes to the low bioavailability of orally administered drugs. It is the process of hepatic biotransformation of a drug during its first passage through the portal circulation before reaching the systemic circulation (Pond & Tozer, 1984).

Other factors that may affect bioavailability include the physicochemical properties, formulation, and route of administration of a drug; concomitant drug therapy; blood flow and gastrointestinal motility; the activity of the intestinal drug-metabolising enzymes; vomiting and diarrhoea; disease states; and drug or food interactions (Thomson, 2000). Absorption is also influenced by bowel length, the permeability and maturation of the mucosal membrane, bile salt formation, intestinal microflora, and dietary composition (Berseth, 1992; Crom, 1994).

The acidity of the gastrointestinal system can influence both the stability and extent of ionisation of a drug. Invariably, this determines the proportion of drug available for absorption and its ability to traverse the cell membranes. In neonates, gastric pH, gastric motility, and intestinal motility are relatively increased (Crom, 1994; Dumont *et al*., 1994). The elevated gastric pH will

increase the absorption of acid-labile drugs, such as penicillin G, erythromycin, ampicillin, and amoxicillin, which results in a greater bioavailability in neonates than in infants, children, and adults (Benedetti *et al*., 2007; Brown *et al*., 1989; Huang *et al*., 1953). By contrast, weakly acidic drugs, such as phenobarbital and phenytoin, are readily absorbed in older children and adults, thereby suggesting a need for increased oral doses for younger children to achieve a therapeutic plasma concentration (Morselli *et al*., 1980; Wallin *et al*., 1974). Basic drugs, such as propranolol, are absorbed more rapidly in neonates, infants, and children than in adults (Bartelink *et al*., 2006).

Gastric emptying and intestinal motility are important factors that influence the rate of drug contact and absorption across the intestinal mucosa. Compared to older children and adults, neonates and young infants experience reduced gastric emptying and intestinal motility (Kearns *et al*., 2003).

Bile salt is required for the absorption of lipophilic drugs, such as diazepam (Serajuddin & Jarowski, 1985). Immature bile secretion and reduced activity of the bile salt and pancreatic juice contribute to the reduced absorption of fat soluble vitamins (i.e., vitamins D and E) in neonates and infants (Boehm *et al*., 1997; Heubi *et al*., 1982). The enzyme responsible for the conjugation of bile salt in neonates is immature and results in both reduced bile salt formation and decreased absorption of lipophilic drugs (Russell, 2003).

Age-related changes in splanchnic blood flow during the early neonatal period may alter the drug concentration gradient across the bowel mucosa, thus influencing absorption rates (Kearns *et al*., 2003). The activity of the intestinal drug-metabolising enzyme, glutathione-S-transferase, is decreased from infancy through early adolescence. Busulfan, a substrate of glutathione-S-transferase,

has a reduced oral clearance in adolescents and adults due to decreased activity of the enzyme (Gibbs *et al*., 1999).

The activity of gut microflora changes from infancy through childhood until adulthood. The gut microflora produces an enzyme that is responsible for the degradation of drugs such as digoxin. The activity of the enzyme is age dependent and higher in adults than in neonates, infants, and young children (Linday *et al*., 1987).

Intramuscular or subcutaneous injections are often erratically absorbed due to variable chemical properties of drugs, differences in the absorptive capacity of the injection site, variability in muscle mass among children, and the hydration status of the patient, the latter of which may result in circulatory failure (Ballard, 1968). Reduced skeletal muscle mass, blood flow, and the inability of the muscle to contract efficiently are responsible for the reduced intramuscular absorption of drugs in neonates (Kearns *et al*., 2003).

Enhanced transdermal absorption of drugs may occur in premature neonates and infants due to their thin outer skin layer (Rutter, 1987). Absorption can also be enhanced by their larger ratio of surface area to body weight (Amato *et al*., 1992; West *et al*., 1981), greater cutaneous perfusion, and increased epidermal hydration compared to adults (Fluhr *et al*., 2000; Okah *et al*., 1995). In children of any age, skin abrasions, dermatosis, and burns tend to increase percutaneous drug absorption.

Transrectal drug administration is useful when oral and intravenous administrations of drugs are impossible. This route is also useful for emergency administration of drugs such as diazepam. The immature hepatic metabolism in neonates and young infants tends to enhance the bioavailability of the drug.

However, expulsion of the drug may be increased in young infants compared to adults, due to the former's larger rectal contractions (Di Lorenzo *et al*., 1995).

1.7.2. Drug distribution

The absorbed drug is distributed into tissue reservoirs and bound to plasma proteins and receptors at the target sites, where it exists as a free or unbound drug in the systemic circulation (Baca & Golan, 2012). Only the bound fraction of the drug to the target site will produce a pharmacological effect. The process following drug absorption is dynamic and complex, because metabolism and excretion coincide with distribution.

The volume of distribution (VD), otherwise called the apparent volume of distribution, of the drug is the hypothetical volume of fluid within which the total drug administered must be distributed to achieve the measured plasma concentration (Thomson, 2000). VD is the ratio of the drug administered (dose) to the plasma concentration (C) of the same drug; VD = dose (mg) / C (mg/L). A large VD influences the half-life ($t_{½}$) of a drug, since its elimination depends on the amount of drug presented per unit of time to the organ of elimination or metabolism. A large VD suggests that a drug is distributed into the extravascular space and is thus unavailable to the excretory organs. Therefore, any condition that increases VD can result in an increase in the $t_{½}$ and prolong the action of the drug (Thomson, 2000). An excessively large VD suggests that a large amount of the drug is sequestered in some tissues or compartments.

The VD of drugs in children is age dependent (Friis–Hansen, 1983). Age-related changes are associated with changing body fluid composition, body fat distribution, and plasma protein binding. The total body water (TBW) is higher in premature neonates (80%) and full-term neonates (70%) than in infants and

adults (60%) (Puig, 1996). This condition requires the use of higher doses (mg/kg body weight) of water-soluble drugs in young children. However, lower doses are required in older children in order to avert toxicity. Age-related changes in the VD of water-soluble drugs has accounted for a relatively higher VD of gentamicin (0.5–1.2 L/kg) in neonates and infants (0.2–0.3 L/kg) than in older children and adults (Kearns *et al*., 2003). The fat content in neonates and young infants is very low and up to 10% to 15% body weight. This condition accounts for the relatively low VD of diazepam in young children compared to adults (Bartelink *et al*., 2006).

The extent to which a drug is distributed into tissues depends on the binding capacity of plasma proteins and tissue binding. Albumin, α_1-acid glycoprotein, and lipoproteins constitute the plasma proteins that bind drugs and limit free drug distribution throughout the body. Acidic drugs bind extensively to albumin, while basic drugs bind extensively to α_1-acid glycoprotein, lipoproteins, or both. Physiological hypoproteinaemia and hypoalbuminaemia occur in neonates and young infants, which results in an increased unbound fraction of drugs. Both the total plasma protein and albumin concentrations approach adult levels during late infancy (Ehrnebo *et al*., 1971). Foetal albumin may persist in neonates and reduce the binding capacity of albumin for weakly acidic drugs (Kearns *et al*., 2003). The plasma levels of endogenous substances, such as bilirubin and free fatty acids, are high in neonates and infants (Fredholm *et al*., 1975). These substances compete with weakly acidic drugs for albumin binding sites, which results in increased plasma-free drug concentrations, prolonged pharmacological effects, and an increased potential for adverse effects. The bilirubin-displacing effect of highly protein-bound drugs, such as sulphisoxazole, has been responsible for the high incidence of kernicterus and mortality reported

in premature infants receiving prophylactic treatment with penicillin and sulphisoxazole (Silverman *et al.,* 1956).

1.7.3. Clearance

Clearance (CL) is the volume of plasma completely cleared of a drug by an organ of elimination (kidneys, liver, bile duct, and others) per unit of time (Toutain & Bosquet–Mélou, 2004). Plasma CL, otherwise called total, systemic, or body CL, refers to the volume of plasma cleared of a drug per unit of time from all elimination systems. CL is dependent on the extraction ratio (*E*) and the rate of blood flow.

Extraction ratio describes the extent to which an organ contributes to drug clearance. It measures the relative efficiency with which an organ clears a drug from the systemic circulation over a single pass through the organ. *E* is thus the ratio of the difference in concentration of a drug immediately before entering (C_{in}) and just after leaving (C_{out}) the organ to the concentration entering the organ. Mathematically, $E = (C_{in}-C_{out}) / C_{in}$ and generally it is in the range of 0 to 1. An organ that contributes substantially to drug CL has an *E* close to 1, while *E* of an organ that has no substantial contribution is be close to zero. Changes in blood flow will produce corresponding changes in the CL of drugs with a high *E*.

The rate of drug delivery to an organ of elimination is dependent on blood flow rate. Therefore, CL (volume/time) has the unit L/h or mL/min. The principles of CL are essential in designing dosage regimens in clinical practice as they relate drug input rate to the maintained level. A drug administered as an infusion at a constant rate will produce a gradual increase in plasma concentration until a steady state (constant plasma concentration) is achieved. At this stage, the rates of drug administration (infusion rate) and elimination are

equal, and the CL becomes the constant that relates the dosing rate to the target steady state concentration (C_{ss}). Therefore, dose rate (mg/hr) = target C_{ss} (mg/L) × CL (L/hr). Altering the dose changes the steady state concentration (Thomson, 2000).

The above rules for infusion drug dosing also apply to other modes of drug administration. In the case of multiple oral doses, dosing rate is substituted for infusion rate, and C_{ss} average (i.e., the mean steady state concentration over the dosage interval) replaces C_{ss}. The steady state concentrations for other routes of administration will change in direct proportion to changes in dose rate. Once C_{ss} is achieved, subsequent doses are those required to replace only the amount of drug that is lost through metabolism and excretion. The maintenance dose rate of a drug is dependent on the drug CL, target C_{ss} average, and dosage interval. Therefore, maintenance dose = target C_{ss} average × CL (Thomson, 2000).

Many clinical and pharmacokinetic factors or concomitant medications can influence CL (Thomson, 2000). These factors may necessitate altering drug dose requirements and include weight of the patient in kilograms or body surface area (Holford, 1996). Other influential factors include hepatic or renal disease, altered protein binding capacity, co-administration of drugs that induce (rifampicin, carbamazepine, and phenobarbitone) or inhibit (cimetidine and ciprofloxacin) hepatic metabolising enzymes, and cardiac or respiratory disease (Thomson, 2000).

1.7.4. Elimination half-life

The rate of drug elimination is proportional to the amount of the drug present in the body, which suggests that a fixed proportion of the drug is eliminated per

unit of time. The decline in concentration-time curve of the drug is exponential. The elimination rate constant (*k*) is dependent upon the volume of fluid cleared per unit of time (clearance) and the volume to be cleared (VD) (*k* = CL [L/hr] / volume [L]) (Thomson, 2000).

The elimination half-life ($t_{½}$) refers to the time required for a plasma concentration of a drug to decrease to one-half of its original value (Toutain & Bousquet–Mélou, 2004). The elimination $t_{½}$ is dependent on the VD and CL. These parameters are related by the equation $t_{½} = \ln 2 \times VD/CL$, ln 2 = 0.693 (natural log constant). Therefore, $t_{½} = 0.693/k$.

The time taken to achieve C_{ss} with multiple-dosing regimens is also dependent on $t_{½}$. The half-life can be used to determine the appropriate dosage interval required to achieve a target concentration time profile.

1.7.5. Drug metabolism

Many organs can metabolise drugs in humans, including the kidneys, liver, gastrointestinal tract, lungs, brain, and skin. The hepatic modification of drug molecules that renders them inactive or facilitates their elimination is referred to as biotransformation, the consequences of which are either (a) activation of an inactive precursor to a pharmacologically active drug (e.g., conversion of L-dopa to dopamine); (b) the further activation of a pharmacologically active drug to another active drug (e.g., conversion of diazepam to oxazepam); or (c) the conversion of a pharmacologically active drug to an inactive drug (e.g., phenobarbital to hydroxylated metabolites). The products of biotransformation are often more water soluble than the original drugs. The reactions involved in drug biotransformation are classified into either phase I (oxidation/reduction reactions) or phase II (conjugation reactions).

The cytochrome P-450 (CYP450) enzymes are involved in phase I reactions, while epoxide hydrolases, glutathione S-transferases, UDP-glucuronosyltransferases, and N-acetyltransferases are involved in phase II reactions. All the enzymes are present in the liver, as well as the intestinal mucosa, kidneys, lungs, brain, and skin (Krishna *et al*., 1994). However, they are mainly concentrated in the liver, the principal site of drug metabolism. Both phase I and II metabolising enzymes constitute the mixed function oxidase and are primarily involved in the metabolism of several drugs in the liver.

On the one hand, phase I reactions include oxidation, reduction, and hydrolysis, all of which may result in the increased, decreased, or unaltered pharmacological activity of a drug. In all phase I reactions a functional group on the drug is either introduced or unmasked to render them polar. On the other hand, phase II reactions consist of synthetic or conjugation processes during which endogenous substrates, such as glucuronic acid and glutathione, are combined with the functional groups derived from phase I reactions to produce a highly polar drug conjugate. Most phase II reactions result in decreased pharmacological activity.

Many drugs first undergo the sequential process of phase I reactions and then the phase II reactions. However, the product of phase I reactions may be sufficiently polar to thus become directly eliminated without the need for phase II reactions. In some instances, however, phase II reactions may precede phase I reactions. Furthermore, a parent drug may be eliminated though unchanged and without having undergone any biotransformation.

Both phase I and II processes involved in drug biotransformation undergo developmental maturation. Some of the metabolic pathways may therefore be more active in children than in adults. The developmental changes affecting the

isoforms of CYP1A; CYP2A, B, C, D, and E; CYP3A; flavin monooxygenase (FMO); and alcohol dehydrogenase (ADH) in phase I drug metabolism have been well documented (Hines & McCarver, 2002). CYP3A isoforms metabolise about 50% of drugs and xenobiotics during phase I reactions, while conjugation via the glucuronic or sulphation pathway predominates during phase II reactions. The developmental changes in the activity of CYP3A, CYP2C, and CYP1A isoforms, as well as UGTs, affect phase I and II drug metabolism.

1.7.5.1. Developmental changes in phase I drug metabolism

Developmental changes in the activity of metabolising enzymes are complex and dynamic. The effect of age on drug metabolism varies throughout the developmental period that spans from birth to adolescence (De Wildt *et al.,* 2003). The total content of CYP450 enzymes in the foetal liver is 30% to 60% of the adult value, which is typically achieved at 10 years of age (Shimada *et al.,* 1996). However, children's enzymatic activity exceeds adult values by the age of 2 to 3 years (Morselli *et al*., 1980; Stewart & Hampton, 1987). Therefore, young children are more able than adults to metabolise drugs via CYP450 dependent metabolic pathways. By puberty, CYP450 enzymatic activity decreases to adult levels.

Pharmacokinetic studies of drugs metabolised predominantly by different CYP450 enzymes, including CYP1A, CYP2C, and CYP3A, suggest an increase in the enzymes' activity with age. The activity of CYP3A4, for instance, is very low at birth but increases with age and reaches adult levels during early infancy (De Wildt *et al*., 2003). The effect of developmental changes in the drug metabolism of CYP3A4 and CYP3A5 has been demonstrated in the CL of intravenous midazolam. Adequate sedation of critical care patients taking midazolam

requires higher infusion rates for older children (De Wildt, 2011) than for preterm neonates (Burtin *et al*., 1994). Another study has showed that children 1 to 4 years old require a higher rate than the maximal recommended rate (0.3 mg/kg/h) when compared to older children (de Gast–Bakker *et al*., 2007). These findings suggest faster CL in children than in neonates, which results in lower midazolam concentrations.

Phenytoin is metabolised predominantly by hepatic CYP2C9 and CYP2C19 enzymes to its major inactive metabolite, para-hydroxy-phenytoin (HPPH) (Bajpai *et al*., 1996). The mean maximal rate of metabolism (V_{max}) of this drug is significantly higher in young children than in adults (Blain *et al*., 1981), progressively decreases during childhood, and reaches adult values after puberty (Anderson, 2000).

CYP1A2 constitutes about 13% of the total CYP450 expression in adult human livers (Shimada *et al.,* 1994). Caffeine is an acceptable probe substrate for the *in vitro* and *in vivo* study of CYP1A2 activity (Tucker *et al.,* 2001). Some *in vitro* studies involving the use of specific immunological probes or a polymerase chain reaction have been unable to detect CYP1A2 expression in specimens of foetal livers at 11 to 24 weeks gestation (Shimada *et al.,* 1996; Yang *et al.,* 1995). This finding has been further corroborated by another study, which demonstrated the absence of CYP1A2 expression during foetal and neonatal periods (Sonnier & Cresteil, 1998). However, increased CYP1A2 expression in infants 1 to 3 months old, as well as a further increase of up to 50% of adult levels in older children, was documented in the same study by Sonnier & Cresteil (1998).

Cazeneuve *et al.* (1994) have reported a significantly lower 1, 3, 7-*N*-demethylation of caffeine in the liver microsomes of foetuses, neonates, and

infants than in those of adults. They also reported that the rate at which foetal microsomes generated the total caffeine metabolites (i.e., dimethylxanthine, paraxanthine, and theobromine) increased with gestational age. Since only CYP3A has been previously identified in human foetal livers, it has been concluded that caffeine metabolism depends on CYP3A during foetal development.

In a study examining the maturation pathways of caffeine during infancy, it was reported that the total caffeine demethylation, as well as *N*3 and *N*7 demethylation, increased exponentially with postnatal age and plateaued by 4 months (Carrier *et al.,* 1988). *N*-1-demethylation was not affected by postnatal age, which suggests a delay in its maturation until reaching 19 months of age. 8-hydroxylation was observed in early and late infancy, which suggests that this pathway reaches maturity at an age as early as 1 month. Pharmacokinetic studies of caffeine disposition (Lee *et al.,* 1997; Thomson *et al.,* 1996) have shown more reduced CL in preterm neonates than in term neonates and infants (Pons *et al.,* 1988a; Pons *et al.,* 1988b). Caffeine CL was significantly influenced by the postnatal age and body weight of new-borns. Studies involving pharmacokinetics of caffeine and CYP1A2 phenotyping have demonstrated lower *N*-3-demethylation activity via CYP1A2 metabolic pathway (Pons *et al.,* 1988a; Pons *et al.,* 1988b) in neonates than in older children (El–Yazigi *et al.,* 1999) and adults (Aranda *et al.,* 1979).

1.7.5.2. Developmental changes in phase II drug metabolism

Phase II metabolism is considerably important, for it is concerned with the excretion of a number of drugs and other xenobiotics as conjugates. Developmental changes affecting the body's ability to conjugate drugs may

therefore result in drug toxicity or an adverse effect. Similar to those of phase I metabolism, phase II metabolic pathways are poorly expressed during the foetal and neonatal periods (McCarver & Hines, 2002). The predominant phase II metabolism involves the conjugation reactions; the most studied pathway is glucuronidation.

The ontogeny of hepatic glucuronidation may influence the CL of a number of drugs in children. The UDP-glucuronyltransferases (UGTs) are part of the important phase II metabolising enzymes that have been extensively studied in regards to developmental changes (Miyagi & Collier, 2011). Several drugs, including morphine, paracetamol, propofol, and chloramphenicol, undergo glucuronidation (De Wildt *et al.,* 1999). These drugs are primarily metabolised via glucuronide conjugation pathways by one or different UGT isoforms, and some may undergo sulphate conjugation by sulphotransferases (SULT-1 and SULT-2 isoforms). The ontology of sulphate conjugation varies with different drugs, thus the effect of developmental changes on the metabolism of paracetamol, propofol, chloramphenicol, and other drugs metabolised via the same pathway may not be uniform.

UGT1, UGT2, UGT3, and UGT8 are the four recognised UGT families capable of catalysing glucuronidation. The members of the UGT1 and UGT2 families are mainly involved in compound detoxification. The UGT1A and UGT2B subfamilies have been the most extensively studied in regards to human glucuronidation reactions.

Morphine is metabolised by the UGT2B7 enzyme to form two metabolites: morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). While the former is the major metabolite and an inactive compound, the latter has some analgesic properties. Sulphate conjugation of morphine has been documented as

a minor metabolic pathway, for it does not contribute to the overall CL of the drug (Choonara *et al.,* 1990). Choonara *et al.* (1990) also documented significantly higher UGT2B7 activity in premature neonates than in children, according to measurements in terms of the ratio of morphine-3-sulphate to morphine in the plasma. This result suggests that morphine sulphation decreases after the neonatal period. However, other studies have documented contrasting results, in which morphine CL was lower in neonates than in young children (McRorie *et al.,* 1992), older children, and adults (Hunt *et al.,* 1999).

UGT1A1 is the enzyme that predominantly metabolises bilirubin. Its expression in the foetal liver is triggered by processes associated with birth, and the levels of activity similar to adults' are attained at 3 to 6 months (Burchell *et al.,* 1989). However, Burchell *et al.* (1989) detected UGT1A3 activity in both foetal and neonatal liver at levels 30% of those observed in adults. This result suggests developmental changes in the expression of UGT1A enzymes.

Paracetamol is another drug metabolised by other UGT isoforms. Its major metabolism is achieved by conjugation via the UGT1A6 metabolic pathway, while metabolism via UGT1A9 and sulphation are its minor pathways (Miller et al., 1976). UGT1A6 is absent in the foetus yet slightly increased in neonates and attains adult levels after 10 years of age (Alam *et al.,* 1977; Rollins *et al.,* 1979). Data from few pharmacokinetic studies have also suggested a higher clearance of paracetamol in neonates, especially in preterm neonates (Allegaert *et al.,* 2004; van Ligen *et al.,* 1999) than in children and adolescents (Würthwein *et al.,* 2005) who invariably have adult CL values. The ratio of glucuronidation to sulphation in the metabolism of paracetamol is significantly lower in children than in adults (Alam *et al.,* 1977). This result suggests that sulphation possibly plays an important role in the metabolism of paracetamol, which contrasts its

minor role in morphine metabolism. Except in paracetamol metabolism, sulphation is typically low and may be unavailable as an alternative metabolic pathway in circumstances when glucuronidation is developmentally low. This is the basis for the development of adverse reactions to chloramphenicol therapy that manifest as gray baby syndrome in neonates. This condition has been attributed to the delayed onset of the UGT2B7 enzyme required for the glucuronidation of chloramphenicol (Becker & Leeder., 2010; Chen *et al.,* 2010), as well as to the lack of alternative metabolic pathway. Consequently, the drug accumulates in the serum and tissue to exert adverse effects (Weiss *et al.,* 1960).

1.7.5.2. Effects of pharmacogenetics on drug metabolism

Pharmacogenetic studies suggest substantial inter-individual variation in the activity of many of the CYP450 enzymes, including CYP3A4, CYP2C9, and CYP1A2 (Lynch & Price, 2007), which may result in 2-to 3-fold variability in the CL of some drugs in children and adults, even when there are no confounding factors that might influence drug metabolism. Since metabolising enzymes are genetically determined and may be absent in some individuals, a defective metabolic pathway may result in individuals who are poor or intermediate metabolisers of numerous drugs (Relling *et al*., 1992). The two phenotypes appear in a considerable proportion of the human population and can result in significantly reduced CL and high drug concentrations in individuals who are deficient in the enzyme required for the drug metabolism. At the same time, normal therapeutic dosages in poor drug metabolisers can result in drug toxicity. Ultra-rapid metabolisers may mean that normal dosage produces insufficient therapeutic concentrations.

Carbamazepine, which is commonly used for children, is a substrate of the CYP3A5 enzyme. Among adults with epilepsy, three alleles of CYP3A5 have been identified and grouped as CYP3A5 expressers (CYP3A5*1/*1 and CYP3A5*1/*3) and CYP3A5 non-expressers (CYP3A5*3/*3). Oral CL of carbamazepine has been documented in adult Koreans with epilepsy; it was significantly higher in CYP3A5 non-expressers than in CYP3A5 expressers (Park *et al.,* 2009). CYP1A2 is substantially involved in the metabolism of drugs such as clozapine, imipramine, caffeine, theophylline, phenacetin, fluvoxamine, paracetamol, tactrine (Levien & Baker, 2003), artemisinin, and thiabendazole (Bapiro *et al*., 2005). Clinical studies of CYP1A2-dependent metabolism of caffeine have demonstrated polymorphic control of the enzyme activity (Basvi *et al*., 2007; Sachse *et al*., 2003). Genetic polymorphisms that can cause changes in the enzymatic activity of the CYP1A2 enzyme have been identified in the wild-type allele (CYP1A2*1); they are the CYP1A2*1C allele, which results from a single point mutation (-3860 G > A), and the CYP1A2*1F allele, which results from a single point mutation (-163C > A). Knowledge of genetic variations in CYP1A2 metabolising activity is therefore useful for identifying individuals who are at risk of adverse drug toxicity with conventional doses of drugs exclusively metabolised by the CYP1A2 enzyme. Individuals who express CYP1A2*1F or CYP1A2*1C variants may demonstrate different pharmacokinetics for different CYP1A2 substrates than normal individuals. Consequently, these individuals may require dose modifications of drugs that are exclusively metabolised by the hepatic CYP1A2 enzyme. Conversely, alternative drugs metabolised independently of the CYP1A2 enzyme may be preferred for patients with potentially impaired CYP1A2 metabolic activity in order to avert adverse drug toxicity.

1.7.6. Drug excretion

Water-soluble drugs are excreted primarily by the kidneys. Though the biliary system contributes to drug excretion, it does so to a lesser degree than the renal system. This finding ensures that the re-absorption of drugs in the gastrointestinal system does not occur. Drugs can also be excreted through breast milk and may affect breastfed infants (Wilson *et al*., 1980).

Hepatic metabolism contributes to the CL and excretion of drugs by increasing the polarity and hydrophilicity of the drugs, which renders the resultant metabolites to be more readily excreted. Renal diseases and compromised or reduced hydration status can also cause reduced renal drug excretion.

Renal excretion of drugs depends on glomerular filtration rate (GFR), as well as tubular secretion and reabsorption. GFR is influenced by renal blood flow (RBF), an age-dependent factor. RBF is 5% to 6% of the cardiac output at birth, increases from 15% to 25% in infancy, and attains adult values during early childhood (Alcorn & McNamara, 2002). The elimination of drugs, such as gentamicin and amikacin, which are exclusively excreted unchanged by the kidneys, depends on GFR efficiency. Neonates and young children are at risk of aminoglycoside toxicity due to their reduced GFR (Zarowitz *et al*., 1992). Ceftazidime and famotidine are excreted exclusively by the kidneys; their plasma CLs are known to correlate with appropriate maturational changes in renal function (James *et al*., 1998; van den Anker *et al*., 1995).

Renal tubular secretion plays an important role in the excretion of drugs such as digoxin (Steiness, 1974). Renal tubular secretion capacity increases from birth through neonatal period and attains adult levels during late infancy (Linday *et al*., 1981). Amiodarone inhibits renal tubular secretion, thus

increasing the plasma concentration of digoxin in infants more than in adolescents and adults (Koren *et al*., 1984).

Polar compounds, consisting mainly of drug metabolites, are not re-absorbable. Drugs in their un-ionised forms are readily reabsorbed from the tubules. Urine pH (4.5–8.0) can influence drug reabsorption and excretion given their potential to ionise. Acidification of urine enhances reabsorption and diminishes the elimination of weakly acidic drugs, decreases reabsorption, and increases the elimination of weakly basic drugs. The opposite effects are produced by urine alkalinisation.

1.8. EFFECTS OF MALNUTRITION ON DRUG DISPOSITION

The nutritional status of individuals, dietary constituents, and many diseases associated with malnutrition (e.g., cancer and HIV) significantly influence the pharmacokinetics and pharmacodynamics of a drug (Kufe *et al*., 2003; Mukonzo *et al*., 2011; Walter–Sack & Klotz, 1996). Infectious diseases, such as malaria, often complicate severe malnutrition and significantly impact the disposition of drugs such as caffeine (Akinyinka *et al*., 2000) and quinine (Pussard *et al*., 1999).

Pathophysiological changes in malnutrition are enormous and complex and affect all organs concerned with drug disposition (Whitehead & Alleyne, 1972). Although some of these changes are adaptive, alterations in the pharmacokinetics and pharmacodynamics of drugs have also been reported (Mehta, 1983). This section thus discusses the impact of pathophysiological changes in malnutrition on the absorption, distribution, metabolism, and excretion of drugs in children.

1.8.1. Drug absorption

Reduced gastric acid secretion, delayed gastric and bowel emptying time (Reddy *et al*., 1976; Shaaban *et al.,* 2004), intestinal mucosa atrophy (Brewster, 2006a; Gilman *et al.,* 1988; Schneider & Viteri, 1972), overgrowth of gut microflora, reduced peristalsis, and intestinal blood flow (Mata *et al*., 1972) have been reported in all types of severe malnutrition. All of these factors may affect the oral absorption of drugs in children with malnutrition. These factors have also been implicated in the reduced oral absorption of carbamazepine (Bano *et al.,* 1986), phenobarbitone (Syed *et al*., 1986), chloroquine (Walker *et al.,* 1987), sulphadiazine (Mehta *et al.,* 1980), and chloramphenicol (Eriksson *et al.,* 1983a; Mehta *et al*., 1975; Samotra *et al*., 1986).

Decreased muscle mass and body fat in malnourished children can impact the absorption of intramuscular drugs. Studies of the effects of malnutrition on the absorption of drugs administered intramuscularly have reported that the absorption rate of quinine (Treluyer *et al*., 1996) was significantly decreased in malnourished children who were undergoing treatment for malaria. By contrast, kwashiorkor had no effect on plasma concentrations of tobramycin following intramuscular injection (Buchanan & Eyberg, 1978).

1.8.2. Drug distribution

Alterations in plasma protein levels, body fluid composition, and tissue fat and protein occur in malnourished children, and these altered parameters can influence drug plasma CL and VD. Protein synthesis is decreased as indicated by decreased serum albumin, prealbumin, and lipoprotein levels (Treluyer *et al*., 1996). By contrast, globulin and α-1-acid glycoprotein synthesis are increased (Pussard *et al*., 1999). Decreased plasma protein, particularly albumin, results in an increase in the unbound fraction of drugs. Changes in the plasma levels of an

unbound drug (f_u) may increase its VD, and the unbound drug may distribute into the tissues, which leads to a decrease in the plasma concentration profile. For rapidly metabolised drugs, CL depends on the hepatic blood flow. However, changes in the f_u of slowly metabolised drugs may directly change CL. Theoretically, acidic drugs, such as phenytoin, carbamazepine, and acetylsalicylate, which bind to plasma proteins, may exhibit potential toxicity in malnourished children when taken at the normal recommended dose.

Total body water (TBW) increases proportionally to the degree of malnutrition (Buchanan & Eyberg, 1978) and is highest in marasmus (Rabinowitz *et al*., 2009). In addition to increased TBW, there is a proportionate increase in the extracellular fluid (ECF) in kwashiorkor due to oedema formation (Buchanan & Eyberg, 1978).

Changes in body fluid may result in changes to the VD of hydrophilic drugs. Oedema, generalised dermatosis, and capillary leakage due to infection are common characteristics of kwashiorkor (Whitehead & Alleyne, 1972). These pathologies contribute to the increased ECF in kwashiorkor and account for the increased volume of distribution and decreased CL of aminoglycosides, such as gentamicin (Bravo *et al*., 1982; Buchanan *et al*., 1979a) and tobramycin (Buchanan & Eyberg, 1978).

Earlier studies have suggested a decrease of adipose tissue in malnourished children (Garrow *et al*., 1965; Waterlow & Mendes, 1957). However, recent body composition studies have shown that malnourished children accumulate more body fat than lean body mass (das Neves *et al*., 2006; Martins *et al*., 2004). Theoretically, the VD of lipid-soluble drugs increases and can result in a decrease in the plasma levels of drugs. Clinically, normal

therapeutic doses of lipophilic drugs may not achieve the desired effects in malnourished children.

1.8.3. Drug metabolism

Biotransformation of drugs occurs mostly in the liver via the microsomal enzyme pathways. The activities of the enzymes for the metabolic pathways may be impaired in PEM. Although many animal studies have reported alterations in the activities of hepatic metabolising enzymes (Narang, 1987; Reen *et al*., 1999), few studies have reported findings regarding malnourished children.

The activity of the bilirubin-uridyldiphosphate (UDP) enzyme, which is responsible for the hepatic metabolism of chloramphenicol, is significantly decreased in children with severe malnutrition (Mehta *et al.,* 1975). The plasma level of paraxanthine, a metabolite of caffeine, was indirectly used to measure the activity of CYP1A2 after caffeine administration to children with kwashiorkor (Akinyinka *et al.,* 2000). CYP1A2 activity, as well as the hepatic clearance of caffeine, was significantly decreased.

A study of the activities of aminopyrine *N*-demethylase, which is responsible for the oxidation of drugs such as antipyrine, and bilirubin UDP-glucuronyl transferase, which is responsible for conjugation of drugs such as chloramphenicol, showed severe impairment of the oxidative process and less impairment of the conjugation process in malnourished rats (Narang, 1987).

Acetylsalicylic acid is metabolised by UDP-glucuronosyltransferase 1A6 (UGT1A6) and cytochrome P450 2C9 (CYP2C9) (van Oijen *et al*., 2005). A study of acetylsalicylic acid in malnourished children with autoimmune disease suggests a decrease in the hydrolysis and oxidation of the drug (Lares–Asseff *et al*., 1999). These indicate that the hepatic metabolising activities of UGT1A6 and CYP2C9 are impaired by malnutrition.

The endocrine system may play a role in drug metabolism in children, since hormones mediate the dramatic physical changes of growth and development and also serve to coordinate metabolic events in diverse tissues (Redmond *et al.,* 1980). Thyroid hormones often considerably affect drug metabolism in children (Saenger *et al.,* 1976; Vesell *et al.,* 1975). A decrease in serum triodothyronine (T3) level, a normal level of thyroxine (T4), and a free thyroxine index (FTI) have been reported in severely malnourished children (Onuora *et al.,* 1983; Turkay *et al.,* 1995). A study of antipyrine metabolism in malnourished children showed both a significant decrease in antipyrine metabolism and also an associated decrease in T3. It has been suggested that the decreased T3 concentration resulted in a decrease in antipyrine metabolism (Homeida *et al.,* 1979).

1.8.4. Drug excretion

Glomerular filtration rate, renal blood flow, and tubular reabsorption are also impaired in severe malnutrition (Alleyne, 1967). The impact of malnutrition on the glomerular filtration rate is most relevant in children receiving drugs primarily excreted by the kidneys (e.g., penicillin and aminoglycosides). Studies in severely malnourished children have reported a significant decrease in renal CL of penicillin (Bolme *et al.,* 1988; Buchanan *et al.,* 1979a), cefoxitin (Buchanan *et al*., 1980b), and methotrexate (Mayhew & Christensen, 1993). Other studies have reported a decreased but not significantly changed renal CL of streptomycin (Bolme *et al.,* 1988), amikacin (Hendricks *et al.,* 1995), gentamicin (Bravo *et al.,* 1982), and ethambutol (Graham *et al.,* 2006).

Considering the wide range of pathophysiological changes due to malnutrition that affect drug disposition, it was necessary to perform an extensive search of studies of drug disposition in children with malnutrition. This

search included a systematic review of studies of pharmacokinetics of drugs in malnourished children and aimed to identify new areas of research that will help improve drug safety in severely malnourished children.

CHAPTER TWO:

SYSTEMATIC REVIEW OF STUDIES OF DRUG PHARMACOKINETICS IN PROTEIN-ENERGY MALNOURISHED CHILDREN

2.1. INTRODUCTION

Absorption, distribution, metabolism, and excretion (ADME) are the four major components of drug pharmacokinetics. Although drug metabolism is an integral part of pharmacokinetic studies, hepatic drug metabolism has not been specifically evaluated in malnourished children. Information regarding hepatic drug metabolism in malnourished children can therefore only be obtained indirectly from pharmacokinetic studies. A systematic review of studies of drug disposition in severely malnourished children is thus crucial in order to identify what work has been performed in this area. A systematic review is a type of literature review that focuses on a research question trying to identify, appraise, select, and synthesise all high quality research evidence relevant to the question. This review is necessary for a proper understanding of drug metabolism in malnourished children.

This systematic review aimed to determine how different types of malnutrition affect absorption, distribution, metabolism, elimination, excretion, and the pharmacodynamics of drugs in children. It involved examining published articles for quantitative and qualitative evidence of the effects of malnutrition on drug disposition in children.

2.2. IDENTIFICATION OF RELEVANT LITERATURE

Searches of the MEDLINE and EMBASE databases covered publications between January 1960 and December 2009. Articles describing drug absorption and disposition in malnourished children were sought using both databases and by reference tracking. The search strategies for both databases involved an initial search of all relevant abstracts using the medical subject heading (MeSH) descriptor 'drug absorption' OR 'drugs absorption', limited to children from 0 to 17 years. Similar searches were performed for all drug pharmacokinetic parameters, which included distribution, metabolism, and clearance (CL). In other searches, the term 'absorption' was substituted with each of the terms 'distribution', 'pharmacokinetics', 'pharmacodynamics', 'metabolism', 'elimination' and 'clearance', respectively. Another search was performed that involved the use of MeSH descriptors 'malnutrition' OR 'mal-nutrition' OR 'undernutrition' OR 'under-nutrition' OR 'underweight' OR 'under-weight' OR 'protein energy malnutrition' OR 'protein-energy malnutrition' OR 'protein calorie malnutrition' OR 'protein-calorie malnutrition' OR 'marasmus' OR 'marasmic-kwashiorkor' OR 'kwashiorkor'. The searches were later combined to produce the desired abstracts for the study. Duplicate abstracts were removed, and abstracts were scanned to determine whether they met the inclusion criteria.

Inclusion criteria sought abstracts that assessed or discussed absorption, distribution, pharmacokinetics, pharmacodynamics, metabolism, elimination, or CL of drugs in children who were underweight and/or marasmic and who had marasmic-kwashiorkor and/or kwashiorkor. Abstracts that assessed or discussed disposition of micronutrients and orexigenic drugs in malnourished children were excluded. Full articles of the relevant abstracts were retrieved, and the references of the retrieved articles were scanned in order to identify additional

appropriate studies. The relevant additional articles meeting inclusion criteria were accessed.

2.3. DATA EXTRACTION

The desired studies were critically reviewed. Each study was independently reviewed by my lead supervisor, a paediatric clinical pharmacologist, and myself. A standard form was used to extract data on the number and year of studies, characteristics of the participants and controls, types of drug studied, and the number of blood samples collected. The pharmacokinetic parameters studied (absorption, distribution, metabolism, and CL were noted. We also extracted data on the methods of PEM classification in each of the studies.

2.4. RESULTS OF THE SYSTEMATIC REVIEW

Results were based on the specific parameters identified in the study.

2.4.1. Publications

The EMBASE search yielded 731 abstracts, while the MEDLINE search yielded 546 abstracts, all of potential use. The results of the search strategy are presented schematically in Figure 2.1. Only 51 abstracts met the inclusion criteria. Most abstracts were excluded because they assessed serum macro-and micronutrients and serum proteins in malnourished children. One abstract written in French was excluded because the translator at the university was unable to assist.

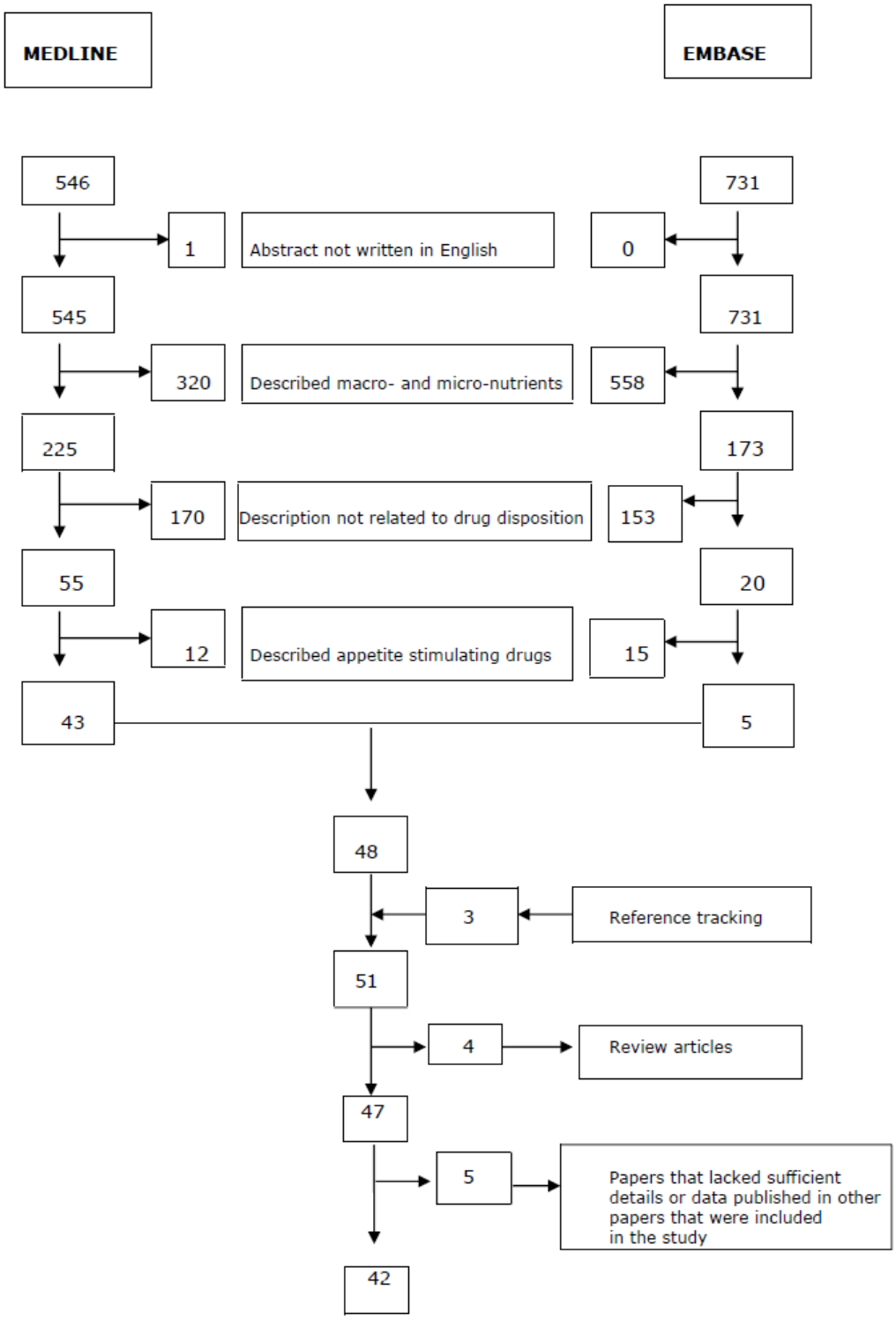


Figure 2.1. Schematic representation of the literature search

The abstracts were mainly original research (41), review articles (4), and letters to the editor (6). Both original research articles and letters to the editor (47 publications) were included in this review. Five publications (Akbani *et al.,* 1977; Buchanan *et al.,* 1976; Eriksson *et al.,* 1988; Lares–Asseff *et al.,* 1993; Seaton *et al.,* 2007) were excluded because there were either insufficient details (no documentation of the mean value of the data or the control data) or the data were published in more detail in a subsequent paper, which was included in the final analysis. Altogether, 42 publications were analysed.

The majority of studies were published in the 1970s (14) and 1980s (18); thereafter, the number progressively decreased over the subsequent two decades (Figure 2.2). Twenty-eight studies involved only one drug. A total of 34 different drugs were studied.

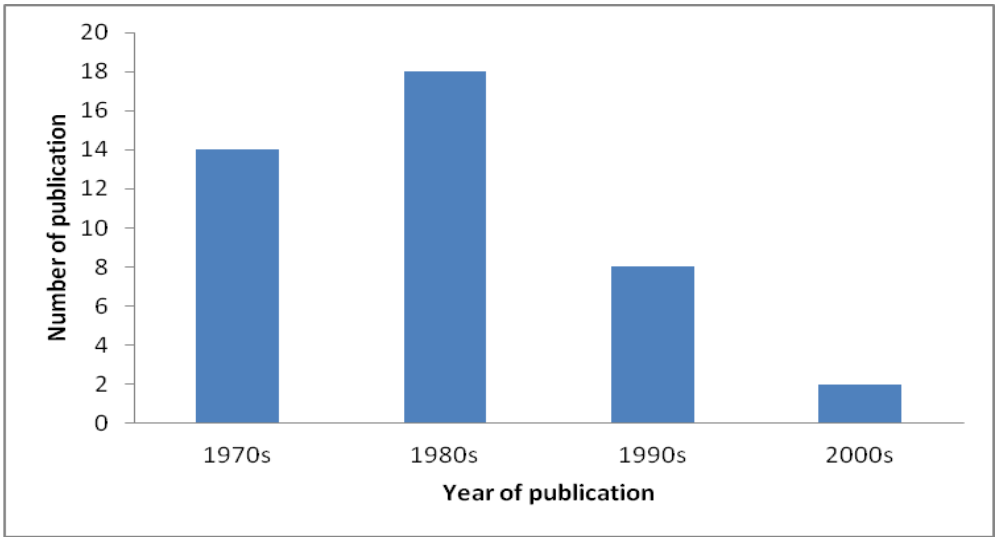


Figure 2.2: Frequency of publication of pharmacokinetic studies of drugs in children with protein-energy malnutrition

2.4.2. Methodology of trials

The age range of the patients and control participants used for the studies was 3 months to 16 years. The number of patients in individual studies varied from 5

to 41. Age-matched, healthy children were the control participants in 24 studies. Nutritionally rehabilitated children were used as additional control participants in four studies; they were also used as the only control participants in seven studies. Five studies had no control participants.

PEM was classified in the participants according to the Wellcome Trust Working Party (WTWP) classification (22 studies) and Gómez classification (seven studies). Coexisting infections, including tuberculosis (4), bacterial sepsis (3), malaria (2), and HIV with tuberculosis (1), were reported in 10 studies. Most studies described in this review used traditional methods to study pharmacokinetics and involved a collection of one to 17 blood samples from the individual children.

2.4.3. Effect of PEM on drug absorption

Drug absorption is commonly described by rate (time dependent) and extent, otherwise called bioavailability (F), which is time independent. The extent of absorption was reported in only one study (Eriksson *et al.,* 1983a). The effect of PEM on the absorption rate and area under curve concentration (AUC) of the drugs are presented in Table 2.1. The drug absorption rate (K_a) was reported for eight drugs, six of which showed no difference in the values of K_a for malnourished children and the control groups. These drugs were gentamicin (Samotra *et al.,* 1995), metronidazole (Lares–Asseff *et al.,* 1992), phenytoin (Bano *et al.,* 1985), chloramphenicol (Samotra *et al.,* 1986), paracetamol (Mehta *et al.,* 1985), and sulphamethoxazole (Bravo *et al.,* 1984). However, the *p*-values were not documented in the studies, except those involving gentamicin ($p > 0.05$) and chloramphenicol ($p > 0.05$).

Table 2.1: Effect of protein–energy malnutrition on absorption rate (K_a) and extent of absorption (AUC) of drugs

Drug	Route of administration	Number of PEM children	Age (years)	Degree of malnutrition	Mean± s.d absorption rate (Ka/h)		Mean± s.d AUC (µg/ml/h)		Controls	*P* value	Reference
					Controls	PEM children	Controls	PEM children			
No statisticallly significant decrease in Ka and/or AUC											
Aspirin	Oral	5	0–2	Undefined	-	-	154.9±59.3[a]	164.2±57.8[a]	NH (n=6)	-	Treluyer *et al.*, 1991
Ethambutol	Oral	15	1–12	U, M	-	-	23.1±9.8[a]	21.3±16.8[a]	–	-	Graham *et al.*, 2006
Gentamicin	IM	6	4–14	Undefined	0.2±0.0	0.1±0.0	22.4±2.3	22.3±2.2	H (n=4)	-	Samotra *et al.*, 1985
Metronidazole	Oral	10	0–4	M-K, M	0.6±0.5	1.0±0.7	-	-	R (*n*=10)	>0.05	Lares-Assef *et al.*, 1992
Penicillin[b]	Oral	8	0–6	U	10.1±4.3	9.5±3.4	493.6±0.0[c]	476.7±0.0[c]	NH (*n*=6)	-	Bolme *et al.*, 1995
	Oral	6	0-6	M	10.1±4.3	8.4±1.5	-	-	NH (*n=6*)	-	
Phenytoin	Oral	5	7–14	Undefined	0.3±0.0	0.3±0.1	277.0±22.4	298.8±24.1	H (*n*=4)	-	Bano *et al.*, 1985
Pyrazinamide	Oral	27	1–14	Undefined	-	-	496.0±407.0[a]	332.7±293.6[a]	–	-	Graham *et al.*, 2006
Quinine	IM	8	0–5	M, M-K	-	-	97.5±31.4[a]	74.9±29.1[a]	NH (n=10)	-	Treluyer *et al.*, 1996
Theophylline	Oral	11	1–16	Undefined	-	-	52.8±6.7	42.0±5.8	–	>0.05	Kumar *et al.*, 1989
Tobramycin	IM	4	0–2	K	-	-	0.4±0.2[d]	0.4±0.2[d]	R (n=4)	-	Buchanan *et al.*, 1978
Chloramphenicol	Oral	8	4–14	Undefined	1.1±0.6	1.0±0.4	-	-	H (n=4)	>0.05	Samotra *et al.*, 1986
Paracetamol	Oral	11	0–6	M, M-K, K	2.9±1.0	2.7±0.3	-	-	R (n=5)	-	Mehta *et al.*, 1985
Sulphamethoxazole	Oral	7	0–2	M	0.7±0.4	1.2±0.9	-	-	NH (n=10)	-	Bravo *et al.*, 1984
Statistically significant decrease in Ka or AUC											
Carbamazepine	Oral	6	6–13	Undefined	-	-	219.0±40.0	72.0±1.0	H (n=6)	<0.05	Bano *et al.*, 1986
Chloroquine	Oral	5	2–4	K	-	-	9.2±3.1	3.3±2.1	NH (n=6)	<0.001	Walker *et al.*, 1987

Table 2.1: Continued

Drug	Route of administration	Number of PEM children	Age (years)	Degree of malnutrition	Mean± s.d absorption rate (Ka/h)		Mean± s.d AUC (µg/ml/h)		Controls		Reference
Penicillin[a]	Oral	6	0-6	K	10.1±4.3	2.5±1.3	-	-	NH(n=6)	-	Bolme *et al.*, 1995
Sulphadiazine	Oral	6	0–5	M, M-K	0.7±0.1	0.5±0.0	-	-	H (n=5)	<0.01	Mehta *et al.*, 1980
Chloramphenicol	Oral	8	4–14	Undefined	-	-	252.0±45.0	128.0±45.0	H (n=4)	<0.01	Samotra *et al.*, 1986
Statistically significant increase in AUC											
Metronidazole	Oral	10	0–4	M-K, M	-	-	140.0±47.0	191.7±101.7	R (n=10)	<0.05	Lares-Assef et al., 1992
Caffeine	Oral	7	1–4	K	-	-	22.7±15.6	67.2±37.5	H (n=5)	<0.05	Akinyinka *et al.*, 2000
Chloramphenicol	Oral	10	0–3	U, M, K, M-K	-	-	222.0±0.0	375.0±0.0	H (n=4)	-	Mehta *et al.*, 1975
Paracetamol	Oral	11	0–6	M, M-K, K	-	-	30.0±5.4	66.1±20.9	R (*n*=5)	<0.05	Mehta *et al.*, 1985
Penicillin[b]	Oral	6	0–6	M	-	-	493.6±0.0[c]	941.7±0.0[c]	NH (*n*=6)	-	Bolme *et al.*, 1995
	Oral	6	0–6	K	-	-	493.6±0.0[c]	975.2±0.0[c]	NH (*n*=6)	-	Bolme *et al.*, 1995
Phenobarbitone	Oral	5	7–10	Undefined	-	-	454.0±45.1	607.1±49.9	H (n=5)	<0.05	Syed *et al.*, 1986
Quinine	Oral	6	1–3	K	-	-	25.0±2.4	37.5±6.8	H (n=7)	<0.001	Salako *et al.*, 1989
Sulphadiazine	Oral	6	0–5	M, M-K	-	-	136.1±15.4	283.9±15.3	H (*n*=5)	<0.01	Mehta *et al.*, 1980
Sulphamethoxazole	Oral	7	0–2	M	-	-	328.2±158.6	573.0±154.5	NH (*n*=10)	<0.001	Bravo *et al.*, 1984

IM = intramuscular; U= underweight; M = marasmus; K = kwashiorkor; M-K = marasmic-kwashiorkor; Undefined = PEM not classified; R- rehabilitated patients; H = healthy children with normal weight; NH = non-healthy children with normal weight; [a]Values in mg/L/h; [b]Drug administered to fasted patients; [c]Values in µg/ml/min; [d]Unit uncertain

In a study involving children with different categories of malnutrition, the absorption rate of penicillin showed no difference in underweight and marasmic children when compared to the control (Bolme *et al.,* 1995) yet decreased in children with kwashiorkor. However, the *p*-values were again undocumented.

Most drugs were administered either orally or intramuscularly, and their AUCs were reported in this review as AUC_{oral} or $AUC_{i.m}$. Although the *p*-values were undocumented, the AUC of seven drugs did not differ for malnourished children when compared to their control groups (Table 2.1). However, there was a statistically significant decrease in malnourished children regarding the AUC of two drugs: carbamazepine ($p < 0.05$) (Bano *et al.,* 1986) and chloroquine ($p < 0.001$) (Walker *et al.,* 1987). By contrast, there was a statistically significant increase in the AUC of six drugs: metronidazole ($p < 0.05$) (Lares–Asseff *et al.,* 1992), caffeine ($p < 0.05$) (Akinyinka *et al.,* 2000), paracetamol ($p < 0.05$) (Mehta *et al.,* 1985), phenobarbitone ($p < 0.05$) (Syed *et al.,* 1986), sulphadiazine ($p < 0.01$) (Mehta *et al.,* 1980), and sulphamethoxazole ($p < 0.001$) (Bravo *et al.,* 1984).

In a study of children with different categories of malnutrition, there was no change in the AUC of penicillin in underweight children. However, AUC was increased in children with marasmus and kwashiorkor when compared to the control group, though it is important to note that the *p*-values were not documented in the study (Bolme *et al.,* 1995).

In another study of children with different categories of malnutrition, Eriksson *et al.* (1983a) did not report the AUCs for chloramphenicol, though the bioavailability *F* (%) was reported. It was not affected in children with marasmus (57.0 ± 12.0), though there were statistically significant decreases in the values in children with marasmic-kwashiorkor (44.0 ± 6.0; $p < 0.05$) and kwashiorkor

(30.0 ± 9.0; $p < 0.01$) when compared to the control participants (85.0 ± 15.0). These results were similar to the statistically significant decrease ($p < 0.01$) in the AUC for children with undefined malnutrition reported by Samotra *et al.* (1986) but in contrast to the increased AUC for all forms of malnutrition reported by Mehta *et al.* (1975).

In addition, contrasting results were also documented for quinine. There was a statistically significant increase ($p < 0.001$) in the AUC of oral quinine in malnourished children (Salako *et al.,* 1989), though PEM had no effect on its intramuscular absorption (Treluyer *et al.,* 1996).

2.4.4. Effect of PEM on drug protein binding and volume of distribution

The effects of PEM on plasma protein binding were identified in nine papers from the systematic review. Seven of these papers were published by Buchanan and his colleagues, who evaluated the plasma protein binding of 19 drugs in seven *in vitro* studies (Eyberg *et al.,* 1974; Buchanan *et al.,* 1976; Buchanan, 1977a; Buchanan & Van der Walt, 1977b; Buchanan & Van der Walt, 1977c; Buchanan & Van der Walt, 1977d; Buchanan & Van der Walt, 1977e). Only two studies (Treluyer *et al*., 1991; Treluyer *et al.*, 1996) evaluated the *in vivo* binding of aspirin (salicylate) and quinine to serum protein. Serum or plasma of children with kwashiorkor was used for studies of protein binding of the drugs in the *in vitro* studies (Eyberg *et al.,* 1974; Buchanan *et al.,* 1976; Buchanan, 1977a; Buchanan & Van der Walt, 1977b; Buchanan & Van der Walt, 1977c; Buchanan & Van der Walt, 1977d; Buchanan & Van der Walt, 1977e). The number of patients studied and their ages were not provided, except for the studies that involved aspirin (Treluyer *et al.,* 1991) and quinine (Treluyer *et al.,* 1996). Fresh pooled adult serum and plasma were used as controls, except for the quinine study in which plasma from healthy children was used as a control. There was a

statistically significant decrease in the protein binding of 17 drugs in kwashiorkor when compared to healthy adults (p-values ranged from <0.0005 to <0.05) (Table 2.2). However, PEM had no statistically significant effect on the protein binding of two drugs: aspirin ($p > 0.05$) and quinine ($p > 0.05$).

Table 2.2: Effect of kwashiorkor on drug protein binding or fraction unbound drug (f_u)

Drug	Percentage free drug (%)	Percentage change in free drug (%)	*P* value
Decreased protein binding (Increased f_u) and statistically significant percentage change in free drug			
Chloramphenicol (Buchanan, 1977a; Buchanan & Van der Walt, 1977b)	80.0	5.9	<0.0005
Cloxacillin (Buchanan, 1977a)	25.1	65.1	<0.01
Gentamicin (Buchanan, 1977a)	96.0	4.7	<0.01
Flucloxacillin (Buchanan, 1977a)	28.9	83.7	<0.01
Penicillin (Buchanan, 1977a)	65.0	8.3	<0.01
Streptomycin (Buchanan, 1977a; Buchanan & Van der Walt, 1977c)	67.6	28.3	<0.0005
Sulphamethoxazole (Buchanan, 1977a)	94.6	31.6	<0.0005
Digoxin (Buchanan, 1977a; Buchanan *et al.,* 1976)	89.2	8.3	<0.01
Ethionamide (Buchanan, 1977a; Buchanan & Van der Walt, 1977c)	78.5	13.6	<0.0005
Para-amino salicylic acid (Buchanan & Van der Walt, 1977c)	96.2	14.4	<0.0005
Rifampicin (Buchanan & Van der Walt, 1977c)	28.2	14.2	<0.0005
Phenobarbitone (Buchanan, 1977a)	84.7	11.4	<0.01
Salicylate (Eyberg *et al.,* 1974)	71.3	54.7	<0.0005
Thiopentone (Buchanan, 1977a; Buchanan & Van der Walt, 1977d)	66.2	21.2	<0.0005
Phenytoin (Buchanan, 1977a)	12.0	27.7	<0.0005
Chloroquine (Buchanan, 1977a; Buchanan & van der Walt; 1977e)	60.3	16.0	<0.05
Dicumarol (Buchanan, 1977a)	9.8	22.5	<0.0005
Unaffected protein binding (Unaffected f_u) and no statistically significant percentage change in free drug			
Ethambutol (Buchanan, 1977a)	93.8	0.0	-
Isoniazid (Buchanan, 1977a)	100.0	0.0	-
Quinine (Treluyer *et al*., 1996)	6.6	0.3	>0.05
Aspirin (salicylate) (Treluyer *et al*., 1991)	9.6	1.3	>0.05

Five drugs (cloxacillin, flucloxacillin, rifampicin, phenytoin, and dicoumarol) were highly protein bound as suggested by their low percentage of the unbound fraction of drugs (Buchanan *et al.,* 1976; Buchanan, 1977a; Buchanan & Van der Walt, 1977c). Of these drugs, cloxacillin and flucloxacillin showed an increase in percentage change of over 50% at the free drug level.

The effects of PEM on the volume of distribution (VD) were evaluated for 14 drugs in 19 papers. For most drugs, PEM had no statistically significant effect on VD (Table 2.3). There were contrasting results for four drugs: gentamicin (Bravo *et al.,* 1982; Buchanan *et al.,* 1979d), quinine (Pussard *et al.,* 1999; Treluyer *et al.,* 1996), streptomycin (Bolme *et al.,* 1988) and theophylline (Eriksson *et al.,* 1983b; Kumar *et al.,* 1989). In the case of gentamicin, a study of children with kwashiorkor documented a statistically significant decrease ($p < 0.05$) in VD (Buchanan *et al.,* 1979d). By contrast, a study of children who were underweight, marasmic, or experiencing marasmic-kwashiorkor had a statistically significant increase ($p < 0.05$) in VD of gentamicin (Bravo *et al.,* 1982).

In the case of theophylline, children with marasmus and kwashiorkor had a statistically significant increase in VD ($p < 0.02$ and $p < 0.01$, respectively) (Eriksson *et al.,* 1983b), whereas children who were underweight presented no change in VD (Eriksson *et al.,* 1983b). Another study in which the degree of malnutrition was undefined showed that PEM had no effect on the VD of theophylline (Kumar *et al.,* 1989).

In the case of streptomycin, children with kwashiorkor exhibited a statistically significant increase ($p < 0.01$) in VD, whereas children with marasmus or who were underweight had no change in VD (Bolme *et al.,* 1988).

Table 2.3: Effect of protein energy malnutrition on drug distribution (*Vd*)

Drug	Number of PEM children	Age (years)	Degree of malnutrition	Mean ±s.d *Vd* (L/kg)		*P* value	Controls	Reference
				Controls	PEM children			
No statistically significant change in *Vd* values								
Acetanilide	5	0–2	K	6.3±0.6	5.3±0.3	-	R (*n*=5)	Buchanan *et al.*,1980
Amikacin	10	1–4	K	<1.0[b]	0.3±0.1	-	H (controls, adults from another study)	(Hendricks *et al.*,1995)
Antipyrine	5	0–5	M, K	0.6±0.1	0.7±0.1	-	R/H (*n*=5)	Narang *et al.*,1977
	15	0–2	K	4.7±1.4	4.4±1.5	>0.25	R (*n*=15)	Buchanan *et al.*, 1979[a]
	8	9–13	Undefined	25.0±3.1	21.7±2.7	-	R (*n*=8)	Homeida *et al.*, 1979
Aspirin	5	0–2	Undefined	0.3±0.1	0.3±0.1	>0.05	H (*n*=6)	Treluyer *et al.*, 1991
Chloramphenicol[a]	8	0–6	M	1.9±0.2	2.1±0.5	-	NH (*n*=8)	Eriksson *et al.*, 1983a
	9	0–6	M-K	1.9±0.2	1.5±0.2	-	NH (*n*=8)	Eriksson *et al.*, 1983a
	8	0–6	K	1.9±0.2	1.3±0.2	-	NH (*n*=8)	Eriksson *et al.*, 1983a
Ethambutol	6	1–12	U, M	510.6±375.5[c]	300±156.1[c]	>0.05	NH (*n*=7)	Graham *et al.*, 2006
Isoniazid	7	0–3	K	14.2±8.0	9.7±3.9	-	R (*n*=7)	Buchanan *et al.*, 1979b
Metronidazole	10	0–4	M-K, M	1.6±1.0	1.5±0.9	>0.05	R (*n*=10)	Lares-Asseff *et al.*, 1992
Penicillin[a]	7	0–6	U	1.4±0.2	1.5±0.6	-	NH (*n*=6)	Bolme *et al.*, 1995
	8	0–6	M	1.4±0.2	0.9±0.1	-	NH (*n*=)	Bolme *et al.*, 1995
	8	0–6	M-K	1.4±0.2	0.9±0.1	-	NH (*n*=6)	Bolme *et al.*, 1995
	8	0–6	K	1.4±0.2	1.2±0.2	-	NH (*n*=6)	Bolme *et al.*, 1995
	8	0–2	K	13.1±7.4	12.8±3.9	-	R (*n*=9)	Buchanan *et al.*, 1979c
Quinine	8	0–5	not K	1.7±0.4	2.0±0.8	>0.05	NH (*n*=7)	Treluyer *et al.*, 1996
Sulphamethoxazole	7	0-2	M	0.6±0.3	0.5±0.1	-	NH (10)	Bravo *et al.*, 1984
Streptomycin	11	0–12	U	0.3±0.0	0.3±0.1	>0.05	NH (*n*=4)	Bolme *et al.*, 1988
	12	0–12	M	0.3±0.0	0.3±0.1	>0.05	NH (*n*=4)	Bolme *et al.*, 1988
Theophylline	11	1–16	Undefined	0.7±0.1	1.0±0.2	>0.05	H (*n*=10)	Kumar *et al.*, 1989
	6	1–s3	U	0.5±0.4	0.5±0.1	-	H (*n*=12)	Eriksson *et al.*, 1983b
Statistically significant increase in *Vd*								
Gentamicin[a]	11	0–1	U, M, M-K	0.4±0.2	0.5±0.3	<0.05	H (*n*=7)	Bravo *et al.*, 1982
Streptomycin	5	0-12	K	0.3±0.0	0.4±0.2	<0.01	H (*n*=4)	Bolme *et al.*, 1988
Theophylline	8	1–8	M	0.5±0.4	0.7±0.1	<0.02	NH (*n*=12)	Eriksson *et al.*, 1983b
	5	1–8	K	0.5±0.4	0.7±0.1	<0.01	NH (*n*=12)	Eriksson *et al.*, 1983b
Statistically significant decreased in *Vd*								
Quinine[a]	10	2–6	Undefined	1.6±1.1	0.6±0.3	<0.05	H (*n*=10)	Pusasard *et al.*, 1999
Gentamicin[a]	6	1–3	K	4.5±1.4[c]	3.9±0.5[c]	<0.05	R (*n*=6)	Buchanan *et al.*, 1979d

[a]Drug administered intravenously; [b]Values for adult reference;, [c]Values in litres; U= underweight; M = marasmus; K = kwashiorkor; M-K = marasmic-kwashiorkor; Undefined = PEM not classified; R- rehabilitated patients; H = healthy children with normal weight; NH = non-healthy children with normal weight

A statistically significant decrease ($p < 0.05$) in the VD of quinine in children with undefined PEM in one study (Pussard *et al.,* 1999) was in contrast to another study involving children with marasmus and marasmic-kwashiorkor in which VD was unaffected (Treluyer *et al.,* 1996).

2.4.5. Effect of protein-energy malnutrition on drug CL and elimination half-life

Table 2.4 shows the effect of PEM on the total CL and elimination half-life of nine drugs that are primarily metabolised in the liver. There was a statistically significant decrease in the total clearance of six drugs: acetanilide ($p < 0.025$) (Buchanan *et al.,* 1980a); antipyrine ($p < 0.05$) (Narang *et al.,* 1977), ($p < 0.0025$) (Buchanan *et al.,* 1979b), and ($p < 0.05$) (Homeida *et al.,* 1979); caffeine ($p < 0.01$) (Akinyinka *et al*., 2000); sulphamethoxazole (Bravo *et al*., 1984); isoniazid ($p < 0.01$) (Buchanan *et al*., 1979c); and metronidazole ($p < 0.01$) (Lares–Asseff *et al*., 1992). There was a corresponding statistically significant increase in the plasma half-life. In two studies of malnourished children who were poorly categorised (Pussard *et al.,* 1999) and experiencing kwashiorkor (Salako *et al.,* 1989), there was a statistically significant decrease ($p < 0.05$ and $p < 0.001$, respectively) in the total clearance of quinine. However, a statistically significant increase ($p < 0.05$) in its total clearance was reported by another study of children with all categories of PEM except kwashiorkor (Treluyer *et al.,* 1996). Similarly, one study reported a statistically significant decrease ($p < 0.01$) in the total CL of chloramphenicol in kwashiorkor or the total CL unaffected in marasmus and marasmic-kwashiorkor (Eriksson *et al.,* 1983a).

Table 2.4: Effect of protein energy malnutrition on total clearance (CL) and half-life ($t_{½}$) of drugs primarily metabolised by the liver

Drug	Number of PEM children	Age (years)	Degree of malnutrition	Mean *CL* (ml/min/kg)		Mean $t_{½}$ (h, except where stated)		*P* value	Reference
				Controls	PEM children	Controls	PEM children		
Statistically significant decrease in *CL* and increase in $t_{½}$									
Acetanilide	5	0–2	K	2.45±0.22[b]	0.92±0.19[b]	1.76±0.13	4.78±1.07	<0.025 for *CL* <0.0025 for $t_{½}$	Buchanan *et al.,* 1980[a]
Antipyrine	10	0–5	M, M-K, K	70.10±7.20[c]	47.2±5.9[c]	6.30±0.50	10.40±0.80	<0.05 for *CL* <0.01 for $t_{½}$	Narang *et al.,* 1977
	15	0–2	K	15.50±8.70[d]	8.40±5.10[d]	4.30±2.30	7.90±5.00	<0.0025 for *CL* <0.0005 for $t_{½}$	Buchanan *et al.,* 1979b
	8	9–12	Undefined	32.00±5.1[e]	19.50±3.20[e]	9.90±1.30	14.80±2.10	<0.05	Homeida *et al.,* 1979
Caffeine	7	1–4	K	4.50±1.90	1.60±1.00	3.70±1.80	13.10±7.90	<0.01 for *CL* <0.05 for $t_{½}$	Akinyinka *et al.,* 2000
Chloramphenicol[e]	8	0–6	K	7.53±1.09	4.16±0.73	2.85±0.26	3.76±0.36	<0.01 for *CL* <0.05 for $t_{½}$	Eriksson *et al.,* 1983[a]
Sulphamethoxazole	10	0-2	M	0.08±0.04	0.04±0.01	4.90±0.50	9.40±2.10	No *P* value for *CL* <0.001 for $t_{½}$	Bravo *et al.,* 1984
Isoniazid	7	0–3	K	329.40±399.66[d]	114.0±75.38[d]	43.86±19.83	61.86±20.13	<0.01 for *CL* <0.0025 for $t_{½}$	Buchanan *et al.,* 1979c
Metronidazole	10	0–4	M-K, M	0.17±0.98[b, f]	0.09±0.05[b, f]	5.68±1.97[f]	11.73±6.10[f]	<0.01 for *CL* <0.05 for $t_{½}$	Lares-Asseff *et al.,* 1992
Quinine	6	1–2	K	108.50±34.80[g]	31.50±8.50[g]	8.00±1.30	15.0±4.40	<0.001	Salako *et al.,* 1989
Quinine[a]	20	2–4	Undefined	4.00±2.10	1.70±1.50	5.10±2.60	7.20±5.90	<0.05	Pussard *et al.,* 1999
Statistically significant increase in *CL* and decrease in $t_{½}$									
Quinine	8	0–5	not K	2.30±1.40	4.40±3.60	10.10±3.40	6.30±1.80	<0.05	Treluyer *et al.,* 1996

Table 2.4

No statistically significant change in *CL* and $t_{½}$									
Chloramphenicol[e]	17	0–6	M	7.53±1.09	8.16±2.29	2.85±0.26	2.88±0.52	-	Eriksson *et al.,* 1983a
	8	0–6	M-K	7.53±1.09	5.39±1.01	2.85±0.26	3.20±0.53	-	Eriksson *et al.,* 1983a
Theophylline	11	1–16	Undefined	129.00±14.20[c]	170.70±20.70[c]	4.10±0.62	5.21±1.19	>0.05	Kumar *et al.,* 1989
	6	1–8	U	1.22±0.20[h]	1.09±0.15[h]	4.93±0.59	5.25±0.55	-	Eriksson *et al.,* 1983b
	8	1–8	M	1.22±0.20[h]	1.45±0.17[h]	4.93±0.59	5.38±0.22	-	Eriksson *et al.,* 1983b
	5	1–8	K	1.22±0.20[h]	1.49±0.22[h]	4.93±0.59	5.61±0.31	-	Eriksson *et al.,* 1983b

[a]Drug administered intravenously; [b]Value in L/h; [c]Value in kg/ml/h; [d]Value in ml/min; [e]Unit of the value was not given; [f]Median value; [g]Value in mg/min; [h]Value in kg/ml/min; U= underweight; M = marasmus; K = kwashiorkor; M-K = marasmic-kwashiorkor; Undefined = PEM not classified

In a study involving poorly categorised children with malnutrition, there was no statistically significant change ($p > 0.05$) in the total CL of theophylline when compared to the controls (Kumar *et al.,* 1999). Similar results were documented in another study of children who were underweight or experiencing either marasmus or kwashiorkor (Eriksson *et al.,* 1983b).

The effects of PEM on the total CL of six drugs primarily eliminated by the kidneys are presented in Table 2.5. In a study involving all categories of PEM, there was a statistically significant decrease in the total CL of penicillin in association with a statistically significant increase in elimination half-life for children with marasmus ($p < 0.001$), marasmic-kwashiorkor ($p < 0.01$), and kwashiorkor ($p < 0.01$) (Bolme *et al.,* 1995). Another study involving children with kwashiorkor demonstrated a statistically significant decrease ($p < 0.0005$) in the CL of penicillin, as well as a statistically significant increase ($p < 0.0025$) in the drug's elimination half-life (Buchanan *et al.,* 1979d).

There was a statistically significant decrease ($p < 0.0005$) in the CL of cefoxitin, though $t_{½}$ was unaffected in children with kwashiorkor (Buchanan *et al.,* 1980b). There was no influence of PEM on the total CL of the two aminoglycosides studied: gentamicin (Bravo *et al.,* 1982; Buchanan *et al.,* 1980b) and amikacin (Hendricks *et al.,* 1995). One study involving all categories of PEM documented a statistically significant decrease ($p < 0.01$) in the renal CL of streptomycin, as well as a statistically significant increase ($p < 0.01$) in the elimination half-life of the drug in children with kwashiorkor but no change in children who were underweight or had marasmus only (Bolme *et al.,* 1988).
Twelve studies calculated the plasma half-life without any CL values (Table 2.6).

Table 2.5: Effect of protein energy malnutrition on *CL* and $t_{½}$ of drugs primarily eliminated by the kidneys

Drug	Number of PEM children	Age (years)	Degree of malnutrition	Mean CL (ml/min/kg)		Mean $t_{½}$ (h)		*P* value	Reference
				Controls	PEM children	Controls	PEM children		
Statistically significant decrease in *CL* and increase in $t_{½}$									
Penicillin[a]	8	0–6	M	22.20±0.90	14.00±0.50	0.65±0.08	0.90±0.04	<0.001	Bolme *et al.,* 1995
	8	0–6	M-K	22.20±0.90	16.90±1.0	0.65±0.08	0.92±0.08	<0.01	Bolme *et al.,* 1995
	8	0–6	K	22.20±0.90	11.60±1.1	0.65±0.08	0.93±0.16	<0.01	Bolme *et al.,* 1995
	8	0–2	K	271.69±75.94[b]	183.43±116.26[b]	0.65±0.08[f]	2.33±0.80[f]	<0.0005 for *CL* <0.0025 for $t_{½}$	Buchanan *et al.,* 1979c
Streptomycin	5	0–12	K	67.00±15.00	49.00±7.00	2.58±0.44[f]	9.69±2.56[f]	<0.01	Bolme *et al.,* 1988
Statistically significant decrease in *CL* but $t_{½}$ unaffected									
Penicillin[a]	7	0–6	U	22.20±0.90	15.10±0.90	0.65±0.08	0.68±0.13	<0.01 for *CL* No *P*-value for $t_{½}$	Bolme et al., 1995
Cefoxitin	6	1–4	K	70.60±16.6[b]	50.70±5.00[b]	0.47±0.07	0.49±0.06	<0.025 for *CL* No *P*-value for $t_{½}$	Buchanan *et al.,* 1980b
No statistically significant change in *CL* and $t_{½}$									
Gentamicin	11	0–1	U, M, M-K	3.57±0.02	3.99±0.01	0.01±0.00	0.01±0.00	>0.05	Bravo *et al.,* 1982
Gentamicin[a]	6	1–3	K	28.70±21.30[b]	13.60±6.00[b]	2.98±1.66	3.82±1.58	-	Buchanan *et al.,* 1980b
Amikacin	10	1–4	K	<0.50±0.00[c, d]	6.19±2.51[d]	<0.50±0.00[e]	2.56±1.00	-	Hendricks *et al.,* 1995
Ethambutol	11	0–12	U	37.84±16.00	52.81±71.89	9.70±4.00	7.40±5.60	>0.05	Graham *et al.,* 2006
Streptomycin	12	0–12	M	67.00±15.00	91.00±14.00	2.58±0.44	2.27±0.24	-	Bolme *et al.,* 1988
	11	0–12	U	67.00±15.00	74.00±8.00	2.58±0.44	2.14±0.26	-	Bolme *et al.,* 1988

[a]Drugs administered intravenously; [b]Value in ml/min; [c]Values for adult reference; [d]Value in L//h/kg, [e]Value in L/h; [f]Significant difference in t ½ values; U= underweight; M = marasmus; K = kwashiorkor; M-K = marasmic-kwashiorkor; Undefined = PEM not classified

Table 2.6: Effect of protein-energy malnutrition on half-life ($t_{½}$)

Drug	Number of PEM children	Age (years)	Degree of malnutrition	Mean $t_{½}$ (h)		*P* value	Reference
				Controls	PEM children		
Statistically significant increase in $t_{½}$							
Chloramphenicol	8	0–5	Undefined	12.00±0.41	≥30.00±2.20	-	Mehta *et al.,* 1982
Paracetamol	11	0–6	U, M, M-K, K	4.18±0.54	6.52±0.80	<0.05	Mehta *et al.,* 1985
Phenobarbitone	5	7–10	M-K, K	30.15±6.10	58.90±9.50	<0.05	Syed *et al.,* 1986
Sulphadiazine	6	0–5	Undefined	21.27±1.51	31.78±3.82	<0.02	Mehta *et al.,* 1980
Sulphamethoxazole[a]	7	0–2	M	4.90±0.50	9.40±2.10	<0.05	Mehta *et al.,* 1980
No statistically significant increase in $t_{½}$							
Chloramphenicol	8	4–14	Undefined	0.80±0.40	0.74±0.30	>0.05	Samotra *et al.,* 1982
Aspirin	5	0–2	Undefined	4.00±2.10	5.60±6.40	>0.05	Treluyer, *et al.,* 1991
Chloroquine	5	2–3	K	195.00±3.10	180.00±73.00	>0.05	Walker, *et al.,* 1987
Gentamicin	6	4–14	Undefined	3.31±0.30	6.21±1.21	>0.05	Samotra *et al.,* 1985
Isoniazid	13	0–7	U	1.82±0.21	1.78±0.28	>0.05	Seifart *et al.,* 1995
Phenytoin	5	7–13	Undefined	21.40±5.90	24.42±3.50	-	Bano *et al.,* 1985
Tobramycin	4	0–2	K	1.12±0.32	0.84±0.16	0.2	Buchanan *et al.,* 1978

[a]Clearance and half-life of the drug were provided in the study; clearance was apparently decreased in PEM children, but the level of significance was not provided when compared with normal control children; U= underweight; M = marasmus; K = kwashiorkor; M-K = marasmic-kwashiorkor; Undefined = PEM not classified

Four studies documented a statistically significant increase in the elimination half-life of four separate drugs: paracetamol ($p < 0.05$) (Mehta *et al.,* 1985), phenobarbitone ($p < 0.05$) (Syed *et al.,* 1986), sulphadiazine ($p < 0.02$) (Mehta *et al.,* 1980), and sulphamethoxazole ($p < 0.05$) (Mehta *et al.,* 1980), while others showed that PEM had no statistically significant effect on the elimination half-life of six of the remaining seven drugs (Bano *et al.,* 1985; Samotra *et al.,* 1985; Seifart *et al.,* 1995; Treluyer *et al.,* 1991; Walker *et al.,* 1987). One study documented an increased elimination half-life of chloramphenicol in children with poorly categorised malnutrition (Mehta *et al.,* 1982), while another study documented no statistically significant effect ($p > 0.05$) (Samotra *et al.,* 1982).

2.5. DISCUSSION

2.5.1. Number of publications and the methodology of the studies

Between 1970 and 2009, only 42 publications had examined the disposition of drugs in malnourished children. These studies are relatively few when compared to the high proportion of children affected with malnutrition globally. Malnutrition, similar to the tropical diseases schistosomiasis, onchocerciasis, lymphatic filariasis, trachoma, hookworm, roundworm, and whipworm, causes severe morbidity and mortality in sub-Saharan Africa yet remains a neglected disease (WHO, 2012). To make matters worse, there has been inadequate international funding to fight malnutrition. At the World Food Summit in Rome in 2009, the world leaders failed to commit to combating malnutrition (FAO, 2009). Also in 2009, the international assistance available to fight malnutrition was $350 million dollars, while the World Bank estimated $11.2 billion as the amount required to adequately fight the disease in the 36 most affected countries

(Médecins Sans Frontières, 2009). Since malnutrition is likely to remain a significant problem for many children, health professionals and scientists therefore have a duty to improve their understanding of how children with malnutrition handle medicines.

To study pharmacokinetics, most studies described in this review used traditional methods, which involve taking multiple blood samples from each patient. However, the ethical issues surrounding multiple blood sampling from children currently necessitate the use of alternative approaches, such as population pharmacokinetics, in which a fewer number of blood samples are collected from a larger number of patients (Thomson, 2006), or non-invasive methods, such as the caffeine breath test (Parker *et al.,* 1997a; Pons *et al.,* 1988a).

In research a power calculation is necessary to determine the minimum sample size required to yield the desired or greater effect, if such an effect truly exists in a population (Raudys & Jain, 1991). Most studies evaluated in this systematic review did not, however, perform power calculations but often instead used sample sizes of fewer than 10 malnourished children. These studies may have therefore been underpowered and unable to detect any difference. Many studies also failed to report the *p*-values comparing the data for malnourished children and their control groups. Some of the *p*-values may have actually approached significance yet significant differences remained undetected because the study population was too small. These limitations recommend that some of their data be interpreted with caution.

2.5.2. Effects of malnutrition on drug absorption

All but three drugs (gentamicin, quinine, and tobramycin) evaluated for their absorption rate in malnourished children were administered orally. Although the

p-values were not documented, the systematic review showed that the absorption rate constant for the majority of the oral drugs was unaffected in malnourished children. However, this rate was significantly decreased for penicillin and sulphadiazine in children with kwashiorkor and marasmus or marasmic-kwashiorkor. Though the physiological changes in PEM have been known to alter the absorptive capacity of the gastrointestinal tract (Gómez *et al.,* 1956b; Schneider & Viteri, 1972), there appears to have been no significant change in the absorption rate of most oral drugs reported in this review. These results contrast the significantly decreased absorption rates of xylose and glucose in PEM reported in other studies (Das *et al*., 2004; James, 1971).

Most drugs are absorbed by the intestine by either passive or facilitated diffusion and thus depend on the permeability of the mucosal membrane. Brewster *et al.* (1997b) assessed the intestinal permeability in malnourished children using a non-invasive lactulose–rhamnose (L–R) test. The geometric mean L–R ratios were significantly higher in malnourished children than in healthy control groups. A L–R ratio greater than 5 suggested a loss of integrity of the intestinal mucosa. The degree of abnormal permeability in PEM reflects the severity of the illness, and children with kwashiorkor are the most affected (Brewster *et al.,* 1997b). This finding likely explains why the absorption rate of penicillin was unaffected in underweight and marasmic children but significantly decreased in children with kwashiorkor.

Of the three intramuscular (i.m.) drugs evaluated, data on absorption rates were available for gentamicin only. Based on the limited data from a single study, it was not possible to draw a conclusion on PEM's effect on the rate of absorption of i.m. drugs.

The extent of absorption (F = $AUC_{oral/i.m}$ / $AUC_{i.v}$) represents bioavailability. This, however, was reported in only one study (Eriksson *et al.,* 1983a). Based on the limited data available to this review, it was difficult to make conclusions regarding the effect of PEM on the extent of drug absorption.

2.5.3. Effect of malnutrition on drug distribution

2.5.3.1. Protein binding

The majority of studies of protein binding presented no information about the number of patients studied and their ages, though patients were primarily children with kwashiorkor and controls were healthy adults. About 85% of the drugs demonstrated a significant decrease in their protein binding in children with kwashiorkor when compared to healthy adults.

The lack of detailed information about the patients, the use of blood samples of adults as controls, and the exclusivity of the studies to only kwashiorkor patients recommend that the data on protein binding in PEM be interpreted with caution. Although information on drug protein binding in older children is lacking, a qualitative and quantitative decrease in plasma protein binding in new-borns compared to adults has been documented (Rylance, 1981). This finding emphasises the significance of age and the use of appropriate controls in studies of protein binding in PEM.

About 70% of the drugs studied for plasma protein binding have low binding capacities (Table 2.2). The percentage change in the unbound fraction in PEM significantly increased for 17 drugs. Five of these drugs (cloxacillin, flucloxacillin, rifampicin, phenytoin, and dicoumarol) were highly protein bound. The efficiency of a drug may be affected by the degree to which it binds to plasma protein. Drugs that bind minimally to plasma protein traverse cell membranes and penetrate tissues better than their highly bound counterparts

(Scheife, 1989). Protein binding can also influence the elimination half-life of a drug, since as the bound fraction may act as a reservoir from which the drug is slowly released as the unbound fraction (Berezhkovskiy, 2010). Although the change in plasma binding was statistically significant, the clinical significance of these findings are likely relevant only to phenytoin and dicoumarol, since they were 80% to 90% protein bound. Slight changes in the binding of these drugs may influence the interpretation of their total concentrations. However, this may not result in significant changes in clinical response or toxicity unless their metabolism is altered. Previous studies have shown that the increased unbound fraction of drugs less than 80% protein bound are of slight clinical importance (Scheife, 1989).

2.5.3.2. Volume of distribution

The VDs of the majority of drugs identified in the systematic review were unaffected in malnourished children. However, malnutrition has varying effects on the VD of quinine, streptomycin, theophylline, and gentamicin (Table 2.3).

According to Thomson (2000), VD is a constant of proportionality relating the quantity of drug in the body to its plasma concentration. Generally, VD is a theoretical, not a physiological, volume into which a drug distributes in order to achieve the measured plasma concentration. VD is dependent on drug lipophilicity, the drug's plasma protein binding capacity (Øie, 1986), and tissue components such as protein (Grover & Benet, 2009). The VD of drugs that distribute primarily in body water may be increased in conditions associated with fluid retention or decreased plasma protein binding (DiPiro *et al.,* 2011). As expected, the VD for drugs of this type may be decreased during dehydration.

Despite the oedema that characterise children with kwashiorkor and the dehydration that complicates severe malnutrition, the VDs for most drugs were

not significantly affected in PEM. It may be that the oedema had subsided in children with kwashiorkor or that other severely malnourished children were well hydrated during the study. However, detailed information about the treatments received remained undocumented in the studies. Since the majority of the drugs studied have low protein binding capacities as well as slight but nonetheless statistically significant changes in their unbound fractions (Table 2.2), their VDs were not likely affected.

There were contrasting results on the effects of PEM on the VDs of quinine and gentamicin. The VD for quinine was unaffected in malnourished children without kwashiorkor (Treluyer *et al.,* 1996). It decreased significantly in malnourished children who were not properly classified (Pussard *et al.,* 1999) and was not reported in kwashiorkor children studied by Salako *et al.* (1989). While Treluyer *et al.* (1996) studied the pharmacokinetics of intramuscular quinine in malnourished and control children with malarial fever; Pussard *et al.* (1999) studied the pharmacokinetics of intravenous quinine in malnourished and healthy children without malaria. Additionally, Salako *et al.* (1989) studied the disposition of oral quinine in healthy children and children suffering from kwashiorkor, none of whom had malaria. The studies by Treluyer *et al.* (1996) and Pussard *et al.* (1999) documented a higher serum level of α_1-acid glycoprotein (AGP) than in healthy afebrile children, while Salako *et al.* (1989) did not assess the AGP level and VD but nevertheless reported decreased total plasma protein and albumin levels. Irrespective of the cause, fever has been shown to modify the disposition of quinine (Shann *et al.,* 1985; Trenholme *et al.,* 1976) and to increase the fixation of quinine to AGP (Silamut *et al.,* 1991). Other studies have documented increased AGP plasma levels in children with bacterial infection (Treluyer *et al.,* 1991) and malaria (Mansor *et al.,* 1991).

Similar to many other basic drugs, quinine binds predominantly to albumin, though AGP level is the major determinant of the variation in quinine protein binding (Wanwimolruk & Denton, 1992). It can thus be concluded that variations in the methodologies of the studies involving quinine disposition have probably accounted for the varying effects of PEM on its VD.

In two studies, it was documented that the VD for gentamicin significantly increased in a group of malnourished children who were underweight or experiencing marasmus or marasmic-kwashiorkor (Bravo *et al.,* 1992), as well as those children with kwashiorkor only (Buchanan *et al.,* 1979d). Both these above studies also documented that the clearance and elimination half-life of gentamicin were not significantly affected. Another study by Samotra *et al.* (1985) documented that the elimination half-life of gentamicin was not significantly affected in malnourished children who were poorly classified. A possible explanation for these findings is that oedema in marasmic-kwashiorkor and kwashiorkor tends to increase the body fluid within which water soluble gentamicin is distributed, given that its renal CL was unaffected. Increased VD has been reported for gentamicin in infantile sepsis (Thomson *et al.,* 2003) and oliguric renal failure (DiPiro *et al*., 2011) as a result of fluid retention that complicated the conditions.

Another study reported that the VD of amikacin was unaffected in children with kwashiorkor, as compared to healthy adult patients (Hendricks *et al.,* 1995). However, Motohiro *et al.* (1987) have documented a lower VD of amikacin in children than in adults. This discrepancy underscores the need for using well-nourished, healthy children as controls for pharmacokinetic studies of malnourished children.

Aminoglycosides are generally eliminated in unchanged forms by the kidneys. The unaffected renal elimination of aminoglycosides and the presence of oedema may explain the increased VD for streptomycin reported in children with kwashiorkor. It may also explain why the VD for streptomycin was unaffected in underweight and marasmic children (Bolme *et al.,* 1998), since both conditions are not commonly associated with oedema.

2.5.4. Effect of malnutrition on the plasma CL of drugs primarily metabolised by the liver

Eight drugs (metronidazole, caffeine, chloramphenicol, paracetamol, phenobarbitone, quinine, sulphadiazine, and sulphamethoxazole) have significantly decreased plasma CLs (Table 2.4) or increased elimination half-lives (Table 2.5) in malnourished children. Most of these drugs were studied using a single-compartment model. About 80% of these drugs are converted by the liver to their metabolites during phase I and II reactions. The disappearance of the drugs from plasma has been used to compute the parameters of $t_{½}$, VD, and CL, all of which provide an indirect estimate of hepatic function.

CYP450 enzymes are involved in the phase I metabolism of most of these drugs (Badyal & Dadhich, 2001). These enzymes are part of the mixed function oxidase reported to be functioning less optimally in malnourished children (González–Hernández *et al.,* 2008). A study using animal models has shown a 50% reduction in the activity of total hepatic CYP450 enzyme in PEM and that individual CYP450 enzymes were affected differently (Cho *et al.,* 1999). Similarly, Lee *et al.* (2004) have reported the suppression of hepatic CYP1A2 by 60%, CYP 2C11 by 80%, CYP 2E1 by 40%, and CYP 3A1/2 by 50% in rats with PEM. Studies of animals with systems closer to those of humans have also documented a significant decrease during PEM in the total microsomal haeme,

phospholipid, and flavin adenine dinucleotide levels (Rumack *et al.,* 1973), all of which are involved in microsomal drug metabolism. Malnourished monkeys have also been reported to have significantly lower quantities of microsomal protein as well as a lower activity of CYP450 reductase than healthy controls (Catz *et al.,* 1970). CYP450 reductase is an important component of mixed function oxidases in drug hydroxylation (Ortiz de Montellano & Correia, 1983).

Two other studies documented a significantly decreased total CL of quinine in poorly categorised malnourished children (Pussard *et al*., 1999) and those experiencing kwashiorkor (Salako *et al*., 1989). However, a different result was reported by Treluyer *et al.* (1996), who documented a significantly increased total CL of quinine in children with all categories of PEM except kwashiorkor. Eriksson *et al.* (1983) reported a significantly decreased total CL of chloramphenicol in children with kwashiorkor. The CL was, however, unaffected in children with marasmus and marasmic-kwashiorkor. The metabolism of both quinine and chloramphenicol depends upon the activity of uridine diphosphate glucuronyltransferase (UDPGT) (Mirghani *et al*., 2003; Miyagi & Collier, 2007). Although data are lacking in regards to humans, there are reports of significantly reduced activities of phase II metabolising enzymes in malnourished rats. Among the enzymes affected are glutathione S-transferase (GST) and UDP-glucuronosyltransferase (UDPGT) (Badary *et al.,* 2003; Zhang *et al.,* 1999). These above studies also documented that enzyme activities were reduced more in severely malnourished animals than in their moderately malnourished counterparts, which may explain the differential effects of marasmus and kwashiorkor on the CL of chloramphenicol reported by Eriksson *et al.* (1983a).

A study of the ultrastructure of hepatic tissue of severely malnourished children has revealed decreased quantity and quality of phase II hepatic

metabolising enzymes (Brooks *et al.,* 1994). Another study of the hepatic-tissue ultrastructure of children and adults with severe malnutrition also showed injuries to the organelles that are important to protein synthesis and hepatic drug metabolism (Tandon *et al.,* 1974). Such damage included dilatation of the rough endoplasmic reticulum, causing it to form vesicles or fragments; abnormality of the mitochondrial content; variation in the shape and size of the rough endoplasmic reticula; and increased glycogen and collagen levels. However, these abnormalities were completely reversible after nutritional rehabilitation.

All the drugs with decreased CLs in PEM have low extraction ratios (E < 0.3) (Hoyumpa & Schenker, 1982). These drugs were not significantly bound to plasma protein, with the exception of quinine (Table 2.2). A hepatic blood-flow change that can occur in PEM is known to have insignificant effects on plasma levels of drugs with a low E. A decrease in the intrinsic ability of the liver to clear a low extraction drug significantly increases its plasma and tissue free levels.

Based on both the decreased CYP450 enzyme activities in PEM and the decreased plasma CL or increased plasma half-life of all drugs primarily metabolised by the liver, it can be suggested that lower doses or, alternatively, less frequent administration of drugs should be observed in PEM.

2.5.5. Effect of malnutrition on the clearance of drugs primarily eliminated unchanged by the kidneys

The total CL of penicillin was significantly decreased in all of the four categories of malnutrition. However, there were varying effects of PEM on the elimination half-life of penicillin; it was unaffected in underweight children but significantly decreased in children experiencing marasmus, marasmic-kwashiorkor, or kwashiorkor. Penicillin is not metabolised by the liver, for it is eliminated

unchanged by the kidneys. Marasmus and kwashiorkor are two types of severe PEM known to be associated with diminished renal blood flow and glomerular filtration rate (GFR) (Alleyne, 1967). However, the altered renal functions are usually reversible after nutritional rehabilitation. The physiological changes in the renal system of severely malnourished children may have accounted for the observed differential results regarding the AUC (Table 2.1) and elimination half-life (Table 2.5) of oral penicillin.

PEM does not appear to affect the total CL of most drugs primarily eliminated by the kidneys, since their values remained almost unchanged in the participants and controls (Table 2.5). This was likely because most of these drugs are not usually metabolised before being eliminated unchanged by the kidneys (Faull & Lee, 2007). Decreased total CL was, however, demonstrated for streptomycin. In light of the six drugs studied, firm conclusions could not be made about the effect of PEM on renal drug elimination. Further studies are therefore required.

2.5.6. Types of drugs evaluated for disposition in PEM

This systematic review has identified pharmacokinetic studies of antimalarials, analgesics/antipyretics, antibiotics, and antitubercular drugs in PEM. Despite the strong association between malnutrition and HIV infection, the pharmacokinetics of antiretroviral drugs has not been studied in regards to PEM, which is likely due to the inaccessibility of these drugs in developing countries during the 1980s, the period when most pharmacokinetic studies were performed. Furthermore, antiretroviral drugs are rarely commenced in the acute phase of PEM management (Fergusson & Tomkins, 2009).

2.6. CONCLUSIONS

Very few studies have evaluated drug disposition in malnourished children despite the high prevalence and burden of the disease worldwide. Some of the studies lacked clear definition of malnutrition, while other studies lumped children with different categories of malnutrition into one group. In fact, only a few studies have recognised malnutrition as a disease spectrum. These limitations likely contributed to some of the variations in the results. Further studies thus need to recognise PEM as a disease spectrum and examine the differential effects of kwashiorkor and marasmus.

The total CL of most of the drugs primarily metabolised by the liver were significantly reduced in PEM, which may reflect decreased activity of the intrinsic hepatic metabolising enzymes and suggest a need to reduce drug dosage in PEM. More studies are therefore required to assess the activities of the hepatic metabolising enzymes in malnourished children.

Previous studies involved the use of traditional pharmacokinetics that required taking multiple blood samples from each patient. Ethical concerns regarding multiple blood samplings in children currently urge the use of alternative and less invasive approaches.

CHAPTER THREE:

PRINCIPLES OF THE CAFFEINE BREATH TEST AND PROTOCOL FOR USING THE METHOD TO STUDY DRUG METABOLISM IN CHILDREN WITH MALNUTRITION

3.1. INTRODUCTION

Despite the high proportion of children affected with PEM worldwide, there have been relatively few pharmacokinetic studies of drugs frequently used for the treatment of these children. Pharmacokinetic studies have since been considered invasive and have required multiple blood sampling. They also involved using both heterogeneous methods to classify PEM and small samples of participants and controls. Both by studying children experiencing coexisting diseases with the potential to influence the disposition of drugs being evaluated and by limiting the study to only one category of malnutrition, these studies were additionally limited (Oshikoya *et al*., 2010). There is thus a preferential need for alternative approaches that address these limitations, as well as examine the differential effects of marasmus and kwashiorkor on drug disposition.

This chapter discusses the aims and objectives, as well as the methodology, of the present research.

3.2. AIMS AND OBJECTIVES

Although some information exists regarding the effects of malnutrition on drug disposition in children, only a few drugs have been investigated, and no previous study has specifically determined the effects of malnutrition on the hepatic metabolism of drugs in children. Furthermore, there are no published data on

the use of probe substrates in studies of the activity of individual cytochrome P450-enzymes in malnourished children. Similarly, non-invasive methods, such as the caffeine breath test, have not yet been explored for studies of drug metabolism in malnourished children.

This study therefore aimed to use the caffeine breath test to determine the effects of different types of malnutrition on the metabolising activity of hepatic CYP450 enzymes, specifically CYP1A2.

3.3. BACKGROUND OF THE CAFFEINE BREATH TEST

Drug metabolism has been studied in adults using probe substrates for different types of CYP450 enzymes (Fontana *et al.,* 1998; Kinirons *et al.,* 1993). However, many tests are inapplicable in paediatric patients since they involve either the administration of radioactive substrates or intravenous drugs followed by multiple blood sampling, thus rendering their methods ethically unacceptable. Although the ratio of urinary 6β-hydroxycortisol:cortisol has been used to measure the activity of CYP3A4 in neonates (Nakamura *et al.,* 1998) and adults (Kinirons *et al.,* 1993; Watkins *et al.,* 1992), the ratio does not always correlate with the disposition of CYP3A substrate drugs. This finding is due to intra- and inter-individual variability in the renal clearance (CL) of cortisol (Furuta *et al.,* 2003; Galteau & Shamsa, 2003). Furthermore, the test lacks validity for use with infants and older children (Johnson, 2002).

The caffeine breath test (CBT) is a non-invasive method of studying drug metabolism, and many studies have validated its use in children (Parker *et al.,* 1994; Parker *et al.,* 1997a; Parker *et al.,* 1997b; Parker *et al.,* 1998; Pons *et al.,* 1988a).

3.3.1. Caffeine metabolism

Caffeine is a 1, 3, 7-trimethylxanthine compound with the structure presented in Figure 3.1.

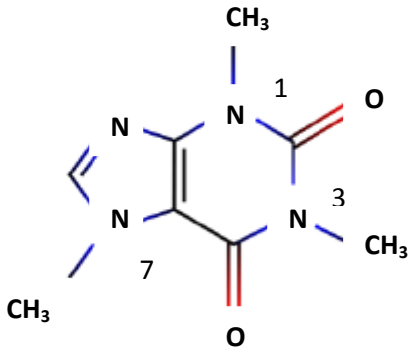


Figure 3.1: Structure of caffeine (1, 3, 7 trimethylxanthine)

Structure adapted from Chem Spider website (Available on http://www.chemspider.com/Chemical-Structure.2424.html; Accessed 25 April 2014) with kind permission of The Royal Society of Chemistry 2014

Its metabolism in the liver is performed by 1-*N*, 3-*N* and 7-*N* demethylation, as well as C-8 hydroxylation to 1, 3, 7-trimethyluric acid (Figure 3.2) (Kalow & Tang, 1993).

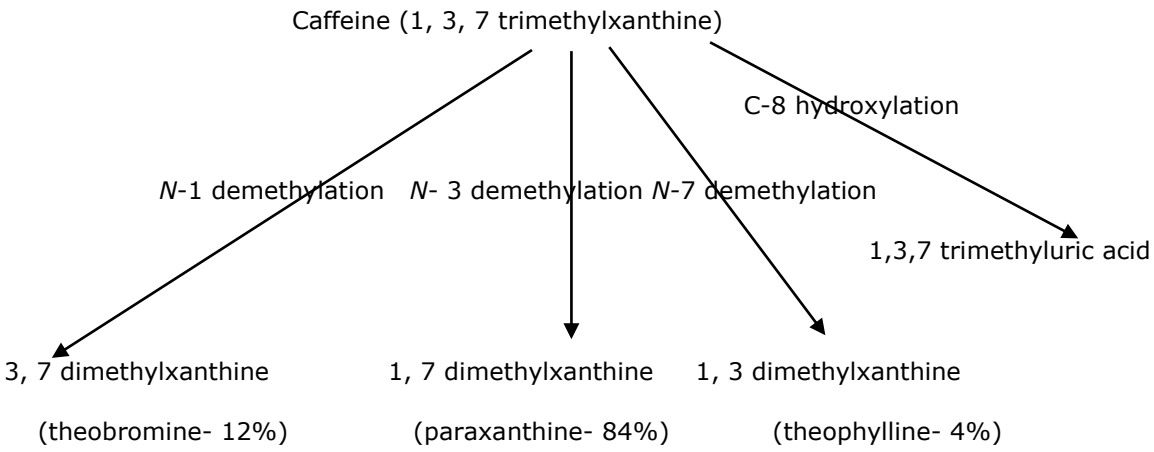


Figure 3.2: The major pathways for caffeine metabolism

Each of these metabolic pathways involves the CYP450 isoenzymes, and the 3-*N* and 7-*N* demethylation pathways account for 88% of caffeine metabolism in

humans (Notarianni *et al.,* 1995). Since enzyme CYP1A2 is responsible for the 3-*N* demethylation of caffeine (Kalow & Tang, 1993; Notarianni *et al.,* 1995), caffeine as a probe substrate has been used to assess CYP1A2 activity in both adults (Blanchard & Sawers, 1983) and children (Akinyinka *et al.,* 2000; Cazeneuve *et al.,* 1994; Fontana *et al.,* 1998; Nakamura *et al.,* 1998; Parker *et al.,* 1994; Parker *et al.,* 1997a; Parker *et al.,* 1997b; Parker *et al.,* 1998).

3.3.2. The caffeine breath test (CBT)

The CBT involves the oral administration of a non-radioactive stable isotope of caffeine (^{13}C replacing the carbon atom on the methyl group attached to *N*-3 on caffeine). The structure of labelled caffeine is presented in Figure 3.3.

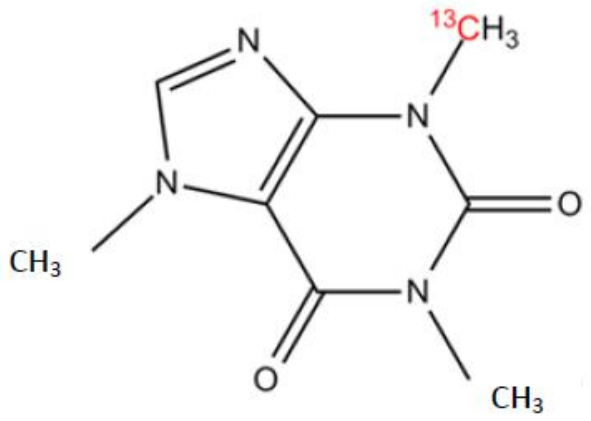


Figure 3.3: Structure of ^{13}C-labelled caffeine

Structure adapted from Chem Spider website (Available on http://www.chemspider.com/Chemical-Structure.2424.html; Accessed 25 April 2014) with kind permission of The Royal Society of Chemistry 2014

A stable isotope includes a non-radioactive atom of the same chemical element, which differs only in the number of neutrons, as there was an extra neutron (Pons & Rey, 1999). Stable isotopes occur naturally, and approximately 1% of carbon occurs in the ^{13}C form instead of the more prevalent ^{12}C. Caffeine undergoes 3-*N*-demethylation in the liver in a CYP450-dependent reaction. After

N-demethylation, the ^{13}C methyl group enters the carbon pool as it is converted to formaldehyde, formate, and bicarbonate (Lambert *et al.,* 1983). The bicarbonate is exhaled as carbon dioxide (CO_2). The exhaled $^{13}CO_2$ correlates with CYP1A2 activity (Parker *et al.,* 1994; Parker *et al.,* 1997a; Parker *et al.,* 1997b; Parker *et al.,* 1998). The CBT is non-invasive and suitable for use in young children and has been used to study the effects of disease states, including cystic fibrosis, gastritis, and epilepsy (Parker *et al.,* 1994; Parker *et al.,* 1997b; Parker *et al.,* 1998), and drug interactions in children.

Studies using the CBT were first performed by Schoeller *et al.* (1977) in the United States during the 1970s. Schoeller *et al.* (1977) showed that the precision of the CO_2 breath test using ^{13}C is limited by natural fluctuations in the ratio of ^{13}C:^{12}C in the expired CO_2. These natural fluctuations increase if the participant had eaten anything either immediately before or during the test. Therefore, Schoeller *et al.* (1977) made the following recommendations:

- The patient should fast overnight before and during the test. If fasting were impossible, the carbohydrate intake should at least be as low as possible.
- The patient should be physically inactive during the test, since activity affects endogenous CO_2 production.
- Serial respiratory CO_2 samples should be collected prior to substrate administration to determine the presence of ^{13}C.
- The collection of CO_2 should be carefully standardised to avoid variation in isotope content due to fractionation effects.

There is an optimal correlation between the plasma clearance of caffeine in infants, older children, and adults and the cumulative excretion of $^{13}CO_2$ at 2 h (r = 0.84–0.90) (Pons *et al.,* 1988b). Studies involving CBT and using three different doses of caffeine (1, 3, 5 mg/kg) have shown that increasing the

caffeine dose beyond 3 mg/kg has no effect on CO_2 excretion during the first 2 to 3 h of the test (Kotake *et al.,* 1982). The optimal dose of caffeine has, therefore, been identified as 3mg/kg, and this dose has been used in various CBT studies in children with no adverse effects (Akinyinka *et al.,* 2000; Parker *et al.,* 1994; Parker *et al.,* 1997a; Parker *et al.,* 1997b; Parker *et al.,* 1998).

CYP1A2 activity may be influenced by the age, sex, and sexual maturation of the patient (Lambert *et al.,* 1986). Such activity was high in prepubertal children and subsequently decreased in either early puberty in females or late puberty in males. In their study of caffeine *N*-demethylation maturation in infants, Pons *et al.* (1988) showed that CYP1A2 activity is minimal in infants during their first month of life. The CL of caffeine in children reaches adult rates by the age of 6 months (Pons *et al.,* 1988b). The effect of age has been addressed in previous studies of the CBT by studying children aged at least 2 years (Parker *et al.,* 1994; Parker *et al.,* 1997a; Parker *et al.,* 1997b; Parker *et al.,* 1998). CBT studies in adults (Kalow & Tang, 1993) and children (Webster *et al.,* 2002) have shown a significant inter-individual variation in CYP1A2 activity; however, no studies have used the CBT to evaluate genetic differences in such activity. A review of the use of caffeine as a probe has confirmed the validity of the CBT as a method for studying CYP1A2 activity (Kalow & Tang, 1993).

3.4. CAFFEINE METABOLISM IN MALNOURISHED CHILDREN

Previous studies have reported alterations in the CL of multi-enzymatic CYP450 substrates in malnourished animals (Adelusi & Salako, 1982; Cho *et al.,* 2001); human adults (Buchanan, 1984; Krishnaswamy & Naidu, 1977); and children (Buchanan *et al.,* 1980a; Salako *et al.,* 1989); all of these results suggest that

malnutrition may alter the activity of the drug-metabolising enzymes. Among the drugs metabolised in the liver by the CYP1A2 enzyme, total CLs were either significantly decreased (i.e., for caffeine and isoniazid) or their elimination half-lives significantly increased (i.e., for paracetamol and phenobarbitone) in malnourished children (Oshikoya *et al*., 2010). These results suggest an alteration in the CYP1A2 metabolic activity in children with malnutrition.

The pharmacokinetics of caffeine has also been studied in children with malaria and kwashiorkor to show that its oral absorption rate, measured as C_{max} and t_{max}, was not significantly affected in children with malaria and kwashiorkor, though hepatic CL significantly decreased (Akinyinka *et al.,* 2000). Paraxanthine is a metabolite of caffeine; its plasma level has been used to measure the CYP1A2 activity after caffeine administration (Akinyinka *et al.,* 2000). The significantly decreased plasma levels of paraxanthine in children with malaria or kwashiorkor suggest a significantly decreased CYP1A2 activity in these patients. Therefore, alternative methods that generate paraxanthine or quantifiable by-products during caffeine metabolism can be used to measure CYP1A2 activity in children with malnutrition.

The activity of CYP1A2 can also be measured by using the production rate of caffeine metabolites through 3-*N*-demethylation as catalysed by CYP1A2. This alternative method is the CBT.

It is important for studies involving the use of a probe substrate to examine the effects of malnutrition on drug metabolism in order to address the differential effects of marasmus and kwashiorkor on drug metabolism. To address this question, the present research devised a method to use the CBT in order to measure CYP1A2 activity and to determine the effects of underweight

malnutrition, marasmus, marasmic-kwashiorkor, and kwashiorkor on caffeine metabolism.

3.5. TRAINING ON BREATH SAMPLE COLLECTION

I undertook observational training on breath sample collection at the pathology clinic of the Royal Derby Hospital in Derby, U.K. The steps involved in breath sample collection are (i) positioning the bottle in the hands, (ii) removing the cap of the exetainer bottle, (iii) placing a straw at the end of the exetainer bottle, (iv) taking a full breath and exhaling through the straw directly into the tube, (v) withdrawing the straw from the exetainer bottle before the exhalation is complete, and (vi) screwing the cap "finger- tight" immediately after its removal from the exetainer bottle.

The breath sample is collected in a specialised container called an Exetainer® vial. Breath samples were collected at 11 time points: -20, -10, -1, 15, 30, 45, 60, 75, 90, 105, and 120 min post caffeine ingestion for each patient. The patient's name or ID number and date of sample collection were recorded on the vials. Each breath sample was collected in duplicate, thus requiring the use of 22 vials per patient.

A straw was placed in the participant's mouth with the Exetainer® vial at the lower end. The participant was instructed to take a full breath and exhale through the straw directly into the vial. It was ensured that the breath was not forced. Immediately prior to the exhalation's being completed, the vial was drawn away from the straw and the vial cap was immediately screwed onto the vial until finger-tight. Overtightening the cap can deform the seal in the cap,

which may cause air leakage.

3.6. TRAINING ON BREATH SAMPLE ANALYSIS

Eight sets of the breath samples collected at the outpatient clinic during my training at the outpatient clinic were analysed at the Pathology Laboratory of the Royal Derby Hospital. The data generated from this observational study were intended only for training and thus excluded from this study.

The ^{13}C enrichment of breath $^{13}CO_2$ was determined by a continuous flow isotopic ratio mass spectrometer (Sercon Automated Breath Carbon Analyser [ABCA-MK5]; Europa Scientific Inc., Crewe, UK). An ABCA-MK5 is an elemental analyser, in which samples of breath nitrogen, oxygen, and CO_2 are separated prior to analysis. Exetainer® vials containing breath samples were arranged in a tube rack with the capacity to hold four sets of samples, which was placed in a tray attached to the machine. A set of eight samples was separated by two reference samples (5%CO_2) previously calibrated against a bicarbonate standard of known ^{13}C enrichment. Breath samples contained within the Exetainer® vials were sampled in a sequential order through a needle attached to the machine. Autosampling involves the machine's automatic injection of 500 µl aliquots of breath sample into its analyser, in which the breath's CO_2 is dried via a water trap and resolved from nitrogen and oxygen by gas chromatography. Using helium as a carrier, the purified CO_2 was passed into an electron impact ion source of the isotope ratio mass spectrometer.

Following ionisation, the ion beams were separated according to their mass/charge (m/z) ratio into masses 44, 45, and 46 m/z and collected by triple collector Faraday cups. For each mass detected, the machine recorded an area proportional to the number of ions contained in real time. The machine

automatically calculated the drift correction between the two sets of references, and the drift correction was applied to calculate the excess atom per cent (AP) of the unknown breath sample. The results were obtained as AP, which represented the absolute ^{13}C enrichment present in the sample. AP was automatically calculated by the software using the formula: A (atom %) = ($Ratio_{sample}$ / (1 + $Ratio_{sample}$) × 100. The difference between the enrichments of the breath samples taken at baseline and plateau were expressed as atom per cent excess (APE).

3.7. PILOT STUDY ON A HEALTHY ADULT VOLUNTEER

A pilot study was conducted and involved a breath sample collection from an adult volunteer. The healthy male volunteer (30 years old, 66kg) was advised to cease eating from 19:00 hours on the night before the study until the study's completion. The weight was measured on the day of the study using a Seca Scale (model 707; Seca Limited, Birmingham, UK); a standiometer was used to measure the height (Holtain, Crymych, UK). After fasting overnight, the participant ingested labeled caffeine solution (4mg/kg, dissolved in distilled water). Prior to the test, the participant abstained from caffeinated products for a period from 48 to 72 h, approximately. The participant neither smokes nor drinks alcohol.

The participant ingested labelled caffeine at 09:00 hours and thereafter blew air via a straw into Exetainer® vials. Three breath samples, in duplicate, were collected before caffeine administration (pre-dose) at -20, -10, and -1 min. Serial breath samples were further collected after caffeine ingestion, in duplicate, at 15 min intervals over a period of 2 h.

A set of the samples was analysed at the Pathology Laboratory of Royal Derby Hospital (A), while the other set was analysed at the Clinical Physiology Laboratory at the Medical School in Derby (B). The results were compared.

The samples were analysed in duplicate 2 to 4 d after collection using a continuous flow isotope ratio mass spectrometry (CF-IRMS), Sercon-Automated Breath Carbon Analyser (ABCA-MK5), and an AP2003 Mass Spectrometer at the Laboratories A and B, respectively. Results were generated for the ^{13}C-enrichment in delta notation. The mean pre-dose (baseline) enrichment was calculated by determining the mean of the enrichments in delta unit at -20, -10, and -1 min (Table 3.1). The mean ^{13}C-enrichment was expressed as atom percent (At%) excess (APE) of ^{13}C concentration excess by subtracting the mean pre-dose enrichment from each of the post-dose enrichments. Medical tracer studies of human physiology are usually reported in units of APE, which indicates the level of isotopic abundance above baseline. The baseline reading in At% was subtracted from the experimental value to give APE.

Table 3.1: Variation in the mean ^{13}C- enrichment of exhaled CO_2 over a 2 hour period

Time (minute)	Mean ^{13}C-enrichment of exhaled CO_2 (At %) Hospital Lab (A)	Mean ^{13}C-enrichment of exhaled CO_2 (At %) Medical School Lab (B)
Baseline	-24.68	-25.73
15	-22.45	-22.93
30	-22.27	-22.73
45	-21.90	-22.39
60	-21.77	-22.42
75	-21.41	-22.56
90	-21.24	-22.40
105	-21.24	-22.40
120	-21.32	-22.44

Table 3.2: Variation in the mean atom percent of [^{13}C] excess in the exhaled CO_2 over a 2 hour period

Time (minute)	Mean^{13}C-enrichment of exhaled CO_2 as At % [^{13}C] excess (A) × 10^{-3} (APE)	Mean^{13}C-enrichment of exhaled CO_2 as At % [^{13}C] excess (B) × 10^{-3} (APE)
15	2.45	3.08
30	2.65	3.30
45	3.06	3.67
60	3.20	3.64
75	3.60	3.49
90	3.78	3.66
105	3.78	3.66
120	3.70	3.62
Mean± S.D	**3.28± 0.52***	**3.52± 0.21***

*There was no significant difference in the mean values, *P*=0.115, using 2-tailed student *t*-test

The ^{13}C-enrichment varies with the time of breath sample collection (Table 3.2). Figure 3.4 shows the time course of the ^{13}C-enrichment in APE for the participant based on data from the two laboratories. The ^{13}C-enrichment in the exhaled CO_2 appeared rapidly, increased gradually, and peaked after 90 min. The curves were similar for both laboratories and typical of the time course reported in previous studies involving a healthy adult (Park *et al.,* 2003). This suggests a rapid hepatic metabolism of caffeine by CYP1A2 in the participant.

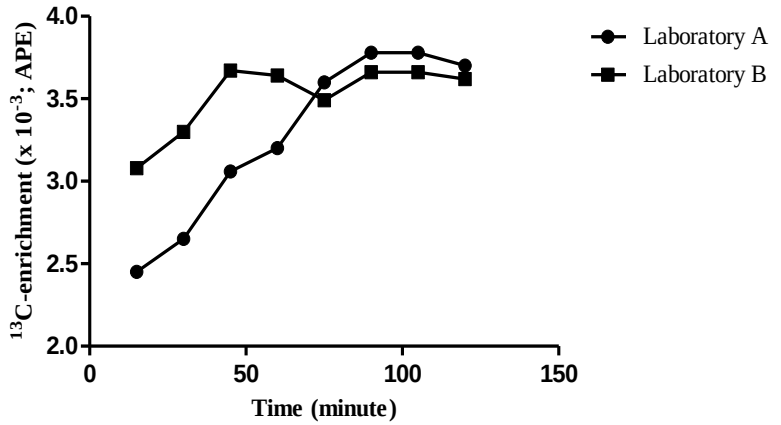


Figure 3.4: Time courses of ^{13}C-enrichment for a healthy adult subject

Abnormal inter- and intra-laboratory variability has been reported in tracer studies when the same breath sample was analysed in the same or a different laboratory (Perri *et al.,* 2003). This variability may, however, affect the CBT results and lead to inappropriate clinical decisions. Although there was an inter-laboratory variability in the results from the two laboratories (A and B), as indicated by the mean ^{13}C-enrichments for the participant ([3.28 ± 0.52] × 10^{-3} APE versus [3.52 ± 0.21] × 10^{-3} APE, respectively), there was no statistically significant difference in the ^{13}C-enrichment values (p = 0.115) using a two-tailed Student's *t*-test.

3.8. SETTING

Malnourished children were recruited from Lagos and Kano, Nigeria. As for the first, Lagos is the smallest but most populous state in Nigeria and, according to the 2006 national census, has an estimated population of 17.5 million (Lagos State Government, 2010). It consists of five administrative divisions: Lagos Island, Ikorodu, Badagry, Ikeja, and Epe. Two teaching hospitals—the Lagos University Teaching Hospital (LUTH) and Lagos State University Teaching Hospital (LASUTH)—and many general hospitals serve the people of Lagos. Children were specifically recruited from four paediatric centres in Lagos: LASUTH; Massey Street Children's Hospital; General Hospital, Ikorodu; and General Hospital, Gbagada. As for the second, Kano is one of the largest and most densely populated Nigerian states. It hosts several general hospitals and two tertiary hospitals: Aminu Kano Teaching Hospital (AKTH) and Murtala Mohammed Specialist Hospital. Malnutrition is rife in Northern Nigeria, particularly in Kano. In order to meet the sample size required for this study, additional patients were recruited from AKTH.

3.9. MANAGEMENT OF MALNUTRITION IN LAGOS AND KANO STATES IN NIGERIA

Children with PEM were managed at all the paediatric centres used in this study according to the 10 steps recommended by the World Health Organization (WHO) (Ashworth *et al.,* 2004). PEM was diagnosed on the basis of patient history, findings from physical examination, and laboratory investigations. Anthropometric measurements (weight and height) and the presence or absence of oedema were used to classify the patients into different categories of malnutrition according to the Wellcome Trust Working Party (WTWP) classification.

Underweight children (i.e., with mild to moderate PEM) were recruited from the outpatient clinics among children who presented features of acute uncomplicated malaria. They were treated for malaria with artemisinin-based combination therapy and nutritionally managed at home by their parents, as well as followed up by their doctors at the outpatient clinic. The severely malnourished children (i.e., with kwashiorkor, marasmic-kwashiorkor, and marasmus) were admitted for specialised hospital ward management.

3.9.1. Acute management

Upon admission, severely malnourished children were routinely investigated for full blood count (FBC); genotype; random blood sugar (RBS); serum protein (total and albumin); electrolyte, urea, and creatinine (EUCr); blood culture; stool for ova and parasites; as well as by urinalysis, chest radiograph, and HIV screening. These investigations enabled the doctors to identify the clinical complications and diseases coexisting with PEM. The investigations were followed by empirical treatment with broad spectrum intravenous antibiotics

(amoxicillin/gentamicin, cefuroxime/gentamicin, or any of the third generation cephalosporins). The choice of antibiotics was informed by the clinical presentation of the patients and the high resistance to ampicillin and chloramphenicol currently experienced in Nigeria.

Due to the high prevalence of parasitic infestation in Nigeria, patients were frequently treated prophylactically with broad spectrum oral antihelminthics, specifically mebendazole.

Patients with poor appetite were passed a nasogastric tube for small and frequent feeds, usually every 2 or 3 h. The diet was made by the hospital nutritionist from pap derived from locally processed maize or guinea corn. The pap was usually fortified with dairy milk, soybeans, dried fish, crayfish, groundnut and palm oil (a diet referred to as 'kwashi pap').

Potassium and micronutrient supplements (vitamin A and trace elements including copper, zinc, selenium, and manganese) were routinely administered orally to patients after feeds. All of these compounds were contained in the Astymin® supplement, which was routinely prescribed to the patients.

3.9.2. Nutritional rehabilitation

After becoming clinically stable, those patients on nasogastric (NG) tubes were initially weaned off of the NG tube and continued oral feeding with a kwashi pap. A week later, they were weaned on adult feeds. All patients were weighed twice weekly at regular intervals and their heights checked weekly in order to monitor their growth. Adult feeds were rich in high calories and protein and were offered daily in increasing amounts. As the clinical condition of the patients improved, their appetites increased and necessitated a further increase in protein and calorie intake, both of which enabled them to approach normal growth patterns.

The duration of hospital admission varied with each patient and the

severity of malnutrition. Patients were hospitalised and rehabilitated nutritionally for an average of 1 month. Thereafter, they were discharged after: (i) exhibiting an excellent appetite; (ii) consistently gaining weight corresponding to height increases; and (iii) attaining a weight-for-age ±3 *SD* of the WHO child growth chart standards, for which the cut-off point for malnutrition is weight-for-age below -3 *SD*) (de Onis *et al.,* 2007; WHO, 2006).

Vulnerable patients whose parents were incapable of taking good care of them after discharge were referred by the attending doctor to a welfare home where they would receive support and care from the government. This group of patients were excluded from the study.

3.9.3. Follow-up

Nutritional rehabilitation continued at home after discharge and after the nutritionist had taught the children's mothers the most ideal and hygienic ways to prepare the child's diet. All patients were followed up at home and at the outpatient clinic by me. During this period, their heights, weights, and physical examinations were routinely checked as indicators of normal growth and well-being. Any of the patients that took ill during follow-up were evaluated and treated accordingly by the attending doctors.

3.10. PATIENT RECRUITMENT

Malnutrition was defined in the patients according to the WTWP classification. Altogether, 60 patients between aged from 3 to12 years who were underweight and/or experiencing marasmus, kwashiorkor, or marasmic-kwashiorkor were recruited. There were 15 patients in each group of malnutrition. The study population was determined from a power calculation based on previous CBT

studies in children (see section 3.16 on statistical analysis). PEM children in the above age range were used for the study given their ability to follow the instructions for each step involved in breath sample collection. Moreover, a similar age group was studied in previous studies of CBT involving children (Parker *et al.,* 1994; Parker *et al.,* 1997a; Parker *et al.,* 1997b; Parker *et al.,* 1998).

Patients with severe PEM (marasmus, kwashiorkor, or marasmic-kwashiorkor) were studied upon admission and during the first week of commencing treatments, so as to allow a recordable improvement in their conditions. Children with kwashiorkor were studied after oedema had subsided, since oedema may exaggerate the actual weight of the patients. The malnourished children also were re-studied after 4 to 8 weeks of nutritional rehabilitation. Underweight children were studied as outpatients.

The clinical signs and laboratory findings of infection are usually masked in PEM; therefore, underweight patients were given oral amoxicillin while other patients with severe PEM received intravenous amoxicillin and gentamicin upon admission for 5 days. This is a standard empiric antibiotic therapy frequently used in Lagos and Kano for treating PEM.

3.11. STUDY DESIGN

Parents who consented to their child's participation in the study or older children who gave their assent were informed about the study procedure, which lasted 2 to 3 h. Parents and older participants were additionally informed that the usual diet would be replaced with Casilan-90® during these hours. Parents were requested to keep their child in bed before and during the study in order to

minimise physical activity. Parents had the option of withdrawing their child at any stage of the procedure.

The participants' demographic and anthropometric parameters, including age, weight and height, were recorded during the first presentation for the study and during follow-up for restudy. All participants abstained from caffeinated products, including cola drinks, chocolate, and herbal medicines containing cola, for at least 20 h (overnight). Ideally, participants would fast for at least 4 h prior to the CBT procedure. This is, however, ethically unacceptable for children with PEM; therefore, all children received Casilan-90® 0.5 h prior to the study. Casilan-90® is a bland, high-quality protein powder derived from milk. It is very low in fat and carbohydrates (both less than 1%) (BNF for Children, 2006). It contains no added sugar, artificial sweeteners, colours, flavours, or preservatives, making it an ideal supplement to many drinks and foods to boost the protein content without altering the flavour.

Underweight participants were studied as outpatients, but since severely malnourished participants required initial hospital admission for acute management, they were studied as inpatients when clinically stable. The stages of severe PEM management and the timing of CBT are presented in a flow chart in Figure 3.5.

An appointment was given to each patient for 08:00 hours on different days between Mondays and Fridays. Participants fasted overnight (i.e., ate no additional food after a light protein diet for supper) and did not consume anything orally on the appointment day. On the day of the study, each participant was attended to in a private consulting room in the presence of the parents, if any. Body weight was measured (to 0.1kg) using a digital Seca scale (model 876, Seca Ltd., Birmingham, UK) as participants wore only underpants.

Height was measured (to within 1mm) using a Holtain stadiometer (Holtain, Crymych, UK) as the participants stood barefoot. Vital signs, including blood pressure, heart and respiratory rates, and temperature, were recorded and monitored throughout the study.

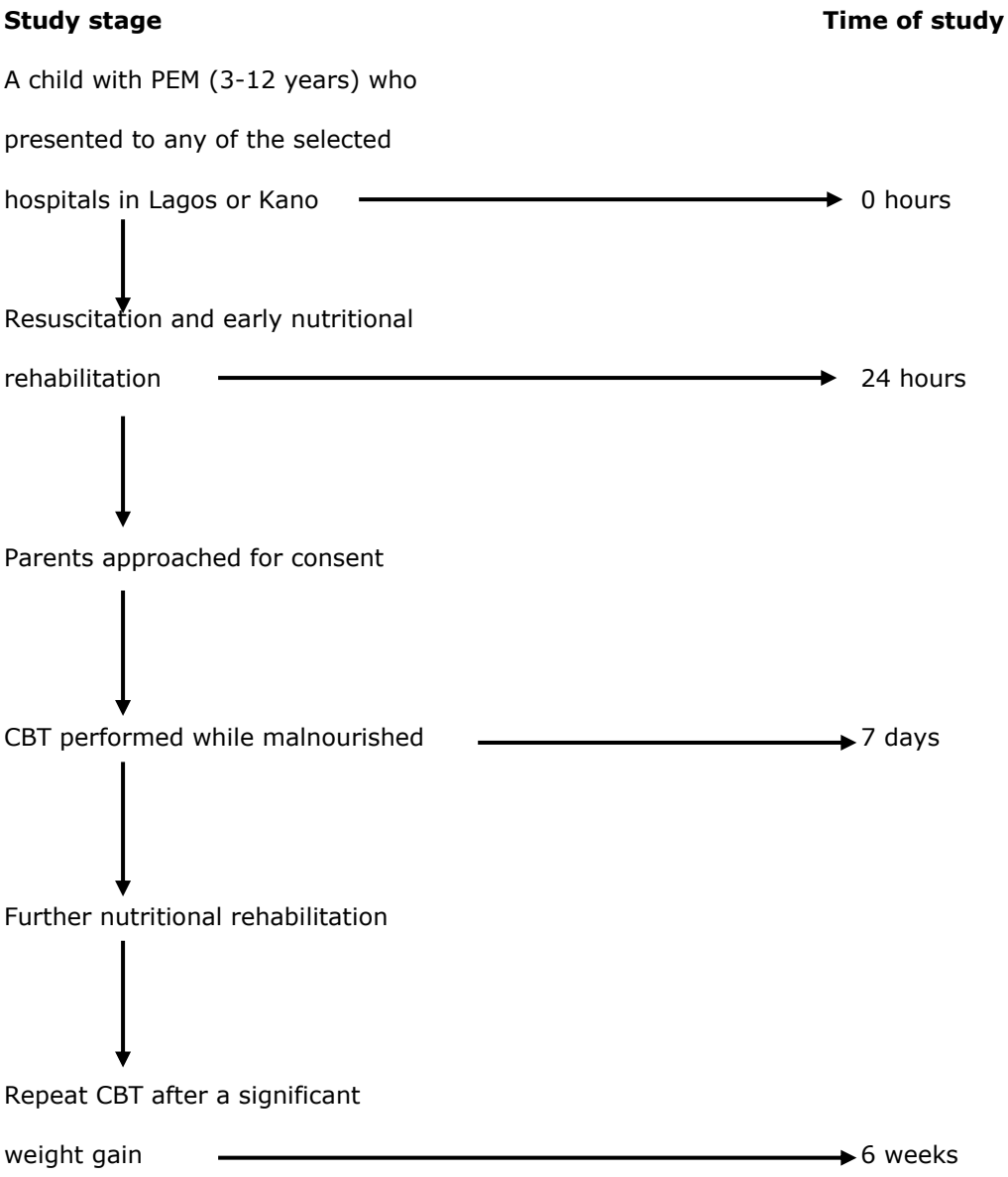


Figure 3.5: Flow chart for the stages of PEM management and time of study

Casilan-90® was administered to each participant 0.5 h prior to caffeine administration, according to the Recommended Dietary Allowance (RDA) (children 3 to 6 years old: [90 kcal × kg^{-1}]; children 7 to 10 years old: [70 kcal × kg^{-1}]) (National Research Council, 1989).

Participants sat quietly for 0.5 h before and throughout the CBT in order to minimise physical activity, which can influence endogenous CO_2 production and affect CBT results. At 09:00 hours, participants ingested 3 mg/kg of [3-methyl-^{13}C] caffeine (99% ^{13}C) obtained in powder form from Cambridge Isotope Laboratories (Cambridge, MA, USA).

Caffeine quantity was determined using an electronic sensitive balance (Ohaus Pioneer Analytical and Precision balance; Ohaus Corporation, Pine brook, NJ, USA). Each sample of caffeine was dissolved in 10 mL of sterile water and mixed with sugar-free squash to mask its bitter taste and followed by a 20 mL water wash of the container. The quantity of caffeine consumed was approximately equivalent to the amount present in cola drinks.

Paired breath samples were collected during normal expiration at -20, -10, and -1 min prior to caffeine (pre-dose samples) and after caffeine ingestion (post-dose samples) at 15 min intervals over a period of 2 h. Breath samples were collected by having each participant blow via a straw into an Exetainer® vial. Participants were observed for signs of caffeine toxicity with a detailed symptom assessment, while blood pressure and respiratory and heart rates were monitored every 0.5 h during testing.

Participants were restudied after nutritional rehabilitation and attainment of weight-for-age and weight-for-height *Z*-scores ±3 *SD* of the WHO child growth chart standards (de Onis *et al.,* 2007; WHO Multicentre Growth

Reference Study Group, 2006). Caffeine administration and breath sample collection during the restudy phase adhered to the aforementioned protocol.

Breath samples were couriered in batches to the Clinical Physiology Laboratory of the Medical School in Derby, University of Nottingham, for analysis.

3.11.1. Inclusion criteria

- Children aged from 3 to 12 years;
- Expression of any of the 3 categories of PEM in a stable clinical condition;
- Informed consent of the participant and/or participant's parent(s).

3.11.2. Exclusion criteria

- Clinical or investigational evidence of tachypnoea, heart failure, overwhelming sepsis, shock, prolonged diarrhoea, and/or vomiting (as recorded during routine medical examination or as documented in the case file of the patient);
- HIV infection;
- Underlying respiratory, cardiovascular, renal, gastrointestinal, or central nervous system disease;
- Use of ventilation support;
- Cerebral palsy, visual or hearing impairment, or other severe complications;
- The participant's or the participant's parent's unwillingness to provide consent.

3.11.3. Withdrawal criteria

- Failure to gain weight-for-age of ±2 *SD* after 6 weeks of nutritional rehabilitation;
- Deliberate withdrawal either by the participant or the participant's parent after the participant's being studied in the acute phase of PEM management.

3.12. ETHICAL CONSIDERATIONS

The study protocol was approved by appropriate ethics committees at the Lagos State Health Service Commission (the body in charge of the management of all the General hospitals in Lagos), Lagos State University Teaching Hospital, and Aminu Kano Teaching Hospital (see Appendices I–III). All parents of the participants received both oral and written information about the study (see appendix IV) and were requested to give their written informed consent (see appendix V) before their child participated in the study. Older children were requested to give their assent (see appendix VI) in addition to the informed consent of their parents.

3.13. ANALYTICAL PROCEDURE

3.13.1. Validation of the stability and reliability of the breath samples

A preliminary analysis of the breath samples from 10 underweight children was performed at the Clinical Physiology Laboratory by the laboratory manager. This analysis involved the use of analytical precision continuous flow isotopic ratio mass spectrometer (AP2003 CF-IRMS; Analytical Precision Products Ltd., Cambridge, UK). A detailed description of the machine is provided in section 3.13.2.1. Data generated from the preliminary analysis were excluded from the final data analysis. The purpose of the preliminary analysis was to check for the stability and reliability of the breath samples. This analysis showed that four of the underweight children had taken either food or caffeinated products prior to the study. This was suggested by the excessively high AP dose of labelled caffeine determined by the breath samples. It was, however, confirmed from the four children affected that they had ingested something prior to the study. This therefore informed the decision to admit the underweight children to the hospital

24 h prior to the study, which enabled their intake to be monitored and ensured that they fasted overnight.

3.13.2. Proper sample analysis

All the samples for the study were analysed at the Clinical Physiology Laboratory at the Medical School in Derby by using the AP2003 CF-IRMS, which has both software and hardware components similar to those of the Sercon Automated Breath Carbon Analyser used to analyse the samples during the pilot study. Their modes of operation are also similar.

3.13.2.1. The breath analyser

The AP2003 CR-IRMS analysed the breath CO_2 isotopically. Its main features are shown in Figure 3.6.

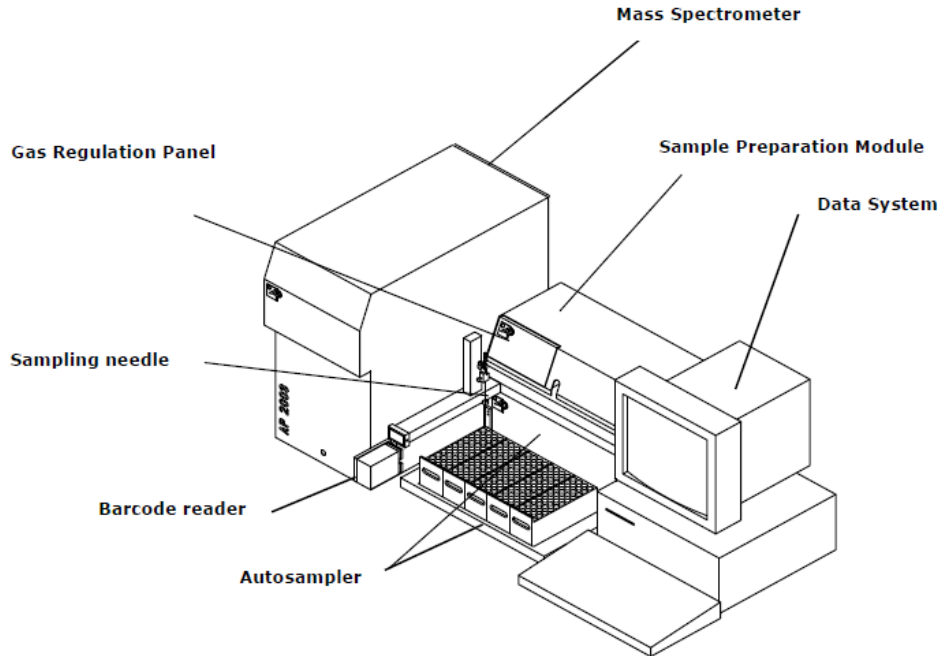


Figure 3.6: The Breath analyser system

It is composed of the mass spectrometer, in which ionisation occurs; the sample preparation module, in which CO_2 is separated from the other constituents of whole breath; the autosampler, onto which the breath sample tubes are

positioned; and the data system, which controls the entire system.

3.13.2.2. Tests prior to running samples

Two important pre-programmed tests (i.e., peak centre and stability tests) were routinely performed before running the samples. These two tests ensured that the mass spectrometer was both working to specifications and stable. Running the peak centre scan yielded the curve shown in Figure 3.7.

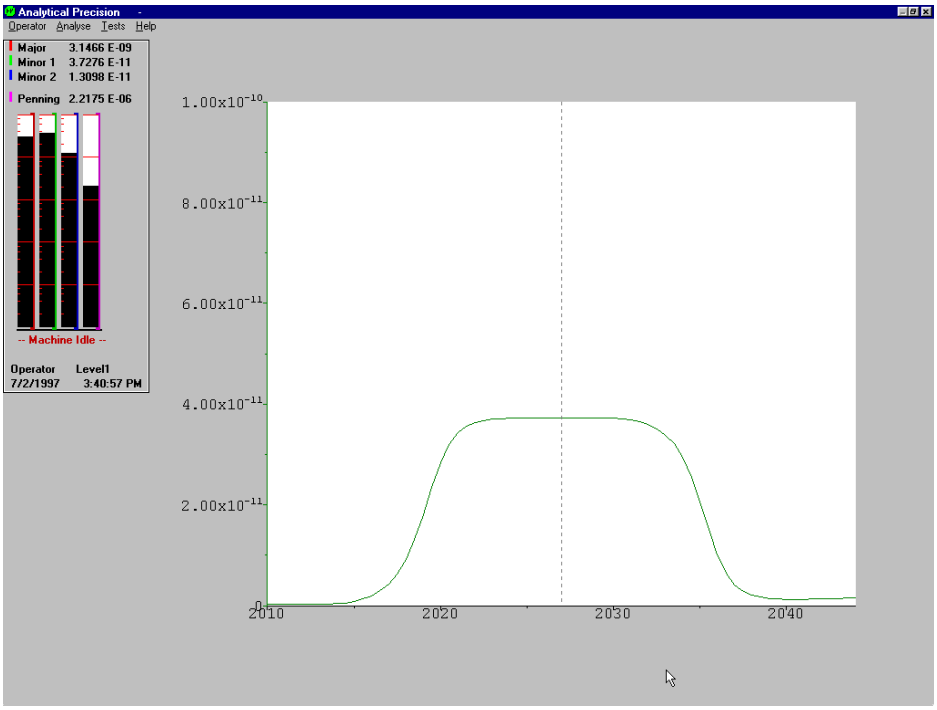


Figure 3.7: Peak centre scan recording

A normal curve shows an approximately symmetrical peak and a flat portion occurring for at least 4 V. The height of the plateau should be approximately 2-6E-11 A, and the vertical cursor should position itself close to the centre of the peak. If any of these conditions were not fulfilled, the help of the laboratory manager was sought. This check was performed at the start of every batch analysis and repeated after every 24 sample analyses during a batch analysis. The stability of the instrument was also tested after every 60 sample analyses.

A stability test was initiated after a satisfactory peak centre scan test. Ten

pulses of reference gas were injected into the mass spectrometer, which later appeared as 10 rectangular peaks equally spaced across the acquisition window as shown in Figure 3.8. This test was performed at the start of every batch analysis. A normal result showed 10 pulses that were rectangular with flat tops, all of the same height, each approximately 5E-9 A. The *SD* of the ratio of the delta values for the two reference gases was automatically calculated by the software. A normal value is usually less than 1E-6. If either of these two conditions was not fully met, the help of the laboratory manager was sought.

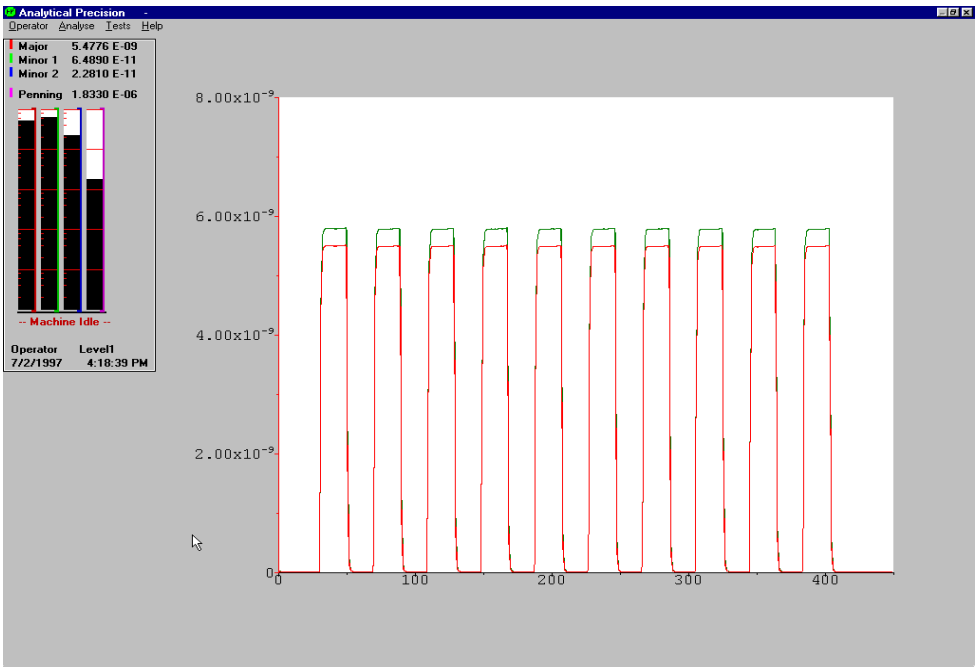


Figure 3.8: Stability test recording

3.13.2.3. Quality control

The machine runs predetermined peak centring and stability checks to optimise analytical parameters to allow large batches of samples to be run without any need for constant monitoring of the instrument. Any fault during an analysis is detected and, since the software either rectifies the fault if it is of a simple nature or aborts the analysis, any wastage of samples is prevented. There was

an additional complete check of the quality of the results throughout the analysis of a batch of samples, thus ensuring their validity. The quality control parameters were defined at the same time each batch was created and were saved with both all other details of each particular batch and the results for the analysis of samples.

The full quality report was checked at the end of every batch analysis or at any necessary time. A sample was declared 'bad' if the height of the CO_2 peak was below the threshold specified in the file for the integration of the peak. This height was normally set to 1E-9 A for the major ion beam. If this situation occurs, the software indicates such with the message 'No sample peak found'. This can occur for a number of reasons, which may not necessarily be due to a system malfunction. Possible causes include no tube loaded in the particular autosampler hole, an improperly filled tube, an empty tube, and the repeated analysis of a tube sample. If three consecutive tubes were 'bad', the malfunctioning instrument was suspected and the run was automatically aborted.

3.13.2.4. End of batch summary

The batch summary was printed automatically for every batch. It provided a sequential list of samples; their data file names, their names, their IDs, their positions, and results. The list was in the order that the samples were analysed. Results from the batch summary included the pre-dose (baseline) and post-dose ^{13}C enrichments in AP.

3.13.2.5. Aliquot sampling of whole breath from an Exetainer® vial

After the routine running tests, the needle was automatically driven down into the Exetainer® vial. The vial was exposed to the pressure set on the sample tube pressure regulator (PR), thus equilibrating with this pressure. After the

pressurisation period, the breath aliquot passed through a water removal device before entering the sample loop. The standard loop size fitted to the system in the sample preparation module has a volume of 120 μl. Correct filling of the sample loop was essential; if partially filled, there may have been an error in the isotopic measurement. The sample loop was constructed of a semi-permeable membrane, which allowed water to pass from the wet gas stream inside the conduit to the outer surface of the membrane, from where it was carried by a dry stream of helium to another vent. The dry breath sample trapped within the volume of the sample loop flowed toward the short chromatography column (GC), which was maintained at room temperature. In the GC, CO_2 was separated from O_2 and N_2, both of which eluted together before the CO_2 gas peak.

A small amount of pre-calibrated CO_2 was delivered into the effluent of a column and moved downstream of the column. A pulse of reference gas was injected in this manner within each sample analysis a short time after the passage of the sample CO_2. The delta value of the sample CO_2 was calculated in comparison to the reference gas pulse. The amount of CO_2 gas delivered to the column effluent depended on the pressure set at the pressure regulator.

Breath samples were run in batches, initially in a single run, which allowed any saturated breath samples to be identified. Saturated breath samples refer to breath samples with excess CO_2 saturating the detector and yielding incorrect isotopic ratios. These breath samples were de-saturated by withdrawing 1 mL to 3 mL of air from the vial with a hypodermic needle connected to a syringe by a two-way valve. Batches of the unsaturated breath samples were then re-run in duplicate to improve data accuracy.

3.14. PRELIMINARY CHECK FOR STABILITY OF THE BREATH SAMPLES IN NIGERIA

The high temperatures in Nigeria require that biological samples be stored by refrigerating, freezing, or using liquid nitrogen. Although previous studies of CBT have indicated that breath samples are stable at room temperature in developed countries (Webster *et al.,* 2002), no such data is available for developing countries. It was therefore necessary to perform a preliminary analysis of the breath samples to check for their stability in Nigeria.

Four underweight children, who were excluded from the main study, were studied on two occasions at 1 week intervals. Participants were studied according to the protocol of the main study (see section 3.11). Breath samples were collected from each participant in duplicate and kept on an open shelf for 6 weeks before being transported to the U.K for preliminary analysis. Breath samples were analysed by the laboratory manager, a senior research fellow in clinical physiology, at the Clinical Physiology Laboratory at the Medical School in Derby within the University of Nottingham. The breath samples were analysed immediately upon arriving in the U.K., as per the analytical procedure outlined in section 3.13.

CBT results for these four participants are presented in Table 3.2. There was no significance difference in the results of the CBT for each participant when compared to the samples collected at a 1 week interval (participant I: p = 0.0030; participant II: p = 0.0002; participant III: p = 0.0078; and participant IV: p = 0.0009) using Student's t-test statistics. These results suggest that the breath samples for each participant were stable over the 6 weeks of open shelf storage in Nigeria.

Table 3.3: Variation in the mean ^{13}C- enrichment of exhaled CO_2 over a 2 hour period by underweight children

Time (minute)	Mean ^{13}C-enrichment of exhaled CO_2 (At %)							
	Subject I Age: 7.25 years weight: 20kg Height: 119 cm		Subject II Age: 5 years weight: 18kg Height: 105 cm		Subject III Age: 3.5 years weight: 11 kg Height: 99cm		Subject IV Age: 4.2 years weight: 15kg Height: 108 cm	
	A	B	A	B	A	B	A	B
Baseline	-24.68	-24.73	-22.31	-22.52	-24.68	-23.74	-23.84	-23.42
15	-22.45	-23.45	-22.43	-22.82	-20.49	-20.86	-19.76	-21.72
30	-22.27	-22.73	-22.33	-22.14	-19.36	-19.75	-17.39	-21.13
45	-21.90	-22.09	-19.15	-18.43	-19.12	-19.15	-18.86	-19.13
60	-21.77	-21.42	-18.06	-19.06	-19.87	-18.39	-19.11	-17.47
75	-21.41	-21.56	-18.44	-18.13	-19.64	-18.46	-17.47	-19.84
90	-21.24	-21.04	-17.50	-18.35	-19.04	-20.21	-18.33	-19.24
105	-21.24	-21.40	-17.37	-17.51	-20.13	-19.09	-19.29	-18.42
120	-21.32	-21.08	-16.21	-17.17	-20.42	-20.51	-17.36	-19.13
P- value	0.352		0.234		0.365		0.157	

A represents first breath test, B represents the second breath test conducted a week later; results of A and B were compared using student's paired *t*-test

3.15. MATHEMATICAL CALCULATIONS AND ASSUMPTIONS

The ^{13}C enrichment of exhaled CO_2 was expressed in AP. The results were expressed as ^{13}C APE (i.e., each of the post-dose enrichments minus the mean pre-dose enrichment). A cumulative $^{13}CO_2$ output was calculated from the measured ^{13}C APE from the eight breath samples taken during the first 2 h following intake of ^{13}C caffeine multiplied by the mean CO_2 output over the period (assumed to be 300 mmol / body surface area [BSA] × time [h]) (Haycock *et al.,* 1978; Shreeve *et al.,* 1970). The value obtained was expressed as a percentage of the caffeine dose.

3.16. STATISTICAL ANALYSIS

Student's paired *t*-test at a significance level of $p < 0.05$ was used to compare data obtained from each participant group both when malnourished and after nutritional rehabilitation. The results of mean percentage of labelled caffeine exhaled as $^{13}CO_2$ both before and after nutritional rehabilitation for children with marasmus, marasmic-kwashiorkor, and kwashiorkor were compared using repeated measures ANOVA at a significance level of $p < 0.05$. Post hoc comparisons, using Tukey's test, were performed if the ANOVA test yielded no significant difference in the mean percentage of labelled-caffeine exhaled as $^{13}CO_2$ both before and after nutritional rehabilitation for all groups of malnutrition. A sample size of 15 patients per group of PEM has a power of 90%, assuming a difference of 2% in the mean ±*SD* score of the 2 h percentage of cumulative ^{13}C from the baseline to 3 to 6 weeks after nutritional rehabilitation to be significant at the 5% level (Parker *et al.,* 1997a). A 5% withdrawal assumption was made to calculate the population size.

3.17. CONCLUSION

This study aimed to determine the effects of different types of malnutrition on drug metabolism by conducting four CBT studies in underweight children and those with marasmus, marasmic-kwashiorkor, and kwashiorkor. The main outcomes measured were the mean percentage of labelled caffeine exhaled as $^{13}CO_2$ both before and after nutritional rehabilitation in each group of malnourished children. The level of significance differences between the mean values both before and after nutritional rehabilitation were compared using a Student's *t*-test and ANOVA and followed by Tukey's post hoc test.

CHAPTER FOUR:

THE CHALLENGES OF CONDUCTING PAEDIATRIC CLINICAL PHARMACOLOGY RESEARCH IN NIGERIA

4.1. INTRODUCTION

Paediatric clinical pharmacology research in Nigeria has been infrequent due to the lack of formal training in this specialisation. The few publications in this specialisation emanating from Nigeria focus on clinical trials and the pharmacoepidemiology of antimalarial drugs (Ayede *et al.,* 2010; Gbotosho *et al.,* 2011; Salako *et al.,* 1989; Walker *et al.,* 1987).

The primary goal of paediatric clinical pharmacology is to advance the clinical use of medicines in children (Hoppu & Kearns, 2013). To improve the use of medicines in children with malnutrition, I conducted a study of drug metabolism in malnourished children using the caffeine breath test (CBT). Previous studies involving the use of CBT to evaluate drug–drug interactions in children to determine the effect of disease on drug metabolism and to assess the developmental changes in CYP1A2 activity were conducted in high-income countries (Levitsky *et al.,* 1989; Parker *et al.,* 1994; Pons *et al.,* 1988b). This trend derives from the high cost of the stable isotope (Webster *et al.,* 2002).

The literature on the challenges of conducting clinical pharmacology research in developing countries focuses mostly on the ethics of clinical trials. Such challenges include delays in obtaining ethical approval for studies (Mbuagbaw *et al.,* 2011), difficulties in obtaining informed consent from participants (Chaisson *et al.,* 2011), an inability to recruit the required number of participants (Alem & Kebede, 2003), a lack of human resources, and inadequate funding (Mbuagbaw *et al*., 2011). In the present research, many

challenges were encountered, most of which related to poverty and illiteracy. Despite the challenges, this non-invasive study was well received by parents and paediatricians.

The challenges and benefits faced during the study are highlighted in Table 4.1 and followed by a brief discussion.

Table 4.1: The challenges and prospects of conducting clinical paediatric pharmacology research in Nigeria

Challenges
Economy and political instability
Delay in getting ethical approval from the institutional review board or ethics committee
Gender dominance of fathers in consenting to child participating in a clinical research
Lack of parental awareness of non-invasive studies involving the use of caffeine breath test in children
Lack of nutritional rehabilitation centres in the local communities
Inadequate manpower and infrastructures
Poverty and financial incapacitation of the parents
Non-compliance to the research protocol, especially feeding and fasting instructions
Lateness in keeping appointments
High cost of clinical research
Prospects
Acceptability of the caffeine breath test to the children and their parents
Opportunity to collaborate and network with local researchers
Safety of the breath samples collected without requiring a special storage system and/or electricity

4.2. CHALLENGES

Prior to commencing the study, I collaborated with paediatricians in the study centres to identify the challenges and to offer solutions in order to ensure the successful conduct of the research in Nigeria. Many of the challenges are characteristic of all resource-poor countries and include economic and political

instability, delays in obtaining research ethical approval, difficulties in obtaining informed consent, participants' lack of knowledge of clinical research, inadequate manpower, poverty, difficulties in following research instructions, and the high cost of research. Other challenges, such as lack of centres for nutritional rehabilitation and tardiness in keeping appointments, are peculiar to Nigeria.

4.2.1. Delays in obtaining ethical clearance

The process of obtaining ethical clearance involved an initial submission of a research proposal to each of the hospitals in October 2010. Unfortunately, the study received ethical approval only five months later in Lagos and seven months later in Kano. Delays in obtaining ethical approval in Lagos mainly derived from bureaucratic slowness and elaborate procedures involved in the review processes. By contrast, the lack of an expert in clinical pharmacology at Aminu Kano University Teaching Hospital (AKTH) who could assess the proposal was responsible for a delay in obtaining ethical approval. It was implied by the secretary of the ethics committee that an expert of clinical pharmacology from another institution had been contacted to assess the proposal.

Alem and Kebede (2002) identified bureaucratic slowness and delays in obtaining the approval of national and institutional research protocol review committees as a major challenge facing researchers in Ethiopia. Delays in obtaining ethical approval not only frustrate many local researchers but also discourage many foreign sponsors from supporting studies in resource-poor countries (Alem & Kebede, 2002; Olopade *et al.,* 2012). In some developing countries, the structures and procedures for ethical review of a research proposal may be lacking or underdeveloped due to a lack of appropriately trained personnel and financial resources. This lack may complicate obtaining an

appropriate or valid ethical review of proposals for clinical research in some resource-poor countries (Nuffield Council on Bioethics, 1999).

Lack of experts in clinical pharmacology at AKTH, therefore, underscores the need for training in paediatric clinical pharmacology in Nigeria and other African countries, which has long been advocated by the World Health Organization (WHO) and experts in paediatric clinical pharmacology in both North America and Europe (Hoppu, 2011). Training in this specialty will hopefully improve and hasten the assessment of future research proposals for paediatric clinical pharmacology for ethical consideration in Nigeria.

4.2.2 Economic and political instability

Rising poverty, decay in public utilities and infrastructures, social tensions, political turmoil, and ethnic conflicts and violence have resulted in economic and political instability in Nigeria (Fagbadebo, 2007). Collecting breath samples earmarked for September 2010 proved infeasible, due to the coincidence of a doctors' strike in public hospitals in Lagos. The doctors demanded improved healthcare facilities in all general hospitals in the state, as well as the full implementation of the new salary structure approved by the Nigerian government (Nigerian Tribune, 2011). Unfortunately, the strike lasted 6 months and ceased only by March 2011. The breath sample collections would have otherwise been completed during this period, had the General Hospitals in Lagos been in operation. However, the study was unavoidably extended by 1 year, which resulted in additional research costs.

In January 2012, Nigeria experienced a national strike of all workforces due to an abrupt increase in the pump price of petrol by the Nigerian government in an effort to hasten the deregulation of the petroleum sector (*BBC News Africa*, 2012). The strike coincided with the finalisation of research in

Nigeria and the dispatch of the breath samples to the U.K by courier. The flight schedules were frequently changed due to the strike and affected my return date, as well as prevented the timely arrival of the samples in the U.K., which consequently delayed the analysis of the breath samples there.

Although industrial strikes are common worldwide, they are more common in resource-poor countries, since general strikes are the only means by which workers express dissatisfaction with their working conditions in order to achieve their goals (Dhai *et al.,* 2011). Similar factors, including civil unrest, incessant and protracted industrial strikes, and incessant power outages, have been recognised as a few of the key challenges affecting the successful conduct of foreign-sponsored clinical research in resource-poor countries (Olopade *et al*., 2012)

Considering these unforeseen circumstances, future clinical paediatric pharmacology research requiring collaboration between high- and low- or medium-income countries, particularly Nigeria, should allow for extra funding and time to accomplish their studies.

4.2.3 Patriarchal dominance in granting consent

Men in many parts of African countries exercise power over women. Men make decisions on behalf of women or revoke their decisions in matters that affect the family (Sen *et al.,* 2007). Often, only the mothers were available to care for their children during hospital admissions. In the present research, the mothers initially signed 42 (70%) of the 60 informed consent forms. Of the 42 consent forms signed by the mothers, the fathers challenged (20; 48%) and revoked (5; 12%) a significant number of the consent forms. Consequently, at the early stages of the study, five patients withdrew and were replaced by another five patients. The dates for sample collections repeatedly changed for eight children

due to a conflict of ideas between the parents. In two cases, a family feud ensued between the parents and resulted in their spousal separation, thus complicating follow-ups with the affected children during nutritional rehabilitation. It is therefore necessary for researchers to be aware of the tension that may arise in families in obtaining a possible participant's parent's consent to be included in clinical research studies.

The autonomy of an individual to consent to participating in a clinical research study may not be present in some developing countries due to the cultural and social structures of their communities (Nuffield Council on Bioethics, 1999). In such communities, an individual is seen as belonging to a family or a community and therefore must meet with the elders, their parents, or other children in the community before consenting to any clinical research or medical treatment (Frimpong–Mansoh, 2008; Nuffield Council on Bioethics, 1999). The present data are in agreement with the report of Marshall *et al.* (2006), who observed that women often obtain the consent of their husbands before they or their children participate in a clinical research study (Hyder *et al.,* 2004). This stands in contrast to the practice of developed countries, where mothers are considered capable of providing consent for themselves and their children (Hyder & Wali, 2006).

A strategy adopted to overcome the problem of informed consent was to inform both parents, or the mother and any of the father's relations, about the study, as well as to ensure that one of the parents signed the consent form. Of the 60 children involved in the study, the fathers (23, 38.3%), mothers (29, 48.3%), or both parents and the father's relation (8, 13.3%) signed the consent form.

4.2.4. Lack of parental awareness of non-invasive studies

The concept of CBT is new in Nigeria, and none of the parents approached for consent had either heard about CBT or seen it performed on adults or children. Therefore, parents were sceptical about the nature of the research, despite being provided with a detailed explanation of the procedure. The level of understanding of the study by sceptical parents was poor due to their low literacy levels.

In sub-Saharan African countries, over 21 million adolescents had dropped out of school in 2007, and only 34% of the secondary school age group were enrolled; this was thus the lowest enrolment ratio in the world (Education for All Global Monitoring Report, 2010). It also has been reported that about 38% of the adult population in sub-Saharan Africa (i.e., 153 million adults) lack the basic literacy and numeracy skills required for everyday activities; over 60% of these illiterate adults were women (Education for All Global Monitoring Report, 2010). Nigeria is among the top 10 countries in the world with the highest levels of adult illiteracy (Education for All Global Monitoring Report, 2010). Assessments of informed consent comprehension in previous clinical trials have shown that many participants were unaware of their being enrolled in the studies due to inaccurate or incomplete understanding of the consent forms (Chaisson *et al.,* 2011; Hill *et al.,* 2008). Factors contributing to the poor understanding included the participants' unfamiliarity with clinical research, poor level of education, anticipation of the clinical benefits of the research, and the verboseness and complexity of the consent forms (Beardsley *et al.,* 2007; Berger *et al.,* 2008; Christopher *et al.,* 2007; Paasche–Orlow *et al.,* 2003). However, the confidence and maximum cooperation of the parents were

successfully gained by providing repeated explanations and demonstrations of the study procedures.

4.2.5. Lack of nutritional rehabilitation centres

Nutritional rehabilitation centres are necessary to the management of malnourished children and have been recommended as part of the management protocol by WHO (WHO, 1999). Unlike other African countries, where many nutritional rehabilitation centres exist to take care of the dietary needs of malnourished children (Brewster *et al.,* 1997b; Giugliani *et al.,* 2010), none are available in Lagos. Therefore, patients were recruited from four general hospitals and a teaching hospital in Lagos, as well as from another teaching hospital in Kano. The task, therefore, involved travelling between the hospitals, despite the distance between them. Given both the high poverty levels and high population of Nigeria, a relatively low proportion of severely malnourished children have presented for hospital care in Lagos, which has led to the erroneous belief of local paediatricians that severe malnutrition is becoming rare in Lagos.

The few cases of severe malnutrition admitted to the study centres had no opportunity to enjoy complete nutritional rehabilitation before being discharged. This trend was due to inadequate bed space for children and the priority of treating children with other life-threatening, acute medical conditions. Admissions were provided only for emergency management of severely malnourished children. They were usually discharged when clinically stable and visited at home for follow-ups. A large proportion of the parents could not adequately feed their children after hospital discharge, thus again putting the children at risk of relapse of malnutrition. Many of the patients (25%) were initially lost to follow-up, though through the provision of incentives to the

parents, such as transport money, mobile phone Top-Up cards, and breakfast, their attitudes to keeping follow-up appointments improved over time.

Inadequate infrastructure has also been identified as a major challenge for the conduct of good clinical research in resource-poor countries (Olopade *et al.,* 2012).

4.2.6. Inadequate manpower

The majority of malnourished children recruited for the present research lived in areas far from the hospitals, which are also poorly accessible. This inaccessibility likely contributed to the initial loss of 20% of patients for follow-up. It, therefore, became necessary to visit the participants' homes for follow-ups and to monitor their feeding habits, weight gain, and well-being. The tasks of performing follow-up visits for the patients in their homes and recruiting new patients from the hospitals were too enormous for a single person to handle. Specific training in clinical research involving the use of CBT is not offered in postgraduate medical education in Nigeria. Furthermore, poor knowledge of clinical research and lack of infrastructure for highly technological research studies have been recognised as negative impacts on the quality of clinical research emanating from resource-poor countries (Olopade *et al.,* 2012).

Based on the lack of both researchers experienced in paediatric clinical pharmacology and paediatricians' specific interest in core paediatric research studies, a research nurse from Lagos assisted with breath sample collections.

4.2.7. Poverty of the parents

Poor social status of the parents of the children studied corroborated that malnutrition is a disease of poverty in developing countries (WHO, 2004). Procuring medicine for these children and for aiding their feeding was difficult due to the poor financial status of the parents. Food and money for medicines

were initially provided to the parents, though these aids were not judiciously utilised. The parents often distributed the medicines and foods to other children in the family, leaving the participants in the study with poor weight gain and delayed recovery from concurrent infections. In the present studies, feeding was later arranged for the participants twice daily in the hospital and feeding between meals at home was suggested to the parents. These practices helped the participants to attain the expected weight in 4 to 8 weeks, which was faster than the 3 to 4 months taken to attain the expected weight for malnourished children nutritionally rehabilitated in Karachi, Pakistan, with locally available foods (Akram *et al.,* 2010).

4.2.8. Noncompliance to feeding and fasting instructions

Many of the children involved in the study lacked adequate supervision from their parents or guardians. A preliminary analysis of the breath samples in the U.K. showed that four of the underweight children had ingested either food or caffeinated products before the study. This was suggested by the excessively high values of per cent dose of caffeine determined in their breath samples. Subsequent interviews with the children revealed that they had ingested food prior to the studies, which partly explains why obtaining accurate data may be more difficult for research studies conducted in resource-poor settings than in high-income countries (Alem & Kebede, 2003).

Through persuasion and repeated dissemination of information to the parents, the children were allowed to be admitted to the hospital for 24 h prior to the study, which enabled their intake to be monitored and ensured that they ingested nothing after their dinners and prior to the time of the study.

4.2.9. Tardiness in keeping appointments

Eight parents refused the admission of their underweight child prior to the study and could not keep the early morning appointments during the study. Reasons alluded for the tardiness included lack of money for transportation, waking up late, lack of time to take care of other children, and uncooperative nature of the child on the day of the study. This resulted in frequent changes to the day of the CBT for individual children. These factors are among those previously identified by Olopade *et al.* (2011) as major setbacks in conducting clinical research in developing countries. However, the problem was overcome by arranging for a taxi to pick up the child and his or her parent on the day of the study, thus further increasing the research costs.

4.2.10. Cost of research

The cost of this study was nearly £20,000 (Table 4.2). The budget for the research was initially £9,000; additional costs (approximately £11,000) were, however, incurred due to the unforeseen circumstances resulting from the prolonged study period.

Table 4.2: Cost of performing caffeine breath test in Nigeria

Item	Cost estimate in Great Britain Pounds (£)
Economy flight to and from Nigeria on three occasions	1,642.88
Labelled-caffeine procurement	4,401.50
Exetainer® bottle procurement	814.68
Weighing balance procurement	823.26
Seca lightweight scale procurement	219.75
DHL to courier breath samples from Nigeria, and to courier Exetainer® bottles and an official letter to Nigeria	1,845.61

Table 4.2: continued

Train ticket from Derby to London on three occasions and a return ticket from London to Derby on one occasion	330.48
Accommodation in Nigeria for 9 months	3,450.00
Nutritional rehabilitation of underweight children	2,100.00
Local flight and transportation in Nigeria, and Casilan® procurement	3,848.30
Total	**£19,476.46**

4.3. BENEFITS

In developed countries, the CBT has been used in various disciplines, such as gastroenterology and clinical pharmacology, to assess the severity of hepatic diseases (Park *et al.,* 2003) and to evaluate the activity of hepatic CYP1A2 in diseases such as cystic fibrosis (Parker *et al.,* 1997a). The approach has been used in the present research to determine the effect of malnutrition on drug metabolism. As such, the present research is the first of its kind conducted in a resource-poor country.

Despite the enormous challenges of conducting clinical research in Nigeria, the data generated in this study will contribute to advancing the knowledge of malnutrition and its therapeutic management. Other positive aspects of the CBT are: (i) its acceptability to children and their parents; (ii) its ability to foster collaboration and networking between a paediatric clinical pharmacologist and other clinical researchers in Nigeria; and (iii) the safety of its breath samples without refrigeration or the use of a special storage system.

4.3.1. Acceptability of the caffeine breath test (CBT)

The fact that the present studies were non-invasive and lasted only 2 h made it acceptable to both the participants and their parents. The approach was also

acceptable to the doctors involved in the management of the children. Similar acceptability has been reported among children and their parents involved in previous CBT studies (Parker *et al.,* 1994; Parker *et al.,* 1997b). The attitudes and knowledge of the parents of the malnourished children improved as the study progressed. Parents were able to discuss the details of the study with other parents whose children did not participate in the study. The parents were eager to learn the outcome of the study and they appreciated our extending the CBT to other childhood diseases. All parents were willing to allow their children to participate in future studies involving the use of the CBT.

4.3.2. Collaboration and networking

The nurses, pharmacists, and doctors involved in the care of the malnourished children in all centres involved with the study applauded the idea of using the CBT for the non-invasive study of drug metabolism in children. Though they found the procedure to be uncommon in Nigeria and other African countries, a considerable proportion of the healthcare professionals expressed a willingness to collaborate in this area of research. One of the doctors suggested that the CBT be extended to children with sickle cell anaemia, due to their nutritional deficiencies. Such collaboration should enhance the use of CBT for future clinical paediatric pharmacology research in Nigeria.

4.3.3. Safety of breath samples

The breath samples required storage over a period of 6 to 12 weeks preceding their transportation to the U.K. for analysis. Despite the fragility of the Exetainer® vials, none broke and no air leaked from the vials during transportation. A preliminary analysis of the breath samples (see section 3.13) yielded reliable data suggesting that the high temperatures in Nigeria did not

adversely affect the breath samples. This is an additional advantage of using the CBT in African countries for the research of paediatric clinical pharmacology.

4.4. CONCLUSION

In summary, though the challenges of effectively conducting paediatric clinical pharmacology research in Nigeria are many, they are surmountable. Studies involving the use of CBT are feasible in Nigeria and acceptable to both children and their parents. The use of the CBT promises to foster collaboration and networking between experts in clinical paediatric pharmacology and other healthcare professionals in paediatrics. There is additionally the prospect of using the CBT to study the effects of other tropical childhood diseases on drug metabolism. However, effective paediatric clinical pharmacology research requires economic and political stability, government commitment, the education of the parents, adequate funding, and collaboration with high-income countries.

CHAPTER FIVE:

RESULTS

5.1. EFFECTS OF UNDERWEIGHT ON CAFFEINE METABOLISM

The demographics and anthropometric parameters of the 15 underweight children are presented in Table 5.1.

Table 5.1: The demographics and anthropometric parameters of the underweight children

Number of subject	Gender	Age (years)	Pre-nutritional rehabilitation		Duration of nutritional rehabilitation (weeks)	Post-nutritional rehabilitation	
			Weight (kg)	Height (cm)		Weight (kg)	Height (cm)
1	F	4.2	13.0	99.3	2	15.0	99.3
2	M	4.3	11.0	92.0	2	13.6	93.5
3	F	4.5	13.0	96.0	3	15.0	97.5
4	F	5.0	14.0	107.0	3	15.0	109.0
5	M	5.0	12.0	98.5	3	14.8	100.0
6	M	5.5	13.0	106.5	3	16.0	107.8
7	M	6.0	13.0	102.0	3	16.6	103.0
8	F	7.5	18.0	119.0	2	20.0	120.0
9	M	8.0	16.0	112.5	4	21.0	113.0
10	M	10.0	22.0	129.5	6	26.4	131.0
11	M	10.0	25.0	133.9	2	27.0	133.9
12	M	10.0	25.0	130.0	4	28.0	130.5
13	M	11.0	23.0	132.0	6	30.2	133.5
14	M	11.5	26.0	137.2	6	31.0	139.0
15	M	12.0	24.0	135.5	6	31.0	136.0
Mean+ s.d		**7.6± 0.9**	**17.9± 5.6**	**115.4± 6.3**	**3.7±1.6**	**21.4± 6.8***	**116.5± 6.2***

*(*P*<0.005; Student's t-test)

Eleven male and four female children were studied. Their mean age was 7.6 ± 2.9 years. Their mean weight significantly increased from 17.9 ± 5.6 kg (before nutritional rehabilitation) to 21.4 ± 6.8 kg (after nutritional rehabilitation) ($p < 0.005$). Similarly, their mean height significantly increased from 115.4 ± 16.3 cm (before nutritional rehabilitation) to 116.5 ± 16.2 cm (after nutritional rehabilitation) ($p < 0.005$). They were restudied after a mean period of 3.7 weeks following nutritional rehabilitation.

The typical time courses of mean ^{13}C-enrichments (substrate in atom per excess [APE]) for the underweight children investigated both before and after nutritional rehabilitation are shown in Figure 5.1. The curves were obtained by plotting the mean ±*SD* of the delta values at 15, 30, 45, 60, 75, 90, 105, and 120 min for the 15 participants against time.

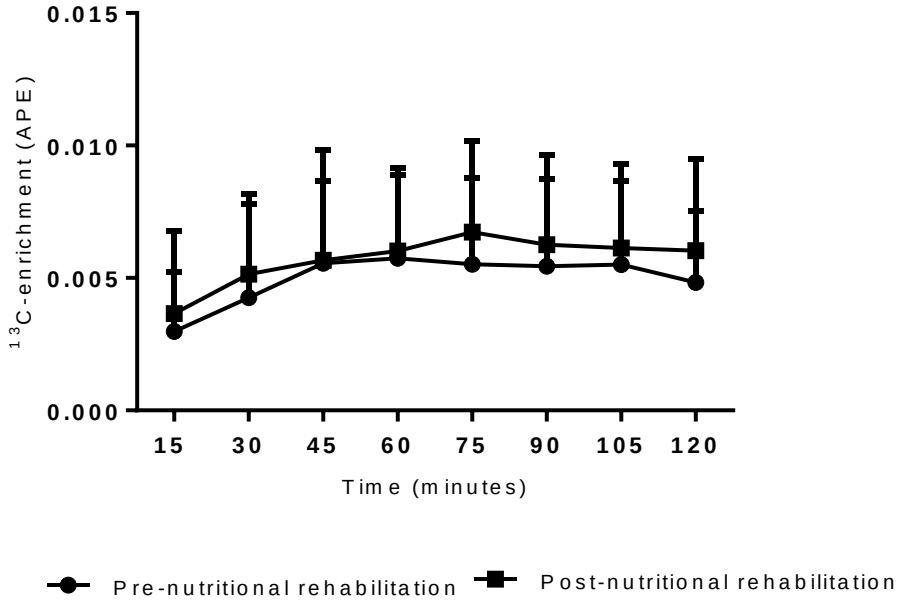


Fig. 5.1. Time courses of mean± SD ^{13}C-enrichments of underweight children, before and after nutritional rehabilitation. APE represents ^{13}C in atom per excess abundance (see Appendix VII for the raw data).

Both curves showed a gradual increase in the mean ^{13}C-enrichments up to the peak values, which was followed by a gradual fall. The mean peak ^{13}C-enrichments were attained at 0.00740 ± 0.00350 APE before nutritional rehabilitation and 0.00694 ± 0.00345 APE after nutritional rehabilitation. There was no significant difference in the mean peak ^{13}C-enrichments (p = 0.691). The peaks were attained at 61.00 ± 23.28 min and 76.00 ± 26.87 min before and after nutritional rehabilitation, respectively. The difference between the two periods in attaining the mean peaks of ^{13}C-enrichments was not statistically significant (p = 0.060).

The mean areas under the enrichment-time curve before and after nutritional rehabilitation were 0.539 ± 0.320 APE min and 0.620 ± 0.322 APE min, respectively. The difference between the two values was not statistically significant (p = 0.528).

The average cumulative per cent ^{13}C-doses recovered (CPDR) in the exhaled CO_2 over the period of 2 h of the underweight children are shown below in Table 5.2. The mean cumulative per cent ^{13}C-dose recovered was 7.56 ± 4.01% and 7.95 ± 3.68% before and after nutritional rehabilitation, respectively, and there was no significant difference in the mean values (p = 0.603). The individual cumulative per cent labelled caffeine dose exhaled as CO_2 in the period of 2 h by each underweight child both before and after nutritional rehabilitation is presented in Figure 5.2.

Table 5.2: Average cumulative percent ^{13}C-dose in the exhaled 2-hour CO_2 of underweight children, before and after nutritional rehabilitation

Number of subject	Pre-nutritional rehabilitation			Time interval between study I and II (weeks)	Post-nutritional rehabilitation			Change after nutritional rehabilitation (B-A) (%)
	Labelled CO_2 output over the 2hr study period (mmol/hr)	Average percent $^{13}CO_2$ exhaled (studyI)	Cumulative percent ^{13}C-dose (Caffeine) recovered in the 2 hr CO_2 exhaled (%) (A)		Labelled CO_2 output over the 2hr study period (mmol/hr)	Average percent $^{13}CO_2$ exhaled (studyII)	Cumulative percent ^{13}C-dose (Caffeine) recovered in the 2 hr CO_2 exhaled (%) (B)	
1	251.44	0.04230	**10.64**	2	232.82	0.05708	**13.29**	2.65 (24.9)
2	283.53	0.01391	**3.94**	2	251.34	0.02519	**6.33**	2.39 (60.7)
3	254.84	0.04083	**10.41**	3	234.51	0.05352	**12.55**	2.14 (20.6)
4	234.57	0.03194	**7.49**	3	224.37	0.05611	**11.34**	3.85 (51.4)
5	263.35	0.04455	**11.73**	3	233.85	0.05029	**11.76**	0.03 (0.3)
6	244.56	0.00332	**0.81**	3	217.67	0.00910	**1.98**	1.17 (14.4)
7	248.78	0.00557	**1.39**	3	217.29	0.00893	**1.94**	0.55 (39.6)
8	196.46	0.03276	**6.44**	2	185.02	0.02886	**5.34**	-1.10 (-17.1)
9	214.02	0.01971	**4.22**	4	184.58	0.02714	**5.01**	0.79 (18.7)
10	170.55	0.05025	**8.57**	6	153.92	0.03742	**5.76**	-2.81 (-32.9)
11	157.12	0.10210	**16.04**	2	150.75	0.05645	**8.51**	-7.53 (-46.9)
12	158.98	0.05382	**8.56**	4	149.35	0.06501	**9.71**	1.15 (13.4)
13	165.26	0.05325	**8.80**	6	142.11	0.05397	**7.67**	-1.13 (-12.8)
14	152.37	0.03678	**5.60**	6	137.90	0.04880	**6.73**	1.13 (20.2)
15	159.86	0.05460	**8.73**	6	139.10	0.07268	**10.11**	1.38 (15.8)
Mean+ s.d			**7.56±4.01**	3.7±1.6			**7.95±3.68**	

The cumulative per cent labeled caffeine dose exhaled as CO_2 showed an increase in 10 children, a decrease in four children, and remained relatively unchanged in one child after nutritional rehabilitation (Table 5.2 and Figure 5.2). There was greater inter-individual variation before nutritional rehabilitation than after such rehabilitation.

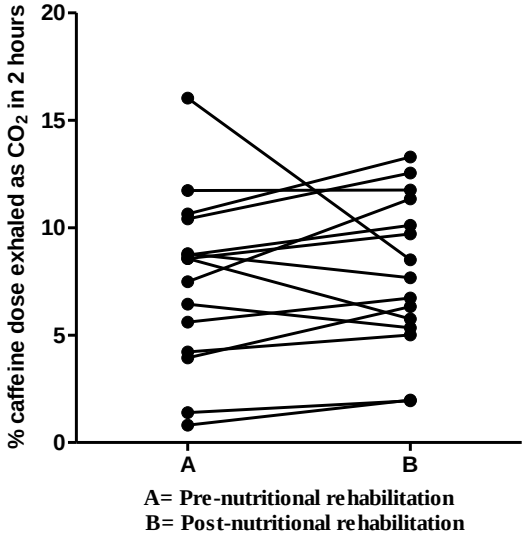
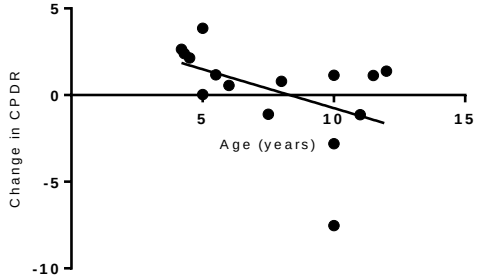
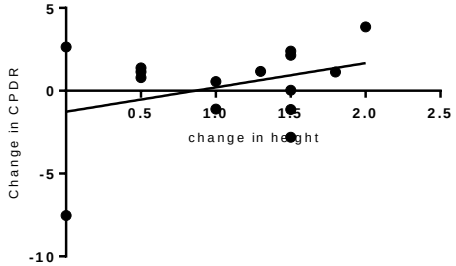


Fig. 5.2. Individual cumulative percent ^{13}C-dose recovery (CPDR) in 2-hours for each underweight child, both before and after nutritional rehabilitation, expressed as a percentage of the oral caffeine dose administered

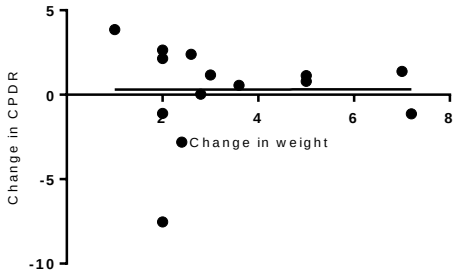
There was no significant correlation between the changes in CPDR during the period of 2 h for underweight children and their age or changes in their weight or height when comparing figures from before and after nutritional rehabilitation (Figure 5.3). The correlation between the change in CPDR and age was r = -0.473 (p = 0.075), while the correlation between the change in CPDR and change in height was 0.341 (p = 0.213). However, there was no clear correlation between change in CPDR and change in weight.



(a)



(b)



(c)

Fig. 5.3. Correlation between changes in cumulative percent ^{13}C-dose recovered (CPDR) in 2-hours for underweight children and (a) their age, (b) changes in their weight or (c) height

5.2. EFFECTS OF MARASMUS ON CAFFEINE METABOLISM

The demographics and anthropometric parameters of the 15 marasmic children are presented in Table 5.3.

Table 5.3: The demographics and anthropometric parameters of the marasmic children

Number of subject	Gender	Age (years)	Pre-nutritional rehabilitation		Duration of nutritional rehabilitation (weeks)	Post-nutritional rehabilitation	
			Weight (kg)	Height (cm)		Weight (kg)	Height (cm)
1	F	3.0	7.5	82.0	5	12.0	84.5
2	M	3.0	7.6	79.5	4	11.0	82.0
3	F	3.5	8.7	90.5	4	12.0	91.0
4	F	3.5	8.7	96.0	4	11.8	97.0
5	F	3.5	9.0	92.0	3	12.0	93.4
6	M	3.8	9.3	94.5	5	13.0	95.8
7	M	4.0	9.2	98.0	6	13.0	99.5
8	F	4.3	9.6	94.0	4	11.5	95.5
9	F	4.5	9.6	102.0	6	13.5	103.5
10	M	4.5	10.0	95.0	6	14.1	95.5
11	F	4.5	10.0	96.0	3	13.0	97.0
12	F	4.5	10.5	100.0	5	14.0	102.0
13	F	5.5	11.3	115.0	5	15.0	116.0
14	M	6.0	11.5	105.0	6	16.0	106.0
15	F	6.5	12.6	114.0	5	16.5	116.0
Mean+ s.d		**4.3± 1.0**	**9.7± 1.4**	**96.9± 9.8**	**4.7±1.0**	**13.2± 1.6***	**98.3± 9.6***

* (*P*<0.005; Student's t-test)

Ten female and five male children were studied. Their mean age was 4.3 ± 1.0 year. Their mean weight significantly increased from 9.7 ± 1.4 kg (before nutritional rehabilitation) to 13.2 ± 1.6 kg (after nutritional rehabilitation) (p < 0.005). Similarly, their mean height significantly increased from 96.9 ± 9.8 cm (before nutritional rehabilitation) to 98.3 ± 9.6 cm (after nutritional rehabilitation) (p < 0.005). The marasmic children were restudied after a mean period of 4.7 weeks following nutritional rehabilitation.

The time courses of mean ±*SD* of the ^{13}C-enrichments for marasmic children studied before and after nutritional rehabilitation are shown in Figure 5.4.

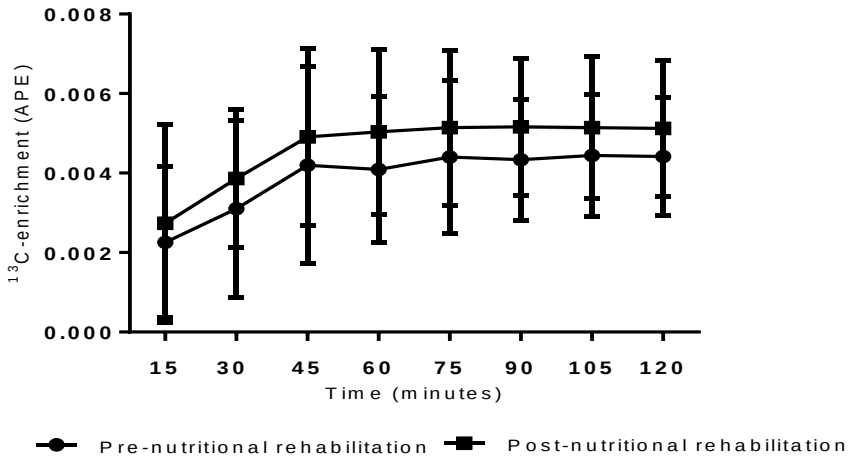


Fig. 5.4. Time courses of mean± SD ^{13}C-enrichments of marasmic children, before and after nutritional rehabilitation. APE represents ^{13}C in atom per excess abundance (see Appendix VIII for the raw data)

In both curves, there was a sudden rise in the mean ^{13}C-enrichments in the first 45 min, followed by a gradual increase until peak values were attained. Thereafter, the mean ^{13}C-enrichments stabilised until the study ended after lasting 120 min. The mean peak enrichment was significantly higher after nutritional rehabilitation (0.00603 ± 0.00250 APE) than before such

rehabilitation (0.00499 ± 0.00221 APE) (p = 0.003). The mean peak ^{13}C-enrichments were attained at 91.00 ± 30.50 min and 90.00 ± 34.49 min before and after nutritional rehabilitation, respectively. There was no significant difference in the mean peak times (p = 0.898). The mean area under the enrichment-time curve was significantly higher after nutritional rehabilitation (0.498 ± 0.186 APE min) than before nutritional rehabilitation (0.419 ± 0.182 APE min) (p = 0.002).

The mean cumulative per cent ^{13}C-dose exhaled as CO_2 during the period of 2 h by the marasmic children is shown in Table 5.4. The mean cumulative per cent ^{13}C-dose significantly increased from 6.80 ± 3.00% (before nutritional rehabilitation) to 7.67 ± 2.81% (after nutritional rehabilitation) (p < 0.001).

The individual cumulative per cent labelled caffeine dose exhaled as CO_2 over a period of 2 h for each marasmic child both before and after nutritional rehabilitation is presented in Figure 5.5. The cumulative per cent labelled caffeine dose exhaled as CO_2 showed an increase in 13 children, a decrease in one child, and remained relatively unchanged in another child after nutritional rehabilitation (Table 5.4 and Figure 5.5). The inter-individual variations before and after nutritional rehabilitation were similar.

Table 5.4: Average cumulative percent ^{13}C-dose recovered in the exhaled 2-hours CO_2 of marasmic children, before and after nutritional rehabilitation

Number of subject	Pre-nutritional rehabilitation			Time interval between study I and II (weeks)	Post-nutritional rehabilitation			Change after nutritional rehabilitation (B-A) (%)
	Labelled CO_2 output over the 2hr study period (mmol/hr)	Average percent $^{13}CO_2$ exhaled (study I)	Cumulative percent ^{13}C-dose (Caffeine) recovered in the 2 hrs CO_2 exhaled (%) (A)		Labelled CO_2 output over the 2hr study period (mmol/hr)	Average percent $^{13}CO_2$ exhaled (study II)	Cumulative percent ^{13}C-dose (Caffeine) recovered in the 2 hrs CO_2 exhaled (%) (B)	
1	364.64	0.02444	**8.91**	5	279.85	0.03187	**8.92**	0.01 (0.1)
2	366.53	0.01724	**6.32**	4	296.77	0.02335	**6.93**	0.61 (9.7)
3	323.76	0.01946	**6.30**	4	271.75	0.03109	**8.45**	2.15 (34.1)
4	316.28	0.03535	**11.18**	4	267.36	0.04346	**11.62**	0.44 (39.4)
5	315.84	0.01681	**5.31**	3	268.96	0.02090	**5.62**	0.31 (5.8)
6	307.04	0.02886	**8.86**	5	255.05	0.03972	**10.13**	1.27 (14.3)
7	304.41	0.01895	**5.77**	6	251.24	0.02937	**7.38**	1.61 (27.9)
8	302.48	0.02893	**8.75**	4	272.77	0.03465	**9.45**	0.70 (8.0)
9	292.84	0.02298	**6.73**	6	242.38	0.03358	**8.14**	1.41 (21.0)
10	294.67	0.00825	**2.43**	6	244.45	0.01030	**2.54**	0.11 (4.5)
11	293.56	0.01516	**4.45**	3	253.79	0.02581	**6.55**	2.1 (47.2)
12	281.27	0.04874	**13.71**	5	239.06	0.05643	**13.49**	-0.22 (-1.6)
13	255.80	0.01927	**4.93**	5	218.91	0.02439	**5.34**	0.41 (8.3)
14	262.70	0.01881	**4.94**	6	219.96	0.02328	**5.12**	0.18 (36.4)
15	242.09	0.01413	**3.42**	5	207.97	0.02577	**5.36**	1.94 (56.7)
Mean+ s.d			**6.80±3.00***	4.7±1.0			**7.67±2.81***	

* Significant difference in the mean values (P=0.001; Student's t-test)

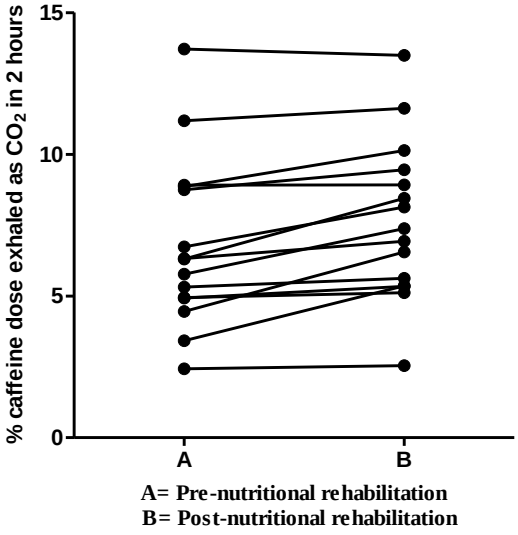
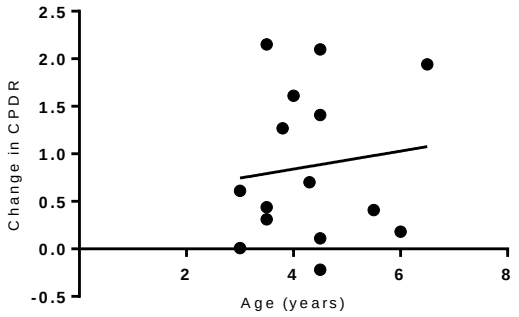
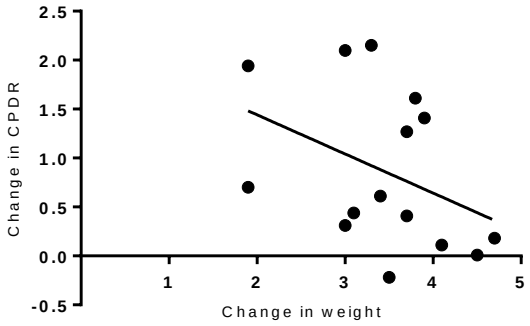


Fig.5.5. Individual cumulative percent ^{13}C-dose recovery (CPDR) in 2-hours for each marasmic child, both before and after nutritional rehabilitation, expressed as a percentage of the oral caffeine dose administered

There was no significant correlation between the changes in either the CPDR during the 2 h period for marasmic children and their age or in their changes in weight or height when comparing data before and after nutritional rehabilitation (Figure 5.6). The correlation between the changes in CPDR and age was r = 0.121 (p = 0.667). There was a negative correlation between the changes in CPDR and both changes in weight (r = -0.200, p = 0.476) and changes in height (r = -0.395, p = 0.145).



(a)



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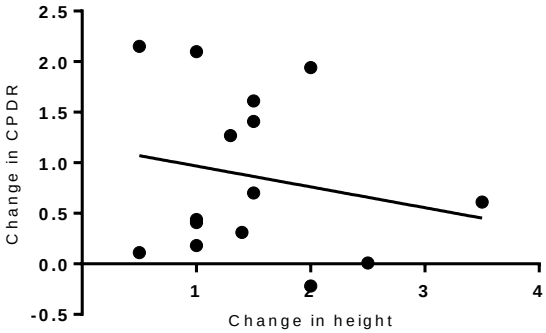


(c)

Fig. 5.6. Correlation between changes in cumulative percent ^{13}C-dose recovered (CPDR) in 2-hours for marasmic children and (a) their age, (b) changes in their weight or (c) height

5.3. EFFECTS OF MARASMIC-KWASHIORKOR ON CAFFEINE METABOLISM

The demographics and anthropometric parameters of the 15 marasmic-kwashiorkor children are presented in Table 5.5.

Table 5.5: The demographics and anthropometric parameters of the marasmic-kwashiorkor children

Number of subject	Gender	Age (years)	Pre-nutritional rehabilitation		Duration of nutritional rehabilitation (weeks)	Post-nutritional rehabilitation	
			Weight (kg)	Height (cm)		Weight (kg)	Height (cm)
1	M	3.00	8.2	90.0	5	11.2	92.2
2	F	3.50	8.2	90	4	12.3	92.3
3	F	3.50	8.8	91.2	4	12.0	92.7
4	F	3.50	9.0	92.3	4	12.5	95.0
5	M	3.75	9.4	96.5	3	12.5	98.0
6	F	4.00	9.2	97.5	5	13.1	99.3
7	M	4.00	9.5	99.2	6	13.5	101.4
8	F	4.50	9.8	98.0	4	13.8	99.50
9	F	4.50	10.1	97.0	6	14.5	98.6
10	F	4.75	10.0	98.0	6	14.2	100.2
11	M	5.00	10.4	110.4	3	14.7	112.3
12	M	5.50	11.0	116.0	5	16.5	118.0
13	F	5.50	11.4	114.5	5	15.5	115.8
14	M	6.00	11.3	105.0	6	16.0	107.6
15	F	6.50	12.5	109.0	5	16.5	116.0
Mean+ s.d		**4.5± 1.0**	**9.9± 1.2**	**100.3± 8.7**	**4.7±1.0**	**13.9± 1.7***	**102.6± 9.0***

* (P<0.005; Student's t-test)

Nine female and six male children were studied. Their mean age was 4.5 ± 1.0 years, which was similar to the age group affected by marasmus. The mean weight of the marasmic-kwashiorkor children significantly increased from 9.9 ± 1.2 kg (before nutritional rehabilitation) to 13.9 ± 1.7 kg (after nutritional

rehabilitation) (p < 0.005). Similarly, their mean height significantly increased from 100.3 ± 8.67 cm (before nutritional rehabilitation) to 102.6 ± 9.04 cm (after nutritional rehabilitation) (p < 0.005). The children were restudied after a mean of 4.7 weeks following nutritional rehabilitation.

The time courses of mean ^{13}C-enrichments of children with marasmic-kwashiorkor studied before and after nutritional rehabilitation are shown in Figure 5.7. Both curves demonstrated a gradual rise in the ^{13}C-enrichments with time.

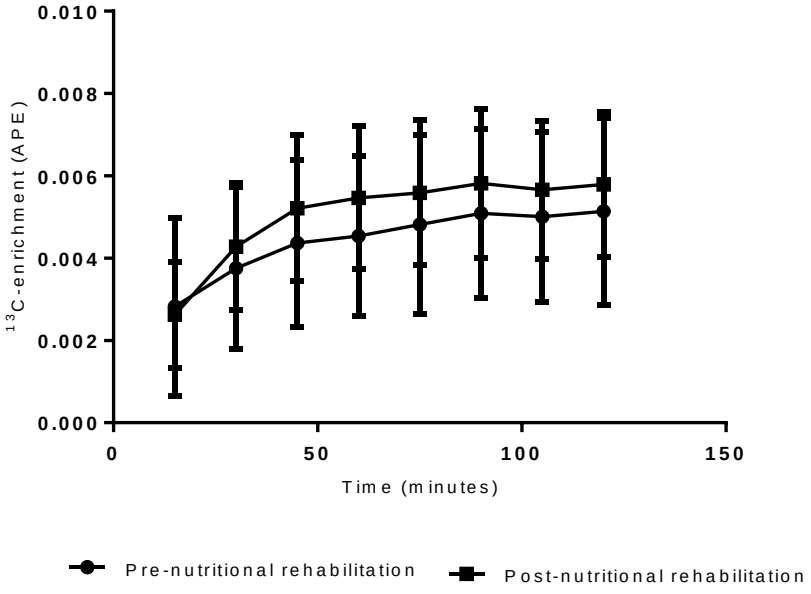


Fig. 5.7. Time courses of mean ± SD ^{13}C-enrichments of children with marasmic-kwashiorkor, before and after nutritional rehabilitation. APE represents ^{13}C in atom per excess abundance (see Appendix IX for the raw data).

The mean peak ^{13}C-enrichments, before (0.00511 ± 0.00178 APE) and after nutritional rehabilitation (0.00577 ± 0.00207 APE), were attained at 87.00 ± 19.80 min and 84.00 ± 33.45 min, respectively, after caffeine administration. There was no significant difference in the mean peak ^{13}C-enrichments in the $^{13}CO_2$ exhaled before and after nutritional rehabilitation (p = 0.102). Similarly,

there was no significant difference in the time to the mean peak ^{13}C-enrichments before and after nutritional rehabilitation (p = 0.783).

The mean areas under the enrichment-time curve before and after nutritional rehabilitation were 0.431 ± 0.144 APE min and 0.480 ± 0.198 APE min, respectively. The difference between the two values was not statistically significant (p = 0.140).

The mean CPDR in the exhaled CO_2 over the 2 h period of the marasmic-kwashiorkor children are shown in Table 5.6. The mean cumulative per cent ^{13}C-dose significantly increased from 6.61 ± 2.26% (before nutritional rehabilitation) to 7.56 ± 2.46% (after nutritional rehabilitation) (p < 0.041).

The cumulative ^{13}C-dose exhaled CO_2 over a period of 2 h for each marasmic-kwashiorkor child before and after nutritional rehabilitation is presented in Figure 5.8. It showed an increase in 13 children and a decrease in two children after nutritional rehabilitation (Table 5.6 and Figure 5.8). The inter-individual variations before and after nutritional rehabilitation were similar.

Table 5.6: Average cumulative percent ^{13}C-dose in the exhaled 2-hour CO_2 of marasmic –kwashiorkor children, before and after nutritional rehabilitation

Number of subject	Pre-nutritional rehabilitation			Time interval between study I and II (weeks)	Post-nutritional rehabilitation			Change after nutritional rehabilitation (B-A) (%)
	Labelled CO_2 output over the 2hr study period (mmol/hr)	Average percent $^{13}CO_2$ exhaled (study I)	Cumulative percent ^{13}C-dose (Caffeine) recovered in the 2 hrs CO_2 exhaled (%) (A)		Labelled CO_2 output over the 2hr study period (mmol/hr)	Average percent $^{13}CO_2$ exhaled post- rehab (study II)	Cumulative percent ^{13}C-dose (Caffeine) recovered in the 2 hrs CO_2 exhaled (%) (B)	
1	336.78	0.01808	**6.09**	5	280.56	0.02784	**7.81**	1.72 (28.2)
2	336.78	0.03317	**11.17**	4	266.66	0.04238	**11.30**	0.13 (1.2)
3	320.79	0.01300	**4.17**	4	269.76	0.02117	**5.71**	1.54 (36.9)
4	315.44	0.01633	**5.15**	4	261.35	0.042097	**5.48**	0.33 (6.4)
5	302.76	0.01691	**5.12**	3	258.15	0.02487	**6.42**	1.30 (21.4)
6	305.03	0.02262	**6.90**	5	250.41	0.02987	**7.48**	0.58 (8.4)
7	297.41	0.02354	**7.00**	6	244.36	0.03851	**9.41**	2.41 (34.4)
8	294.24	0.01410	**4.15**	4	243.30	0.03153	**7.67**	3.52 (84.8)
9	290.69	0.02167	**6.30**	6	237.77	0.02730	**6.49**	0.19 (3.0)
10	291.06	0.03673	**10.69**	6	238.93	0.05261	**12.57**	1.88 (17.6)
11	271.84	0.01229	**3.34**	3	224.16	0.01847	**4.14**	0.80 (24.0)
12	258.64	0.02099	**5.43**	5	206.56	0.03321	**6.86**	1.43 (26.3)
13	255.03	0.03223	**8.22**	5	215.23	0.02718	**5.85**	-2.37 (-28.8)
14	265.20	0.02990	**7.93**	6	217.83	0.05031	**10.96**	3.03 (38.3)
15	247.49	0.03022	**7.48**	5	218.66	0.02433	**5.32**	-2.16 (28.9)
Mean+ s.d			**6.61±2.26***	**4.7±1.0**			**7.56±2.46***	

* Significant difference in the mean values (P=0.041; Student's t-test)

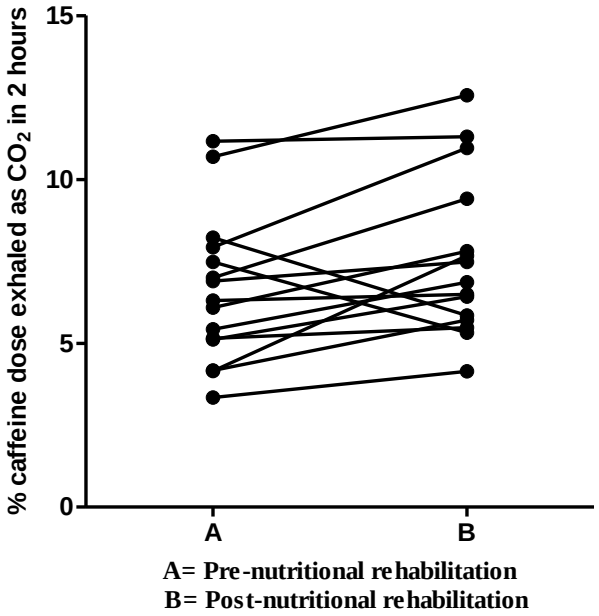
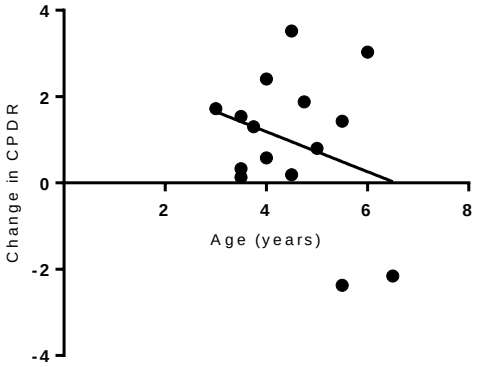
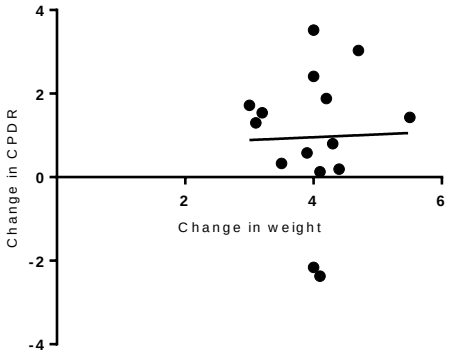


Fig.5.8. Individual cumulative percent ^{13}C-dose recovery (CPDR) in 2-hours for each marasmic-kwashiorkor child, both before and after nutritional rehabilitation, expressed as a percentage of the oral caffeine dose administered

There was no significant correlation between either the changes in CPDR during the 2 h period for children with marasmic-kwashiorkor and their age or the changes in their weight or height when comparing the data before and after nutritional rehabilitation (Figure 5.9). Change in the CPDR did not significantly correlate with age (r = -0.291, p = 0.293), changes in weight (r = 0.0264, p = 0.926), or changes in height (r = -0.426, p = 0.113).



(a)



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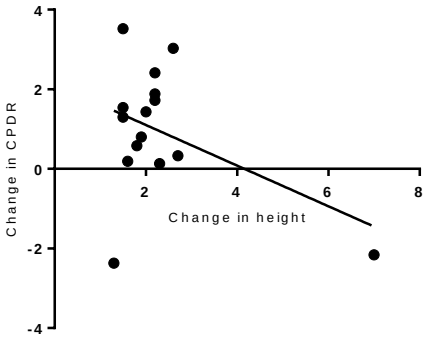


(c)

Fig.5.9. Correlation between changes in cumulative percent ^{13}C-dose recovered (CPDR) in 2-hours for marasmic-kwashiorkor children and (a) their age, (b) changes in their weight or (c) height

5.4. EFFECTS OF KWASHIORKOR ON CAFFEINE METABOLISM

The demographics and anthropometric parameters of the 15 kwashiorkor children are presented in Table 5.7.

Table 5.7: The demographics and anthropometric parameters of the kwashiorkor children

Number of subject	Gender	Age (years)	Pre-nutritional rehabilitation		Duration of nutritional rehabilitation (weeks)	Post-nutritional rehabilitation	
			Weight (kg)	Height (cm)		Weight (kg)	Height (cm)
1	M	3.5	9.8	97.8	6	11.9	98.2
2	M	5.8	13.9	116.8	5	15.4	118.3
3	F	4.5	11.2	101.0	6	13.5	102.2
4	F	5.0	10.8	108.5	8	14.5	110.0
5	F	3.0	9.4	97.5	4	11.0	98.0
6	M	4.0	9.9	99.0	7	13.1	99.3
7	F	6.5	12.6	118.4	8	17.2	119.0
8	F	3.2	10.1	95.0	4	11.4	96.5
9	M	5.5	12.2	116.2	5	14.9	117.6
10	M	4.5	10.7	100.1	6	13.5	101.2
11	M	4.8	10.6	110.4	7	14.0	112.3
12	M	6.0	13.0	116.0	5	16.5	118.0
13	F	3.5	9.5	94.2	5	11.9	95.8
14	M	4.5	11.3	102.0	6	13.8	103.2
15	F	3.8	9.6	96.8	5	12.4	98.3
Mean+ s.d		**4.5± 1.1**	**11.0± 1.4**	**104.7± 8.9**	**5.8±1.3**	**13.7± 1.8***	**105.8± 8.9***

* (P<0.001, Student's t-test)

Eight male and seven female children were studied. Their mean age was 4.5 ± 1.1 years, which was similar to the age of groups affected by marasmus and marasmic-kwashiorkor alike. The mean weight of the children with kwashiorkor significantly increased from 11.0 ± 1.4 kg (before nutritional rehabilitation) to 13.7 ± 1.8 kg (after nutritional rehabilitation) (p < 0.005).

Similarly, their mean height significantly increased from 104.7 ± 8.9 cm (before nutritional rehabilitation) to 105.8 ± 8.9 cm (after nutritional rehabilitation) (p < 0.005). The children were restudied after a mean period of 5.8 weeks following nutritional rehabilitation.

The time courses of mean ±*SD* ^{13}C-enrichments of the kwashiorkor children studied before and after nutritional rehabilitation are shown in Figure 5.10.

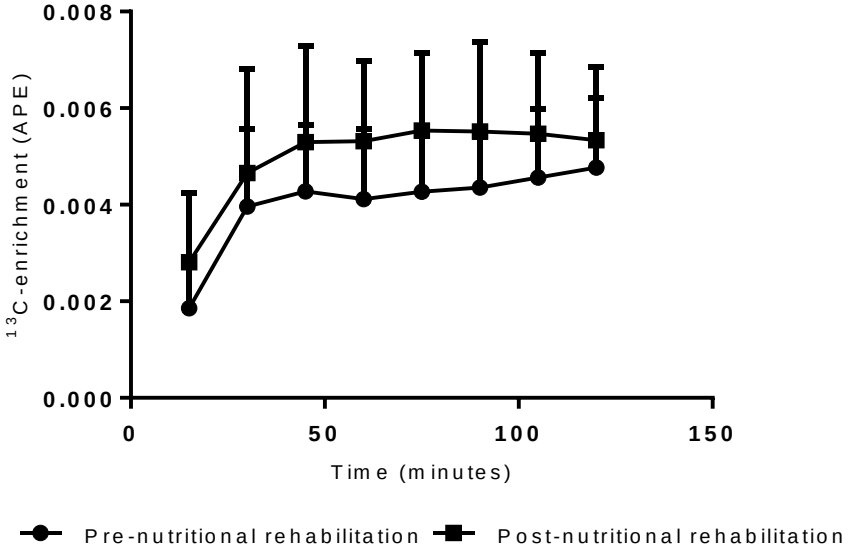


Fig. 5.10. Time courses of mean± SD ^{13}C-enrichments of children with kwashiorkor, before and after nutritional rehabilitation. APE represents ^{13}C in atom per excess abundance (see Appendix X for the raw data).

Both curves showed a rapid rise in the ^{13}C-enrichments during the first 30 min. There was a gradual rise until 45 min, followed by minor changes until the mean peak ^{13}C-enrichment was attained. Thereafter, the curve for post-nutritional rehabilitation gradually fell. By contrast, the curve for pre-nutritional rehabilitation gradually rose after 45 min to the mean peak ^{13}C-enrichment at the end of the study.

The mean peak ^{13}C-enrichments were 0.00544 ± 0.00156 APE and 0.00647 ± 0.00163 APE before and after nutritional rehabilitation, respectively. There was no significant difference between these mean values (p = 0.104). The mean peak ^{13}C-enrichments were attained at 110.00 ± 29.17 min and 84.00 ± 37.09 min before and after nutritional rehabilitation, respectively. The difference between these mean time periods was statistically significant (p = 0.049).

The mean area under the enrichment-time curve was significantly higher after nutritional rehabilitation (0.538 ± 0.160 APE min) than before nutritional rehabilitation (0.433 ± 0.118 APE min) (p = 0.0001).

The mean cumulative per cent ^{13}C-doses exhaled by the kwashiorkor children and labelled CO_2 over a period of 2 h are shown in Table 5.8. The CPDR in the exhaled caffeine labelled CO_2 of the 2 h period significantly increased from 6.29 ± 1.06% (before nutritional rehabilitation) to 7.13 ± 1.72% (after nutritional rehabilitation) (p < 0.002) (Figure 5.11).

The mean cumulative per cent ^{13}C-dose exhaled over the period of 2 h by each kwashiorkor child both before and after nutritional rehabilitation is presented in Figure 5.11. The cumulative per cent ^{13}C-dose showed an increase in 13 children, a decrease in one child, and remained relatively unchanged in another child after nutritional rehabilitation. There was greater inter-individual variation after nutritional rehabilitation.

Table 5.8: Average cumulative percent ^{13}C-dose exhaled in 2-hours CO_2 of kwashiorkor children, before and after nutritional rehabilitation

Number of subject	Pre-nutritional rehabilitation			Time interval between study I and II (weeks)	Post-nutritional rehabilitation			Change after nutritional rehabilitation (B-A) (%)
	Labelled CO_2 output over the 2hr study period (mmol/hr)	Average percent $^{13}CO_2$ exhaled (study I)	Cumulative percent ^{13}C-dose (Caffeine) recovered in the 2 hr CO_2 exhaled (%) (A)		Labelled CO_2 output over the 2hr study period (mmol/hr)	Average percent $^{13}CO_2$ exhaled (study II)	Cumulative percent ^{13}C-dose (Caffeine) recovered in the 2 hr CO_2 exhaled (%) (B)	
1	294.48	0.02289	**6.74**	6	264.86	0.03243	**8.59**	1.85(27.5)
2	227.44	0.02014	**4.58**	5	214.16	0.02330	**4.99**	0.41(9.0)
3	270.60	0.02095	**5.67**	6	243.60	0.02701	**6.58**	0.91(16.0)
4	268.22	0.02647	**7.10**	8	227.68	0.03176	**7.23**	0.13(1.8)
5	301.52	0.02096	**6.32**	4	276.52	0.02300	**6.36**	0.04(0.6)
6	291.47	0.01942	**5.66**	7	250.41	0.03027	**7.58**	1.92(33.9)
7	238.00	0.02744	**6.53**	8	201.73	0.03495	**7.05**	0.52(8.0)
8	293.10	0.02167	**6.35**	4	272.92	0.02635	**7.19**	0.84(13.2)
9	244.47	0.02679	**6.55**	5	218.51	0.03373	**7.37**	0.82(11.1)
10	278.32	0.02641	**7.35**	6	244.55	0.03378	**8.26**	0.91(12.4)
11	269.07	0.02631	**7.08**	7	230.12	0.03707	**8.53**	1.45(20.5)
12	236.42	0.02631	**6.22**	5	206.56	0.03331	**6.88**	0.66(10.6)
13	303.93	0.02323	**7.06**	5	267.47	0.03204	**8.57**	1.51(21.4)
14	268.26	0.02788	**7.48**	6	239.81	0.03945	**9.46**	1.98(26.5)
15	298.98	0.01205	**3.60**	5	258.95	0.00904	**2.34**	-1.26(35.0)
Mean+ s.d			**6.29±1.06***	5.8±1.3			**7.20±1.80***	

* There was a significant different in the mean values (Student's *t*-test, *P*=0.002)

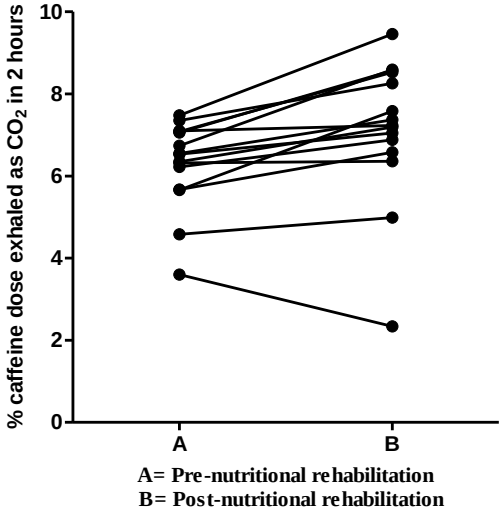
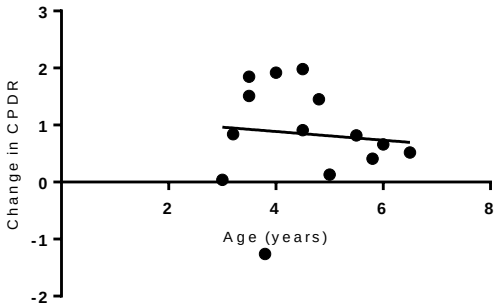
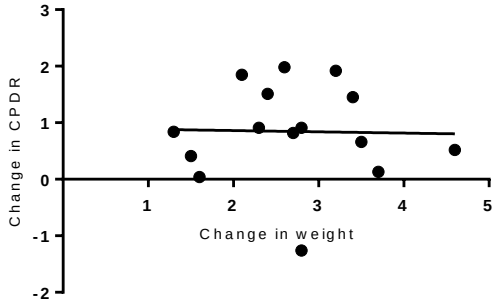


Fig.5.11. Individual cumulative percent ^{13}C-dose recovery (CPDR) in 2-hours for each kwashiorkor child, both before and after nutritional rehabilitation, expressed as a percentage of the oral caffeine dose administered.

There was no significant correlation between either the changes in CPDR over the period of 2 h for children with kwashiorkor and their age or changes in weight or height when comparing data before and after nutritional rehabilitation (Figure 5.12). The correlation between the changes in CPDR and age was r = -0.0953 (p = 0.735). The correlation was r = -0.0235 (p = 0.934) with changes in height and r = -0.2533 (p = 0.362) with changes in weight.



(a)



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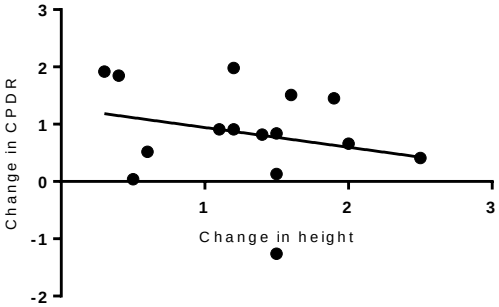

(c)

Fig.5.12. Correlation between changes in cumulative percent ^{13}C-dose recovered (CPDR) in 2-hours for children with kwashiorkor and (a) their age, (b) changes in their weight or (c) height

5.5. SUMMARY OF RESULTS

The present studies assessed the activity of CYP1A2 in underweight and severely malnourished children both during acute malnutrition and after nutritional rehabilitation by using the CBT. The activity of CYP1A2 was determined for each group of malnutrition, both before and after nutritional rehabilitation, by the cumulative CO_2 exhaled over a period of 2 h. The time courses of mean ±*SD* ^{13}C-enrichments of all four categories of malnourished children studied before and after nutritional rehabilitation are shown in Figure 5.13 for comparison.

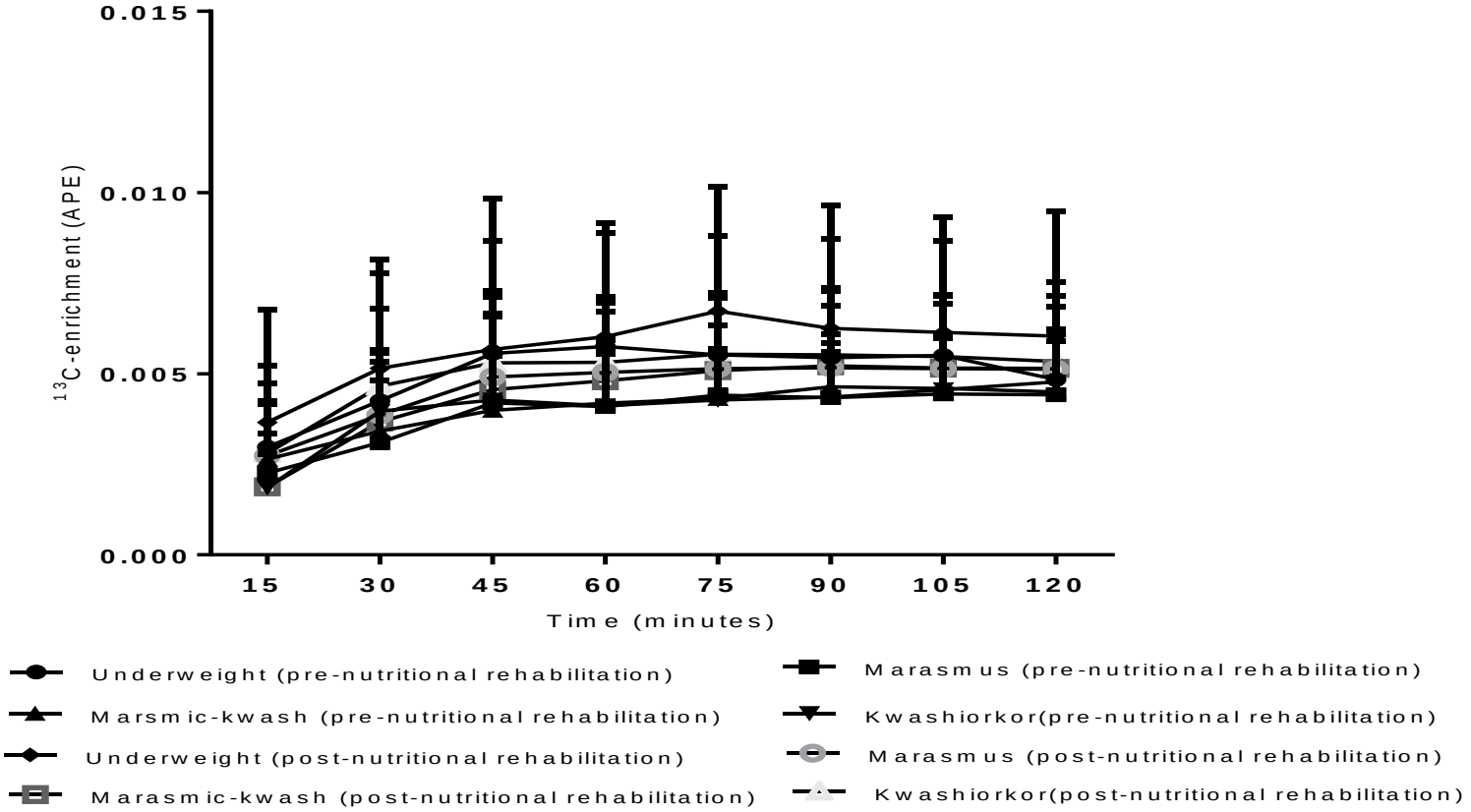

Fig. 5.13. The time courses of mean ±*SD* ^{13}C-enrichments of all four categories of malnourished children studied before and after nutritional rehabilitation. APE represents ^{13}C in atom per excess abundance.

The patterns of the curves were dissimilar for underweight malnourished children and the other three categories of malnutrition before and after nutritional rehabilitation. The curves for underweight malnourished children before and after nutritional rehabilitation, however, were characterised by a steady rise to a peak point and, subsequently, a gradual fall. The pre- and post-nutritional rehabilitation curves for underweight children were consistently higher than those for children with marasmus, marasmic-kwashiorkor, and kwashiorkor. When the three severe forms of protein-energy malnutrition (PEM) were compared, the post-nutritional rehabilitation curves were consistently higher than those for pre-nutritional rehabilitation. However, the curves for marasmus, marasmic-kwashiorkor, and kwashiorkor both before and after nutritional rehabilitation plateaued after reaching the peak levels, and there were no clear differences between them.

The CBT parameters derived from the enrichment-time curves for all the four groups of PEM before and after nutritional rehabilitation are summarised in Table 5.9. When each CBT parameter was compared for the four groups of malnourished children, the mean AUCs did not differ significantly before (p = 0.346, ANOVA; Tukey's post hoc test, not statistically significant) or after (p = 0.389, ANOVA; Tukey's post hoc test, not statistically significant) nutritional rehabilitation. However, there were significant differences in the mean peak ^{13}C-enrichments (Cmax) before nutritional rehabilitation (p = 0.0474, ANOVA; Tukey's post hoc test, not statistically significant) but showed no significant difference after nutritional rehabilitation (p = 0.574, ANOVA; Tukey's post hoc test, not statistically significant). The mean time (Tmax) to attain the mean peak enrichments did not significantly differ for all categories of malnutrition before (p = 0.675, ANOVA; Tukey's post hoc test, not statistically significant) or after (p =

0.295, ANOVA; Tukey's post hoc test, not statistically significant) nutritional rehabilitation.

Table 5.9: Comparison of the CBT parameters from the enrichment-time curves for different types of malnutrition, before and after nutritional rehabilitation

CBT parameters	Type of malnutrition			
	Underweight	**Marasmus**	**Marasmic-kwashiorkor**	**Kwashiorkor**
AUC				
Pre-nutritional rehabilitation	0.539±0.320	0.419±0.182	0.431±0.144	0.433±0.118
Post-nutritional rehabilitation	0.620±0.322	0.498±0.186	0.480±0.198	0.538±0.160
	(*P*= 0.528)	(*P*= 0.002)*	(*P*= 0.140)	(*P*= 0.0001)*
	Post hoc power= 10.2%		Post hoc power= 11.8%	
Cmax				
Pre-nutritional rehabilitation	0.00740±0.00350	0.00499±0.00221	0.00511±0.00178	0.00544±0.00156
Post-nutritional rehabilitation	0.00694±0.00345	0.00603±0.00250	0.00577±0.00207	0.00647±0.00163
	(*P*= 0.691)	(*P*= 0.003)*	(*P*= 0.102)	(*P*= 0.104)
	Post hoc power= 5.5%		Post hoc power= 15.3%	Post hoc power= 42.4%
Tmax				
Pre-nutritional rehabilitation	61.00±23.28	91.00±30.50	87.00±19.80	110.00± 29.17
Post-nutritional rehabilitation	76.00±26.87	90.00±34.49	84.00±33.45	74.00±29.17
	(*P*= 0.060)	(*P*= 0.898)	(*P*= 0.783)	(*P*= 0.049)*
	Post hoc power= 37.2%	Post hoc power= 3.0%	Post hoc power= 4.8%	

* There was a significant difference in the mean values (Student's *t*-test, $P < 0.05$); CBT represents caffeine breath test; AUC represents area under the enrichment-time curve; Cmax is the mean peak ^{13}C-enrichment; Tmax is the mean time to attain the mean peak ^{13}C-enrichment

The mean percentage labelled caffeine in the CO_2 exhaled over a period of 2 h before and after nutritional rehabilitation was compared for underweight children and/or children experiencing marasmus, marasmic-kwashiorkor, and kwashiorkor in Table 5.10.

Table 5.10: Comparison of the mean percentage of labelled caffeine in CO_2 exhaled over a two hour period, before and after nutritional rehabilitation, for different types of malnutrition

Type of malnutrition	Mean percentage labelled caffeine in CO_2 exhaled over a two hour period (%)		*P* value (Student's *t*-test)	Post hoc power
	Pre-nutritional rehabilitation	Post-snutritional rehabilitation		
Underweight	7.56±4.02	7.95±3.68	0.603	4.6%
Marasmus	6.80±3.00	7.67±2.81	0.001	-
Marasmic-kwashiorkor	6.61±2.26	7.56±2.46	0.041	-
Kwashiorkor	6.29±1.06	7.20±1.80	0.002	-

Among the four groups of malnourished children, there was no significant difference in the mean CPDR either before (p = 0.634, ANOVA; Tukey's post hoc test, not statistically significant) or after (p = 0.865, ANOVA; Tukey's post hoc test, not statistically significant) nutritional rehabilitation. However, for the individual group of underweight children, there was no significant change in the mean percentage labelled caffeine in the exhaled CO_2 following nutritional rehabilitation, but there were significant changes in marasmus, marasmic-kwashiorkor, and kwashiorkor following nutritional rehabilitation.

CHAPTER SIX:

DISCUSSION

6.1. INTRODUCTION

This section compares the caffeine breath test (CBT) results for each type of malnutrition with those of previous studies that evaluated the hepatic metabolism of drugs in malnourished adults, children, and animals. It also provides insights into the possible mechanisms for the altered drug metabolism in malnourished children, as well as discusses the clinical implications of the findings of the studies. Furthermore, the results of the four groups of malnutrition are related to one another in order to compare their similarities and differences. Lastly, the limitations of the methodology of the studies are also discussed.

6.2. UNDERWEIGHT AND CAFFEINE METABOLISM

Only a few studies have evaluated drug disposition in underweight children. Limited pharmacokinetic data emanating from a systematic review suggest impaired drug metabolism in underweight children (Oshikoya *et al.,* 2010).

In the present study underweight children were studied before and after nutritional rehabilitation. Nutritional rehabilitation for 2 to 6 weeks enabled the underweight children to attain the expected weight-for-height of well-nourished children before they were restudied, thus serving as normal weight controls. The ^{13}C-enrichment curves for the underweight children before and after nutritional rehabilitation were similar. The rates (as indicated by the C_{max} and T_{max} values) of labelled caffeine recovered in the $^{13}CO_2$ exhaled in the 2 h period by the

underweight children before and after nutritional rehabilitation also were similar. There was also a similarity in the extents of recovery of labelled caffeine (as indicated by the AUC values) in the $^{13}CO_2$ exhaled in the 2 h period by underweight children both before and after nutritional rehabilitation. The results suggest that the metabolism of labelled caffeine was not significantly affected in children when they were underweight compared to when they had attained normal weight after nutritional rehabilitation.

The similarities observed in the ^{13}C-enrichment curves for the underweight children before and after nutritional rehabilitation are comparable to results of similar studies. Park *et al.* (2003) used the CBT to compare the metabolising activities of CYP1A2 in healthy adult controls and adults with hepatitis. They observed that the time to peak ^{13}C-enrichment, as well as the peak enrichment level, both after oral labelled-caffeine administration, was not significantly different between the two groups. A study comparing the disposition of labelled caffeine in healthy adult controls and those with liver cirrhosis has also documented no significant difference in the absorption rates (Desmond *et al.,* 1980). Similarly, the absorption rates of labelled caffeine did not differ significantly when its disposition in healthy controls was compared to children suffering from malaria (Akinyinka *et al.,* 2000). It may thus be concluded that disease states, as well as underweight malnutrition, appear to not significantly affect the oral absorption of caffeine.

The CBT was first used by Lambert *et al.* (1986) to determine the activity of CYP1A2 in children. They reported that the time to peak rate of $^{13}CO_2$ exhalation occurred in the first 2 h after labelled caffeine administration. In the present study the mean peak ^{13}C-enrichment values were attained for underweight children before and after nutritional rehabilitation in less than 2 h.

These results were quite similar to the 60 min generally taken to attain the peak ^{13}C-enrichments in healthy adult controls and adults with chronic hepatitis (Park *et al.,* 2003). A pharmacokinetic study also has documented a time of 50 to 60 min to attain the peak plasma concentration of caffeine in healthy children and children suffering from malaria in Nigeria (Akinyinka *et al.,* 2000).

Previous studies involving the use of CBT in children (Lambert *et al.,* 1986; Parker *et al.,* 1994) and adults (Butler *et al.,* 1989; Kalow and Tang, 1993) have shown that the cumulative $^{13}CO_2$ exhaled in the 2 h period reflects a 3-*N*-demethylation of caffeine, which is a CYP1A2-dependent reaction. Two different studies have established the 2 h period's cumulative exhalation of $^{13}CO_2$ as a standard for determining CYP1A2 activity (Kotake *et al.,* 1982; Park *et al.,* 2003), which was based on the observation that a maximal correlation (r = 0.90) between the cumulative percent ^{13}C-caffeine doses recovered (CPDR) in the breath samples and its plasma clearance (CL) occurred at 2 h after the oral intake of labelled caffeine. The present study has established that the mean CPDR in the breath samples collected during the 2 h period before and after nutritional rehabilitation were similar. This result suggests that the metabolising activity of CYP1A2 was unaffected by underweight malnutrition.

Considering the small number of underweight children evaluated before and after nutritional rehabilitation, the present study may not have been powered enough to detect a statistically significant difference in the results. Such a difference would have been the basis for validating the results in a larger population of underweight children. However, a previous study involving the use of CBT has shown that a smaller sample size of 11 children with gastritis was adequately powered to demonstrate a lack of inhibitory effect of cimetidine on caffeine metabolism (Parker *et al.,* 1997b). In another study involving 12

healthy and six underweight children, the lack of significant change between plasma CLs of oral theophylline was documented (Eriksson *et al.,* 1983). Both caffeine and theophylline are methylxanthines predominantly metabolised by the CYP1A2 enzyme via the same pathway. Therefore, it may be concluded that the *N*-demethylation pathway was not significantly affected in underweight children.

The unaffected CYP1A2 activity observed in the underweight children is also comparable to the findings of Tranvouez *et al*. (1985), who determined that the plasma CL of antipyrine (a drug that is partially metabolised by CYP1A2) in underweight adults showed significant change after the adults' nutritional rehabilitation. However, three other studies have shown decreased CL of different drugs in underweight patients. Specifically, antipyrine CL was decreased in underweight adults (Krishnaswamy & Naidu, 1977), while the CLs of acetylsalicylic acid and penicillin decreased in underweight children (Bolme *et al.,* 1995; Lares–Asseff *et al.*, 1999). Different types of CYP450 enzymes are responsible for the metabolism of caffeine and acetylsalicylic acid, while penicillin is usually eliminated without undergoing hepatic metabolism. Acetylsalicylic acid is metabolised by CYP3A4, while caffeine is predominantly metabolised by CYP1A2. Variation in the activity of the metabolising enzymes may account for the differences in the disposition of caffeine and other drugs in underweight children.

6.3. MARASMUS AND CAFFEINE METABOLISM

The mean CPDR exhaled as CO_2 which measures the activity of CY1A2 in the 2 h period was significantly lower in marasmic states than after nutritional recovery. Caffeine is a probe substrate of CYP1A2 enzyme activity which has been

previously used to predict the pharmacokinetics of tactrine in patients with Alzheimer's disease (Fontana *et al.,* 1998). Since the CBT directly assesses the metabolic activity of CYP1A2 enzyme, it may be appropriate to compare the results with those of the plasma CLs of drugs in malnourished children. The decreased metabolism of labelled caffeine observed in marasmic children is consistent with the decreased plasma clearance of sulphamethoxazole and quinine previously reported in a similar group of children (Bravo *et al.,* 1984; Pussard *et al.,* 1999).

Other studies have similarly documented decreased plasma CLs of antipyrine (Homeida *et al.,* 1979; Narang *et al*., 1977) and metronidazole (Lares–Asseff *et al.,* 1992) in marasmic children. Like caffeine, antipyrine and metronidazole are metabolised by oxidation. Although all three drugs are metabolised by CYP1A2, other hepatic cytochrome P450 enzymes (CYP2B6, CYP2C8, CYP2C9, CYP2C18, and CYP3A4) are involved in the metabolism of antipyrine and metronidazole (Engel *et al*., 1996; Loft, 1991). Both CYP1A2 and all other cytochrome P450 enzymes constitute the hepatic mixed-function oxidase system (Watkins, 1990). Limited data have indicated that the metabolising activities of the mixed-function oxidase system are affected by malnutrition (Cho *et al.,* 1999; González–Hernández *et al.,* 2008; Krishnaswamy & Naidu, 1977; Narang, 1977).

My findings regarding marasmic children also agree with those of Tranvouez *et al.* (1985), who investigated the total CL of antipyrine in 49 global protein-calorie malnourished adults, 25 well-nourished control groups, and 27 malnourished adults who had been rehabilitated nutritionally with artificial nutrition for a month. The metabolic CL rate was significantly lower in the control and nutritionally rehabilitated groups. However, the lack of a significant

difference between the metabolic CL rates of the control and nutritionally rehabilitated groups suggested that the activity of the metabolising enzymes reverted to normal after nutritional recovery. Pantuck *et al.* (1985) also found that parenteral nutritional replenishment in adult patients with protein-calorie malnutrition for a week resulted in the increased CL of antipyrine by 87%. A similar result was obtained after changing to oral diets rich in both carbohydrates and proteins.

Unlike the present CBT results, another study has indicated no significant difference in the plasma CL of theophylline in marasmic children compared to their healthy controls (Eriksson *et al*., 1983b). This study was conducted in eight children only and estimated the plasma CL of theophylline instead of the metabolic CL by CYP1A2. Moreover, the marasmic children were not restudied as their own controls after nutritional rehabilitation. The results of the theophylline study may, therefore, be prone to bias from a wide inter-participant variation in CYP1A2 activity. Plus, the small sample size may have underpowered the study's capacity to detect a significant difference in the results.

Eriksson *et al.* (1983a) also studied the total plasma CL of chloramphenicol in eight children with marasmus and eight healthy control groups to show no significant difference in the results. Chloramphenicol is metabolised predominantly by glucuronidation via a conjugation process. This metabolic pathway is quite different from the oxidative process evaluated in the present studies. The enzymes involved in both metabolic processes may have been affected differently by marasmus. However, another study that examined the metabolism of chloramphenicol in seven marasmic children before and after 4 to 6 weeks of nutritional rehabilitation demonstrated a significantly decreased plasma CL in patients with malnutrition compared to these patients after they

had received nutritional rehabilitation (Mehta *et al.,* 1975). Liver biopsies from two of these marasmic children demonstrated a reduction of the glucuronyl transferase enzyme to one-half and one-third, respectively, of the normal value.

The mechanism involved in the altered CYP1A2 activity and decreased caffeine metabolism in marasmic children remains poorly understood. However, it has been suggested that marasmus, as an adaptation to insufficient energy intake, results in reduced total microsomal haeme, phospholipids, and flavin adenine dinucleotide levels, normal levels of which are all necessary for microsomal drug metabolism (Rumack *et al.,* 1973).

In the present studies the patterns of ^{13}C-enrichment curves for marasmic children before and after nutritional rehabilitation were similar. However, the curves were clearly different from those for adults with or without hepatic disease studied by Park *et al.* (2003). They also differed from those reported in adult smokers and non-smokers (Kotake *et al.,* 1982), and sham-operated rat controls (Schaad *et al.,* 1995). A typical ^{13}C-enrichment curve is characterised by a gradual rise to a peak level and then a steady fall over the study period (Park *et al.,* 2003; Kotake *et al.,* 1982; Schaad *et al.,* 1995). By contrast, after attaining the peak levels in the present studies, the ^{13}C-enrichment curves remained relatively constant in marasmic children before and after nutritional rehabilitation. These patterns are similar to the CBT curves observed in rats that underwent a two-third resection of the liver, bile duct ligation, or portocaval anastomosis (Schaad *et al.,* 1995). The hepatic injuries in the animal study were associated with a significantly decreased liver mass and reduced CYP1A2 activity, as indicated by 50% to 60% reduction in the fraction of labelled caffeine dose recovered over the study period. The study of animal models also indicated

that the 3-*N*-demethylation of caffeine was saturated faster when CYP1A2 activity was reduced.

The present research's CBT curves for marasmic children also were similar to those described by Kotake *et al.* (1982) for adult non-smokers administered 5mg/kg labelled caffeine. After attaining the peak ^{13}C-enrichment level in the first hour, the enrichment levels remained relatively constant, since the saturation of CYP1A2 enzyme occurred at a lower dose of 3mg/kg.

The quantity and quality of metabolising enzymes are known to decrease in severe malnutrition (Mehta *et al.,* 1975; Narang, 1977). It is therefore postulated that the 3-*N*-demethylation of caffeine by CYP1A2 enzyme is saturated faster in marasmic children than in well-nourished children. This suggestion may explain the current pattern of CBT curves for children in marasmic and nutritional recovery states. This hypothesis is supported by the following points: (i) decreased oxidative phosphorylation in the liver homogenates of malnourished infants, which was less pronounced in marasmus than in kwashiorkor (Waterlow, 1970); (ii) reduced activities of the enzymes involved in amino acid metabolism in the liver of marasmic infants (Stephen & Waterlow, 1968); and (iii) decreased capacity and affinity of CYP1A2 enzyme in the liver homogenates of juvenile and adult rats that were malnourished (Mao *et al. ,* 2006). Moreover, the biochemical and physiological normalities had been reported to lag behind nutritional recovery in malnourished children, which may not be regained until several weeks later (Golden, 2009). Despite the rapid weight gain and early attainment of normal weight-for-height, malnourished children are deficient in functional tissue during the early stages of nutritional recovery (Cheek *et al.,* 1970).

The rate and extent of labelled caffeine recovery in the exhaled $^{13}CO_2$ were significantly lower in the marasmic state than after nutritional recovery. The percentage decrease in these parameters was much lower than those reported in partially hepatectomised rats and their control groups (Schaad *et al.,* 1995).

6.4. MARASMIC-KWASHIORKOR AND CAFFEINE METABOLISM

In the present studies caffeine metabolism, as determined by the mean CPDR in the CO_2 exhaled in 2 h, significantly decreased in children with marasmic-kwashiorkor compared to the amount exhaled after their nutritional rehabilitation. This finding suggests a decreased CYP1A2 activity in children with marasmic-kwashiorkor. The mechanism involved in the altered CYP1A2 activity and decreased caffeine metabolism in these children is not yet fully understood. As an intermediate severe malnutrition, marasmic-kwashiorkor is characterised by some of the clinical features of marasmus and kwashiorkor. The pathophysiological changes affecting drug metabolism are therefore likely to be less severe in marasmic-kwashiorkor than in marasmus and kwashiorkor. The metabolic alteration in marasmic-kwashiorkor has been attributed mainly to changes in the levels of microsomal membrane proteins, especially that of cytochrome P450 (Hayes *et al,* 1978; Thabrew, 1983) and decreased fluidity of the phospholipid bilayer of the endoplasmic reticulum membrane, which impairs the interactions among NADPH-cytochrome P450 reductase, cytochrome P450, cytochrome b, and other specific drug metabolising enzymes (Suzuki *et al.,* 1980; Thabrew, 1983).

Few data are available on the hepatic CL of drugs in children with marasmic-kwashiorkor (Eriksson *et al.,* 1983a; Lares–Asseff *et al.,* 1992; Narang *et al*., 1977). The findings of the present research are consistent with the reports of reduced plasma CL of antipyrine and metronidazole in children with marasmic-kwashiorkor compared to healthy control children (Lares–Asseff *et al.,* 1992; Narang *et al*., 1977). Although the CBT assessed caffeine metabolism by CYP1A2 enzyme, other studies have assessed antipyrine and metronidazole metabolism by other cytochrome P450 enzymes, including CYP2B6, CYP2C8, CYP2C9, CYP2C18, and CYP3A4.

The findings of the present research, however, contrast the lack of significant difference between the total CL of chloramphenicol in nine children with marasmic-kwashiorkor and eight healthy controls (Eriksson *et al.,* 1983a). This difference may derive from the separate pathways involved in the metabolism of chloramphenicol and caffeine. Caffeine is metabolised by an oxidation process, via an *N*-demethylation pathway, while chloramphenicol is metabolised in young children predominantly by conjugation to the production of glucuronic acid (Glazko, 1966). The oxidation of chloramphenicol thus concerns a minor pathway. Bolme *et al.* (1995) reported a significantly decreased plasma CL of penicillin in eight children with marasmic-kwashiorkor compared to six healthy controls. Penicillin rarely undergoes hepatic metabolism, for it is eliminated unchanged by the kidneys primarily, while caffeine is predominantly metabolised in the liver by CYP1A2 enzymes. The variations in the metabolic pathways of both penicillin and caffeine may explain the differences in the results of the two studies.

6.5. KWASHIORKOR AND CAFFEINE METABOLISM

In the present research the mean cumulative per cent ^{13}C-doses exhaled as labelled CO_2 over a 2 h period significantly decreased in children with kwashiorkor compared to data following their nutritional rehabilitation. Since the labelled caffeine recovered in the 2 h breath samples measures CYP1A2 activity, it can be concluded that the enzyme activity was reduced in children with kwashiorkor.

Of the four categories of malnutrition, pharmacokinetic studies have been most often conducted in children with kwashiorkor (Oshikoya *et al.,* 2010). However, the majority of the medicines evaluated were metabolised through pathways other than those catalysed by CYP1A2.

When CYP1A2 activity from the present CBT data was compared to the activities of other CYP450 enzymes previously assessed by drug plasma CLs in children with kwashiorkor, some similarities and discrepancies were observed. The findings of the present research agree with the significantly reduced plasma CL of caffeine reported in children with kwashiorkor (Akinyinka *et al.,* 2000). Another study of adults with or without liver cirrhosis also documented a significantly decreased plasma CL of caffeine in patients with decompensated liver cirrhosis compared to the healthy controls (Scott *et al.,* 1988). This study also reported that patients with compensated liver cirrhosis and the healthy controls metabolised caffeine similarly. Both Akinyinka *et al.* (2000) and Scott *et al.* (1988) involved six to 10 participants and five to eight controls compared to the 15 participants or controls evaluated in the four present studies. However, contrasting results have been reported regarding the metabolism of theophylline in children with kwashiorkor. Its plasma CL was significantly reduced in kwashiorkor compared to the healthy controls (Eriksson *et al*., 1983b). Only five

participants and 12 controls were studied, which is less than the number of participants or controls evaluated in the present studies' CBT. The small sample size may have underpowered the study's capacity to detect a significant difference in the plasma CLs of theophylline, which may have contributed to the varied effects of kwashiorkor on different drugs metabolised by the CYP1A2 enzyme.

The findings of this study are also similar to the effects of kwashiorkor on the plasma CLs of isoniazid, antipyrine, quinine, and chloramphenicol (Buchanan *et al.,* 1979; Buchanan *et al.,* 1980; Eriksson *et al.,* 1983b; Salako *et al.,* 1989). The plasma CLs of these four drugs were significantly reduced in kwashiorkor. As aforementioned, caffeine is metabolised predominantly by CYP1A2 enzyme, yet other drugs are metabolised by different metabolising enzymes. Isoniazid is metabolised by *N*-acetyltransferase-2 (NAT2), CYP2E1, and glutathione S-transferase (GST) (Sotsuka *et al.,* 2011); antipyrine is metabolised by CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C18, and CYP3A4 (Engel *et al.,* 1996); the metabolism of quinine is catalysed predominantly by CYP3A4 (Zhao *et al.,* 1996); and chloramphenicol is metabolised predominantly via glucuronidation by the UDP-glucuronyl transferase enzyme (Park *et al.,* 2003). These findings could suggest that multiple metabolising enzymes are impaired in children with kwashiorkor.

Penicillin, streptomycin, and cefoxitin, all of which are primarily eliminated unchanged by the kidneys without undergoing hepatic metabolism, have shown decreased plasma CLs in children with kwashiorkor (Bolme *et al.,* 1988; Bolme *et al.,* 1995; Buchanan *et al.,* 1979c; Buchanan *et al.,* 1980b). The findings of the present research concur regarding children with kwashiorkor. Contrasting reports have, however, been documented on the plasma CLs of gentamicin and

amikacin (Buchanan *et al.,* 1980b; Hendricks *et al.,* 1995). Both drugs demonstrated no significant changes in their plasma CLs in children with kwashiorkor. These varying effects of kwashiorkor on the hepatic metabolism and renal elimination of drugs may derive from the pathophysiological changes that affect all organs of drug disposition (Krishnaswamy, 1983).

A number of potential mechanisms have been documented to explain the altered drug metabolism in children with kwashiorkor. The activity of the metabolising enzymes may be suppressed or reduced (Eriksson *et al.,* 1983a; Narang, 1987). The quantity of the metabolising enzymes may also be decreased (Mehta *et al.,* 1975) or their quality decreased (Chatterjee & Mukherjee, 1968; Rumack *et al.,* 1973). Kwashiorkor has been linked to diminished kinetics of microsomal enzymes (Mgbodile *et al.,* 1973). Micronutrient deficiencies are constituent features of children with kwashiorkor (Krishnaswamy, 1983; Sauerwein *et al.,* 1997), and the absences of these deficiencies are necessary cofactors to the metabolising enzymes' optimal performance. The growth hormone required for the modulation of the metabolising enzymes (Wilson, 1973) is known to be significantly increased in kwashiorkor (Laditan, 1983; Van Der Westhuysen *et al.,* 1975).

Limited data have also indicated that impairment of drug metabolism in children with kwashiorkor may be related, at least in part, to maladaptation and nutritional deprivation (Franco *et al.,* 1999). Hepatomegaly is a common feature of kwashiorkor, though liver function tests have shown normal results in at least two studies (Porta & Hartroft, 1970; Tandon *et al.,* 1974). However, ultrastructural studies of liver biopsies of patients with kwashiorkor have shown fatty changes, abnormal rough endoplasmic reticula and mitochondria, and decreased peroxisomes (Brooks *et al.,* 1992; Brooks *et al.,* 1994; Tandon *et al.,*

1974), all of which were completely reversible after nutritional rehabilitation (Buchanan *et al.,* 1979a).

6.6. DIFFERENTIAL EFFECTS OF ALL CATEGORIES OF MALNUTRITION ON CAFFEINE METABOLISM

This section aims to compare the similarities and differences among the effects of each category of malnutrition on caffeine metabolism.

The enrichment curves for the underweight children before and after nutritional rehabilitation were similar and comparable to those of healthy adults (Park *et al.,* 2003), adult non-smokers (Kotake *et al.,* 1982), and sham-operated rat controls (Schaad *et al.,* 1995). These results suggest that the activity of the enzyme involved in the metabolism of labelled caffeine was not significantly affected in underweight children compared to results after they had attained normal weight following nutritional rehabilitation.

The ^{13}C-enrichment curves for children with marasmus, marasmic-kwashiorkor, and kwashiorkor differed from those of the underweight children and were characterised by plateaus after attaining peak levels. These are similar to the shape of the CBT curves described for rats that had undergone a partial hepatectomy, bile duct ligation, or portocaval anastomosis (Schaad *et al.,* 1995). The rats of the experimental group lost approximately 30% of their liver mass in association with a reduction of approximately 60% in their CYP1A2 activity. The significance of the altered curves remains uncertain.

The mean cumulative per cent labelled-caffeine in the CO_2 exhaled over a period of 2 h showed no significant difference between the four groups of malnutrition before and after nutritional rehabilitation. This result would suggest

that the activity of CYP1A2 enzymes were similar in underweight children and those experiencing marasmus, marasmic-kwashiorkor, or kwashiorkor. The studies were not, however, powered well enough to detect a significant difference in CYP1A2 activities between the four groups of malnutrition. Therefore, caution is recommended in interpreting the results. A larger study is needed to validate the results presented in this study.

6.7. COMPARISON WITH OTHER STUDIES

When the results of the present CBT are compared to those of pharmacokinetic studies in healthy controls and those patients experiencing marasmus, marasmic-kwashiorkor, or kwashiorkor, there were some similarities and differences (Bolme *et al.,* 1995; Bolme *et al.,* 1988; Eriksson *et al.,* 1983a; Eriksson *et al.,* 1983b). The data from the three groups of severely malnourished children stood in contrast to the lack of significant difference in the plasma CLs of streptomycin and theophylline reported in similar groups of patients (Bolme *et al.,* 1988; Eriksson *et al.,* 1983b). However, other studies have documented a significant decrease in the plasma CLs of penicillin and chloramphenicol in children with marasmus, marasmic-kwashiorkor, and kwashiorkor compared to their respective healthy control groups (Bolme *et al.,* 1995; Eriksson *et al.,* 1983a).

The variation in the present CBT results and those of these previous pharmacokinetic studies may be explained by methodological differences, the involvement of other hepatic cytochrome P450 enzymes in the metabolism of drugs other than caffeine, and the elimination pathways of some of the drugs. The present research evaluated malnourished children who also served as their

own controls after nutritional rehabilitation. Their nutritional statuses were determined from anthropometric parameters only. Furthermore, they were treated empirically for infections prior to the study, and the CBT was used to assess the activity of their CYP1A2 enzyme. By contrast, participants in the previous pharmacokinetic studies were either children or adults; malnutrition was secondary to coexisting gastrointestinal diseases, such as Crohn's disease, ulcerative colitis, and bowel cancer; diagnosis of malnutrition was based on weight loss of 10% and reduced serum albumin concentration; participants either served as their own controls or age, sex-matched control groups were used for comparison; plasma CLs of the drugs were determined; and the drugs studied were metabolised by cytochrome P450 enzymes other than CYP1A2 (Krishnaswamy & Naidu, 1977; Tranvouez *et al*., 1985). Previous studies have shown that coexisting diseases, such as malaria, can influence the disposition of caffeine and quinine in children (Akinyinka *et al.,* 2000; Pussard *et al.,* 1999).

The percentage decrease in CYP1A2 activity, as indicated by the mean cumulative per cent labelled caffeine in the CO_2 exhaled in 2 h was 4.9% in underweight children. This contrasted the 11.3%, 12.5%, and 12.6% observed in marasmus, marasmic-kwashiorkor and kwashiorkor, respectively. These percentage changes in CYP1A2 activity for the three groups of severely malnourished children were relatively similar but much lower than the 55.5% decrease by ciprofloxacin in children with cystic fibrosis (Parker *et al.,* 1994). It is believed that variation in the pathophysiology of malnutrition and cystic fibrosis and the potential interaction of ciprofloxacin with caffeine may have caused the differences in the results. On its own, cystic fibrosis has been reported to enhance xanthine oxidase activity required in caffeine metabolism

(Hamelin *et al.,* 1994), while ciprofloxacin is a known CYP1A2 inhibitor (Levien & Baker, 2003).

6.7.1. Appropriateness of the sample size

The sample size of 15 participants per group of malnutrition type is substantially larger than any of those involved in the previous studies that evaluated total plasma CLs of drugs exclusively metabolised in the liver of malnourished children (Oshikoya *et al.,* 2010).

Only 5 to 10 malnourished children were studied for the clearances of caffeine (Akinyinka *et al.,* 2000), theophylline (Eriksson *et al.,* 1983b), chloramphenicol (Eriksson *et al.,* 1983a), acetanilide (Buchanan *et al.,* 1980a), quinine (Salako *et al.,* 1989), antipyrine (Narang *et al.,* 1977), and metronidazole (Lares–Asseff *et al.,* 1992). Based on a power calculation, it was determined that the 15 patients who participated in each CBT study were adequate to minimising bias caused by the small sample size characterising previous pharmacokinetic studies of malnourished children. However, post hoc analysis of the results that yielded no significant difference between the malnourished and nutritionally restored states showed that a sample size of 15 may not be adequately powered to detect any significant difference in the results. Therefore, a sample size greater than 15 may be necessary to validate some of the results presented in this study.

6.7.2. Age group and gender of the patients

Previous studies have shown low 3-*N*-demethylation of caffeine in infants younger than 6 months (Pons, *et al.,* 1988). The CBT is therefore appropriate to use in children older than 6 months as a method to study the effect of diseases on drug metabolism (Parker *et al.,* 1994). Malnutrition affects infants and young children (6 to 60 months old) more frequently than older age groups (Nnakwe,

1995; WHO, 2000). The severely malnourished children evaluated in the present CBT study were between 3 and 6.5 years old—ages which are in keeping with the age group most affected by malnutrition. However, children younger than 3 years were excluded, because they may not have been able to follow the instructions required for reliable breath sample collection. Similar exclusion criteria have been used in previous CBT studies of children (Parker *et al,* 1994; Parker *et al,* 1997a; Parker *et al,* 1997b). All the previous CBT studies involved children who were 6 to 17 years old. The children tolerated the test for 2 h and cooperated with the investigators throughout its duration.

The participants for the present studies were not balanced by gender. No balance was considered necessary, because the participants served as their own controls. In addition, the gender effect of CYP1A2 on caffeine metabolism has not been determined in children, though there have been inconsistent reports in adults (Horn *et al.,* 1995; Ou–Yang *et al.,* 2000). In one study the activity of CYP1A2, as indicated by the metabolic CL of caffeine, was reported to be higher in men than in women (Ou–Yang *et al.,* 2000). In another study, which used CBT to assess the activity of CYP1A2 enzyme, there was no statistically significant difference between the enzyme activity in men and women (Horn *et al.,* 1995).

6.7.3. Focusing on CYP1A2 enzymes only

CYP1A2 accounts for about 13% of the total human hepatic cytochrome P450 enzymes (Shimada *et al.,* 1994) and metabolises a variety of clinically important drugs. Nevertheless, only the dispositions of caffeine, theophylline, antipyrine, aminopyrine, metronidazole, and propranolol have been studied in malnourished children. The present studies focused on the metabolising activity of CYP1A2 enzymes only, thus precluding the use of CBT to evaluate the metabolism of

other clinically relevant drugs that operate independently of CYP1A2 enzymes in malnourished children.

Given the spectrum of medicines used for treating malnourished children that are not metabolised by the *N*-demethylation pathway only, there is a need to develop or explore other methods for studying drug metabolism in this sect of the population. Such methods include a population pharmacokinetic study involving a larger paediatric sample size with a few blood samples (Thomson, 2006). Salivary samples or dried blood spot tests may replace the venous blood to render the procedure as non-invasive as possible.

6.8. SUMMARY

Four studies involving the use of CBT were conducted to evaluate the effect of underweight malnutrition, marasmus, marasmic-kwashiorkor, and kwashiorkor on ^{13}C-caffeine metabolism and CYP1A2 activity. Altogether, 60 malnourished children—15 participants per group of malnutrition—were studied, both in their acute malnourished states and after their nutritional rehabilitation.

The ^{13}C-enrichment curves for underweight children before and after nutritional rehabilitation were similar to those previously reported in the literature regarding healthy adults. The curves showed both the same pattern and similarities in the rates and extents of recovery of labelled caffeine in the $^{13}CO_2$ exhaled in 2 h before and after nutritional rehabilitation. Furthermore, the mean CPDR in the expired CO_2 in 2 h by underweight children both before and after nutritional rehabilitation did not differ significantly. These results suggest that the metabolism of labelled caffeine, as well as the activity of CYP1A2 enzyme, was not significantly affected in underweight children compared to

findings when they had attained normal weight after their nutritional rehabilitation. The findings were, however, based on a small number of underweight children, which may not be powered enough to detect any significant difference in the results before and after nutritional rehabilitation.

Severely malnourished children (i.e., experiencing marasmus, marasmic-kwashiorkor, and kwashiorkor) also demonstrated significantly decreased CYP1A2 metabolising activities compared to findings when they had recovered nutritionally. This was made clear by the decreased mean CPDR in their expired CO_2 in 2 h during acute malnutrition.

Upon comparing the four groups of malnutrition both before and after nutritional rehabilitation, there was no significant difference in their mean CPDRs. This result suggests that the activity of CYP1A2 enzymes is similar in underweight children and those experiencing marasmus, marasmic-kwashiorkor, or kwashiorkor. The four studies, however, were not powered enough to detect a significant difference in CYP1A2 activities between the four groups of malnutrition.

CHAPTER SEVEN:

CONCLUSION

7.1. INTRODUCTION

This research initially involved performing a systematic review of pharmacokinetic studies in children with protein-energy malnutrition (PEM), followed by the caffeine breath test (CBT) to assess the activity of CYP1A2 enzyme in four study samples of child participants. The major findings from the systematic review and the CBT are summarised below.

7.2. THE SYSTEMATIC REVIEW

The systematic review identified that different groups of malnutrition affect drug disposition differently. The absorption of oral drugs was generally not significantly impaired in malnourished children compared to the control groups. However, in a study involving children with different categories of malnutrition, there were variations in the oral absorption of penicillin. Its absorption rates were similar for underweight and marasmic children when compared to the controls (Bolme *et al.,* 1995) but decreased in kwashiorkor. There were insufficient data on the absorption rate of intramuscular (i.m.) drugs, thus precluding any firm conclusion regarding the effects of PEM on the absorption rate of i.m. drugs. Similarly, there was a scarcity of data on the extent of absorption (i.e., bioavailability) of drugs in PEM. It is, therefore, difficult to conclude on this topic.

Malnutrition appeared to not affect the volume of distribution (VD) of oral and i.m drugs identified in the systematic review, likely since the majority of the drugs have low protein-binding capacities and slight but statistically significant

changes in their unbound fractions. However, while malnutrition showed varying effects on the VD of quinine, streptomycin, theophylline, and gentamicin, the values remained unaffected or decreased and/or increased.

About a half of the drugs identified in the systematic review had significantly decreased plasma clearances (CL) or increased elimination half-lives in malnourished children. However, two drugs (chloramphenicol and quinine) demonstrated variations in their plasma CLs in different groups of malnutrition. For chloramphenicol, total CL was significantly decreased in children with kwashiorkor but unaffected in children with marasmus and marasmic-kwashiorkor (Eriksson *et al.,* 1983a). Similarly, the total CL of quinine was significantly decreased in children with kwashiorkor (Salako *et al.,* 1989) but increased in those who had marasmus or marasmic-kwashiorkor (Treluyer *et al.,* 1996). The pathophysiological changes affecting different types of malnutrition appeared to have contributed to the variations in the results.

7.3. THE CAFFEINE BREATH TEST (CBT)

Caffeine metabolism was not significantly impaired in underweight children as suggested by the similarity in their mean cumulative per cent ^{13}C-dose recovered in the 2 h CO_2 exhaled, both before and after nutritional rehabilitation. Overall, this finding suggests that underweight malnutrition did not significantly influence CYP1A2's metabolising activity. However, post hoc power analysis showed that the study was inadequately powered to detect a statistically significant difference in the activity of CYP1A2 in underweight children and when they had been nutritionally rehabilitated. Further studies with larger sample sizes are therefore required to validate the present results.

There were contrasting results for underweight children regarding the effects of the three types of severe malnutrition (marasmus, marasmic-kwashiorkor, and kwashiorkor) on caffeine metabolism. The mean cumulative per cent ^{13}C-dose recovered in the exhaled CO_2 over the period of 2 h significantly decreased in children with marasmus, marasmic-kwashiorkor, and kwashiorkor, compared to when they were nutritionally rehabilitated. It is uncertain why there was plateauing of the ^{13}C-enrichment curves for each group of severe malnutrition after attaining peak levels before and after nutritional rehabilitation. However, it is possible that the *N*-demethylation pathway was saturated early in the three forms of severe malnutrition.

The decreasing trend in the mean cumulative per cent ^{13}C in the CO_2 exhaled over the period of 2 h observed from underweight to kwashiorkor children, before and after nutritional rehabilitation, would suggest that different groups of malnutrition affect drug metabolism differently. However, the lack of a significant difference in the mean cumulative per cent ^{13}C recovered in the exhaled CO_2 of the underweight and severely malnourished children, before and after nutritional rehabilitation, was not powered enough to enable a firm conclusion regarding the differential effects of malnutrition on caffeine metabolism.

7.4. CLINICAL IMPLICATIONS

Data emanating from the systematic review have indicated a significantly reduced total CL of drugs predominantly metabolised in the liver of children with marasmus, marasmic-kwashiorkor, or kwashiorkor. Data from the present CBT have demonstrated a decreased metabolising activity of the CYP1A2 enzyme in severely malnourished children. Clinicians may need to consider reducing the

dose of clinically relevant drugs such as paracetamol, theophylline, diazepam, naproxen, propranolol, ondansetron, amiodarone, cimetidine, omeprazole, ciprofloxacin, clarithromycin, erythromycin, nalidixic acid, ketoconazole, arthemether/lumefantrine, thiabendazole, carbamazepine, phenobarbital, phenytoin, isoniazid, rifampicin, and ritonavir (Levien & Baker, , 2003), all of which are metabolised via the *N*-demethylation pathway. However, population pharmacokinetic studies are required to determine the therapeutically safe doses of these drugs in severely malnourished children.

The altered hepatic drug metabolism in all groups of severely malnourished children is reversible after nutritional rehabilitation, therefore drug dosages and dosing regimens should be revised for patients continuing drug therapy after nutritional recovery.

Both using the CBT to further study caffeine metabolism in large populations of severely malnourished children and validating those results with population pharmacokinetic and pharmacodynamic studies are necessary to inform future international guideline recommendations.

7.5. FUTURE WORK

This research recognises that even more research needs to be done in the area of malnutrition and drug metabolism. There is a particular need for more population pharmacokinetic studies to define drug dosages and dosing regimens of clinically relevant drugs yet unstudied in malnourished children. The CBT can be extended to study the effects of concomitant diseases in malnourished children on CYP1A2 activity.

7.5.1. Additional pharmacokinetic studies

A significant limitation of traditional pharmacokinetic studies is the need for multiple blood samples. This need poses ethical and practical challenges for obtaining pharmacokinetic data in malnourished children. Antitubercular drugs (isoniazid, rifampicin, pyrazinamide, and ethambutol), and antiretroviral drugs (nevirapine, efavirenz, stavudine, lamivudine, zidovudine, abacavir, didanosine, and lopinavir/ritonavir) are other therapeutically relevant drugs for malnourished children. Many of these drugs may not be metabolised by the CYP1A2 enzyme, and their metabolism could not be studied with the CBT. An alternative approach that involves the use of population pharmacokinetics in which a few blood samples are collected from a large number of patients (Thomson, 2006) may be necessary. The dried blood spot (DBS) technique that involves the collection of a few drops of blood onto a filter paper and is used in combination with pharmacokinetic modelling techniques (Patel *et al.,* 2010) is another useful approach. Salivary samples have been compared with venous and capillary blood samples in the investigation of pharmacokinetics of paracetamol (Rittau & McLachlan, 2012). The results were promising and may be useful for future pharmacokinetic studies in malnourished children, especially if there were an associated mild-to-moderate anaemia.

7.5.2. Using the CBT to study the effects of concurrent infections with malnutrition on caffeine metabolism

It may be possible to use the CBT to evaluate the combined effects of malnutrition and concurrent diseases, such as malaria, tuberculosis, and HIV infection, on caffeine metabolism. Previous pharmacokinetic studies have indicated decreased plasma CLs of caffeine and quinine in children with malaria

(Akinyinka *et al.,* 2000; Pussard *et al.,* 1999). HIV infection or stage of infection in adults has been reported to alter the activities of phase I and II drug metabolising enzymes (Jones *et al.,* 2010). HIV infection has also been associated with an increased variability of CYP1A2, CYP2D6, N-acetyltransferase 2 (NAT2), and xanthine oxidase (XO) compared to uninfected control groups. Malnutrition is also frequently associated with HIV infection. Concomitant malnutrition causes significant increases in mortality rate and diminished response to therapies, including antiretroviral therapy (ART) (Fergusson & Tomkins, 2009). The feasibility of using the CBT to determine the combined effects of malnutrition and HIV infection on drug metabolism in children should be explored in the future.

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APPENDICES

1. Appendix I: Participant information form.
2. Appendix II: Consent form for the parents.
3. Appendix III: Assent form for participants older than six years.
4. Appendix IV: Ethical approval from Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria.
5. Appendix V: Ethical approval from Lagos State Health Service Commission, Lagos, Nigeria.
6. Appendix VI: Ethical approval from Aminu Kano Teaching Hospital, Kano State, Nigeria.
7. Appendix VII: Raw data for the mean± SD 13C-enrichments of underweight children, before and after nutritional rehabilitation.
8. Appendix VIII: Raw data for the mean± SD 13C-enrichments of marasmic children, before and after nutritional rehabilitation.
9. Appendix IX: Raw data for the mean± SD 13C-enrichments of marasmic-kwashiorkor children, before and after nutritional rehabilitation.
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Appendix I

Participant Information Sheet

This is a document containing a summary of the study "**USING THE BREATH TEST TO STUDY CAFFEINE METABOLISM IN PROTEIN-ENERGY MALNOURISHED CHILDREN**"

1. Children suffering from protein-energy malnutrition (PEM) are at risk of other health problems that may require multiple drug therapies. The body of these children handles drug differently from that of other children. Only a few studies have looked at drug metabolism in PEM children, they all involved collection of multiple blood samples.
2. We plan to study drug metabolism in your child without taking any blood sample. The study hopes to improve the safety and dosing of medicines for children with the same illness as your child.
3. The study will last 2-3 hours, commencing at 9a.m. During the study, the usual diet of your child will be replaced with Casilan®.
4. Your child will abstain from caffeinated products for at least 20 hours and remain in bed, before and during the study, so as to minimise physical activities.
5. We plan to administer, in a small quantity, a substance called caffeine to your child a few days after hospital admission and between 4-6 weeks after an appreciable recovery.
6. Caffeine is harmless to children and is abundant in many of their daily foods and drinks such as candies, cookies, chocolate bars and drinks, coffee drink, soda drinks, and diary drinks. 3 mg/kg dose have been used in previous studies involving children without any adverse effects. The caffeine will be dissolved in a small quantity of water with a little squash added for your child to drink.
7. We will request your child to blow through a straw into a special bottle. This will be done at 20, 10 and 1 minute before taking the caffeine drink and at every 15 minutes after taking the caffeine drink over a 2 hour period.
8. We will continue to monitor your child to be sure that everything is fine during and after the procedure.
9. There is no monetary reward for participating in the study.
10. Your child is free to opt out of the study at any point should you find the procedure intolerable or for any reason best known to you.
11. All the information collected about your child will be treated with utmost confidentiality.

Appendix II

USING THE BREATH TEST TO STUDY CAFFEINE METABOLISM IN PROTEIN-ENERGY MALNOURISHED CHILDREN

Consent Form for Children below 6 Years Old

Investigators: Dr Kazeem Adeola Oshikoya, Dr Helen Sammons and Prof. Imti Choonara (Academic Division of Child Health, University of Nottingham)

The parent/caregiver of the participant is requested to complete the whole of this sheet himself/herself.

Please cross out as necessary.

1. Have you read or the researcher has read to you and you understood the participant information sheet **YES/NO**
2. Have you had the opportunity to ask questions and discuss the study? **YES/NO**
3. Have all the questions been answered satisfactorily? **YES/NO**
4. Have you received enough information about the study? **YES/NO**
5. Do you understand that your child/ward is free to withdraw from the study at any time? **YES/NO**
6. Do you understand that your child/ward is free to withdraw from the study without having to give a reason? **YES/NO**
7. Do you agree to your child/ward participating in the study? **YES/NO**

"This study has been explained to me to my satisfaction, and I agree to my child/ward taking part. I understand that my child/ward is free to withdraw at any time."

Signature of the child's parent/caregiver ……**Date**……

Name of the child (in block letters)……..

I have explained the study to the parent/caregiver of the above child/participant and he/she has agreed to allow the child to participate.

Signature of the researcher ……..**Date** ……..

Appendix III

USING THE BREATH TEST TO STUDY CAFFEINE METABOLISM IN PROTEIN-ENERGY MALNOURISHED CHILDREN

Assent Form for Children over 6 years Old

My name is **Dr. Kazeem Oshikoya**. I am trying to learn about how drugs work and how they are removed from the body of kids who are not well fed because inadequate nutrition tends to affect the work and removal of drugs from the body of kids. Please let me know if you are willing to participate in this study.

If you decide to be in this study, you will be asked to blow air with your mouth, through a straw, into a special bottle. You will be asked to blow the air into the bottle at every 15 minutes over a period of 2 hours. After taking your first three breath samples, you will be given a medicine called **Caffeine**, at a dose appropriate for your weight, to drink. The medicine will be dissolved in water mixed with squash drink to give the medicine a pleasant taste. You cannot take any food or other drink during the time of the study. I will need to take your age, weight and height before the study begins.

Taking part in the study will enable us to know that the doses of medicine given to children who are not well fed will not cause them any harm. At the end of the study, the **caffeine** medicine may make you not to go to bed early or make your heart beat faster than normal. I want to assure you that these are not serious problems and may happen only for a short time.

If you decide to be in the study, I will not tell anyone else what you say or do in the study. When I tell other people about the study, I will not use your name, so no one can tell who I am talking about.

Your parents or guardian have to say it's OK for you to be in the study. After they decide, you get to choose if you want to do it too. If you don't want to be in the study, no one will be mad at you. If you want to be in the study now and change your mind later, that's OK. You can stop at any time.

My telephone number is **08090684327**. You can call me if you have questions about the study or if you decide you don't want to be in the study any more.

Agreement

I have decided to be in the study even though I know that I don't have to do it. Signing here means that I have read this form or have it read to me and I am willing to be in this study.

_____ _____

Name and Signature of Study Participant **Date**

_____ _____

Name and Signature of Researcher **Date**

Appendix IV: Ethical approval from Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria

LAGOS STATE UNIVERSITY TEACHING HOSPITAL, IKEJA

1-5, OBA AKINJOBI ROAD, IKEJA, LAGOS. P.M.B. 21005. TEL: 01-4710670, 4975739
www.lasuth.org. e-mail.dsct@lasuth.org.

DIRECTORATE OF CLINICAL SERVICES AND TRAINING

HEALTH RESEARCH AND ETHICS COMMITTEE

REG.NO. NHREC 04/04/2008

PROJECT TITLE: A STUDY OF DRUG METABOLISM IN CHILD REN WITH PROTEING ENERGY MALNUTRITION USING THE CAFFEINE BREATHE TESTS

REF: NO.: LREC/10/06/139

PRIN. INVESTIGATOR: DR. KAZEEM ADEOLA OSHIKOYA

ADDRESS: ACADEMIC DIVISION OF CHILD HEALTH MEDICAL SCHOOL, UNIVERSITY OF NOTHINGHAN ROYAL DERBY HOSPITAL, DERBY UK.

DATE OF RECEIPT OF VALID APPLICATION: 03\06\10

DATE OF APPROVAL: *28/07/2010*

This is to inform that the research described here in the submitted protocol, the consent forms, advertisements and other participant information materials have been reviewed and given full approval by the Health Research & Ethics Committee.

This approval dates from ***28/07/2010 to 28/07/2012***. If there is any delay in starting the research, please inform the HREC so that the dates of approval can be adjusted accordingly. Note that no participant accrual or activity related to this research may be conducted outside of these dates. All inform consent forms used in this study must carry the HREC assigned number and duration of HREC approval study. In multiyear research, endeavor to submit your annual report to the HREC early in order to obtain renewal of your approval and avoid disruption of your research.

The National code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the code including ensuring that all adverse events are reported promptly to the HREC. No changes are permitted in the research without prior approval by the right HREC except in circumstances outlined in the code. The HREC reserves the right to conduct compliance visit to your research site without previous notification.

[signature]

PROF. O. OGUNDIPE.

Chairman, HREC-LASUTH

PS The protem approval is given to enable you get permission to come home. On arrival please let us have a Yoruba [illegible] English translation of the participants information sheet [illegible]

DR. OLAROGE V. OGUNBANJO
BCS, FWACS, FICS
Director Of Clinical Services and Training

DR. FEMI OLUGBILE

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Appendix V: Ethical approval from Lagos State Health Service Commission, Lagos, Nigeria

LAGOS STATE GOVERNMENT
HEALTH SERVICE COMMISSION

1, Ganiu Smith Street
Lagos Island
Lagos.

Telephone: 2637140, 892305
Fax No. 01-2637140
E-mail: .....
Website: .....

Ref. No. SHMB/728/VOL.VII/1117A

Date: 23rd February, 2011

DR. KAZEEM ADEOLA OSHIKOYA,
Academic Division of Child Health,
Medical School in Derby,
University of Nottingham,
Royal Derby Hospital,
United Kingdom.

RE: RESEARCH ON DRUG METABOLISM IN PROTEIN ENERGY MALNOURISHED CHILDREN

I am directed to convey the approval of the Permanent Secretary - Lagos State Health Service Commission to you, to carry out your research.

Please forward copy of your findings to Lagos State Health Service Commission.

DR. (Mrs.) A. M. Onayiga
For: Permanent Secretary.

cc:
The Medical Director's,
Massey Street Children Hospital,
General Hospital Gbagada,
General Hospital Ikorodu,
General Hospital Orile-Agege.

MISSION STATEMENT:- To provide Proactive Management Service that will engender Qualitative Health Care Delivery Service.

Appendix VI: Ethical approval from Aminu Kano Teaching Hospital, Kano State, Nigeria

Dr Kazeem A Oshikoya
Academic Division of Child Health
Medical School in Derby
University of Nottingham
Royal Derby Hospital
Uttoxetter Road
Derby DE22 3 DT
United Kingdom

Re: Ethical Approval
Re: Using the Caffeine Breath Test to Study Caffeine Metabolism in Protein Energy Malnourished Children

Further to your application in respect of the above titled research proposal and your subsequent response to the committee's enquiries; the committee considered your proposal and noted same as a prospective study. Similarly, you are required to provide a consent form to clearly include consent section for parents/guardians in addition to the assent section. You are advised to seek the corporation of paediatric department.
In view of this, Ethical Approval is hereby granted to conduct the research. However, the approval is subject to periodic reporting of the progress of the study and its completion to the committee.

Best Regards
Bara'atu Kabir (Mrs)
Secretary
For: Chairman Ethical Committee,
Aminu Kano University Teaching Hospital,
Kano State, Nigeria.

Appendix VII: Raw ^{13}C-enrichment (APE) data for underweight children

Raw data for underweight children before nutritional rehabilitation

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
15 minutes	0.002625	0.000254	0.000982	0.002035	0.009300	0.003100	0.004830	0.001940	0.001600	0.003660	0.000670	0.004770	0.003280	0.003980	0.001610
30 minutes	0.003058	0.000623	0.000320	0.001773	0.015100	0.003900	0.006760	0.003700	0.004350	0.003620	0.001850	0.003340	0.004570	0.006840	0.004000
45 minutes	0.005647	0.000194	0.000976	0.002089	0.015500	0.009800	0.010920	0.007150	0.003890	0.003040	0.000070	0.003910	0.006110	0.007350	0.006780
60 minutes	0.006502	0.000285	0.003660	0.004163	0.013400	0.004500	0.006530	0.005070	0.006410	0.003770	0.001490	0.006060	0.008720	0.007780	0.007900
75 minutes	0.002414	0.001220	0.002090	0.002133	0.013600	0.004900	0.006110	0.005030	0.006260	0.004130	0.003450	0.006430	0.008960	0.008590	0.007460
90 minutes	0.003740	0.000751	0.003700	0.002068	0.012300	0.005500	0.008830	0.006080	0.003820	0.004410	0.000542	0.005490	0.006780	0.008970	0.008680
105 minutes	0.003267	0.001270	0.002950	0.002262	0.011900	0.005300	0.005280	0.005390	0.006160	0.005070	0.000700	0.009150	0.006340	0.009090	0.008390
120 minutes	0.004682	0.001010	0.004850	0.003188	0.011000	0.005300	0.005340	0.006470	0.004290	0.005060	0.000794	0.005400	0.005490	0.001220	0.008430

Raw data for underweight children after nutritional rehabilitation

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
15 minutes	0.00723	0.001001	0.002708	0.001266	0.003083	0.000296	0.00224	0.00183	0.000976	0.01036	0.009880	0.003959	0.00260	0.00413	0.00321
30 minutes	0.00809	0.001011	0.004737	0.002670	0.005876	0.002930	0.00317	0.00295	0.001001	0.01056	0.010027	0.005746	0.00389	0.00781	0.00668
45 minutes	0.00878	0.001012	0.006580	0.003130	0.006941	0.002276	0.00582	0.00446	0.001028	0.01072	0.010082	0.006084	0.00419	0.00739	0.00655
60 minutes	0.01030	0.001023	0.006966	0.003082	0.007658	0.002668	0.00671	0.00526	0.001050	0.01087	0.010119	0.005975	0.00456	0.00766	0.00636
75 minutes	0.01250	0.001027	0.006315	0.003082	0.007658	0.010558	0.00670	0.00531	0.001043	0.01111	0.010085	0.005749	0.00473	0.00790	0.00714
90 minutes	0.01260	0.001031	0.006402	0.003637	0.007307	0.002859	0.00704	0.00548	0.001044	0.01118	0.01008	0.005673	0.00476	0.00821	0.00649
105 minutes	0.01200	0.001031	0.006256	0.003738	0.006193	0.004510	0.00624	0.00529	0.001044	0.01119	0.010105	0.005969	0.00442	0.00773	0.00632
120 minutes	0.01290	0.001031	0.006603	0.003828	0.006158	0.002362	0.00606	0.00486	0.001041	0.01127	0.010128	0.006106	0.00457	0.00773	0.00587

Appendix VIII: Raw ^{13}C-enrichment (APE) data for children with marasmus

Raw data for children with marasmus before nutritional rehabilitation

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
15 minutes	0.001157	0.000296	0.001715	0.005910	0.001900	0.000539	0.000818	0.001906	0.006940	0.000931	0.001350	0.001770	0.003590	0.002390	0.002670
30 minutes	0.002120	0.000784	0.003266	0.010100	0.003180	0.002374	0.001866	0.003353	0.001150	0.001955	0.002210	0.002240	0.005360	0.002820	0.003747
45 minutes	0.002734	0.001049	0.004279	0.009760	0.004300	0.003079	0.002615	0.003947	0.009360	0.002204	0.002360	0.002780	0.005120	0.003760	0.005556
60 minutes	0.003562	0.001522	0.004079	0.009440	0.003910	0.003907	0.003458	0.003542	0.005940	0.003090	0.002610	0.002630	0.004920	0.003740	0.005039
75 minutes	0.003556	0.001862	0.003073	0.009420	0.004800	0.004477	0.004029	0.003977	0.007330	0.004104	0.002590	0.003000	0.004950	0.003280	0.005641
90 minutes	0.003643	0.001976	0.003598	0.007560	0.005310	0.004542	0.004411	0.003075	0.006340	0.004110	0.002760	0.002990	0.005590	0.003350	0.005750
105 minutes	0.003539	0.002046	0.003922	0.007710	0.005460	0.004942	0.004461	0.003073	0.006200	0.004571	0.002870	0.002660	0.005560	0.003830	0.005804
120 minutes	0.003645	0.001885	0.003754	0.007780	0.005050	0.004633	0.004686	0.003472	0.005850	0.005130	0.002870	0.002990	0.004990	0.003580	0.005960

Raw data for children with marasmus after nutritional rehabilitation

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
15 minutes	0.0013100	0.0006410	0.0010500	0.0041270	0.0014720	0.0019130	0.0007010	0.0037700	0.0107620	0.0012800	0.0021600	0.0025900	0.0039400	0.0023700	0.0029200
30 minutes	0.0026100	0.0012780	0.0024700	0.0065520	0.0030370	0.0048310	0.0034620	0.0046700	0.0057450	0.0022900	0.0035800	0.0016300	0.0069200	0.0037300	0.0051000
45 minutes	0.0032300	0.0017750	0.0029800	0.0102050	0.0045300	0.0081140	0.0049840	0.0053400	0.0058040	0.0029800	0.0034500	0.0036600	0.0069700	0.0039100	0.0056900
60 minutes	0.0033600	0.0019920	0.0031400	0.0109740	0.0048630	0.0057040	0.0054800	0.0052500	0.0059400	0.0038500	0.0039800	0.0044000	0.0066200	0.0038400	0.0061100
75 minutes	0.0034900	0.0020410	0.0033500	0.0106010	0.0051590	0.0056380	0.0056530	0.0057500	0.0055370	0.0042700	0.0038500	0.0045000	0.0061600	0.0044200	0.0067000
90 minutes	0.0035900	0.0018270	0.0033600	0.0090720	0.0051410	0.0058840	0.0059460	0.0048700	0.0054860	0.0041600	0.0046300	0.0048300	0.0067600	0.0047700	0.0071300
105 minutes	0.0036501	0.0019371	0.0033501	0.0096091	0.0051391	0.0057491	0.0061011	0.0042601	0.0058451	0.0040201	0.0047801	0.0049801	0.0062701	0.0044701	0.0069801
120 minutes	0.0035019	0.0022109	0.0033519	0.0087909	0.0052839	0.0059998	0.0066849	0.0037019	0.0056619	0.0048419	0.0047019	0.0050419	0.0061019	0.0036719	0.0072719

Appendix IX: Raw ^{13}C-enrichment (APE) data for children with marasmic-kwashiorkor

Raw data for children with marasmic-kwashiorkor before nutritional rehabilitation

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

Raw data for children with marasmic-kwashiorkor after nutritional rehabilitation

15 minutes	0.000612	0.002918	0.003680	0.001126	0.003564	0.002190	0.001906	-0.000036	0.002640	0.002480	0.001720	0.004450	0.001280	0.002140	0.008860
30 minutes	0.001487	0.005101	0.004122	0.003524	0.002750	0.004450	0.003353	0.000892	0.003560	0.003460	0.002520	0.006760	0.003120	0.003640	0.002570
45 minutes	0.002520	0.005687	0.005154	0.003942	0.003268	0.004620	0.003947	0.001590	0.003540	0.004180	0.002550	0.007800	0.003490	0.004540	0.002970
60 minutes	0.003497	0.006114	0.004650	0.003832	0.003252	0.004770	0.003542	0.001520	0.003290	0.004810	0.002840	0.008080	0.004470	0.004420	0.003660
75 minutes	0.003705	0.006699	0.005049	0.003920	0.003694	0.004720	0.003977	0.001940	0.003370	0.004690	0.003120	0.007580	0.004280	0.004280	0.003710
90 minutes	0.004319	0.007134	0.005608	0.004382	0.003935	0.005280	0.003757	0.002170	0.003420	0.005140	0.003870	0.008020	0.004040	0.004100	0.004410
105 minutes	0.004066	0.007090	0.005455	0.004294	0.004012	0.005040	0.003073	0.002560	0.003540	0.005160	0.003560	0.008270	0.003780	0.004170	0.004780
120 minutes	0.004209	0.007473	0.005275	0.002149	0.004202	0.004000	0.003472	0.002570	0.003310	0.005270	0.003630	0.007940	0.004330	0.004430	0.005210

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
15 minutes	0.001494	0.000252	0.004627	0.001823	0.001283	0.001553	0.002332	-0.000310	0.001243	0.003173	0.000973	0.004533	0.000803	0.000893	0.003463
30 minutes	0.005161	0.001995	0.005490	0.003063	0.003283	0.002973	0.003853	0.000693	0.002693	0.005673	0.001443	0.008253	0.002273	0.002783	0.005453
45 minutes	0.006302	0.003868	0.006988	0.004143	0.004673	0.004493	0.004283	0.001643	0.003023	0.005523	0.001803	0.009423	0.002603	0.004193	0.005443
60 minutes	0.006049	0.005534	0.007306	0.003733	0.004593	0.004463	0.004843	0.001573	0.003083	0.006163	0.002463	0.008763	0.002813	0.004993	0.005663
75 minutes	0.007080	0.006392	0.007425	0.004393	0.004723	0.004083	0.005223	0.001673	0.002903	0.006873	0.002523	0.009523	0.002853	0.004733	0.005833
90 minutes	0.006634	0.007062	0.008313	0.004573	0.005153	0.004153	0.004963	0.002043	0.003593	0.006313	0.002733	0.009423	0.002923	0.004693	0.005753
105 minutes	0.006624	0.007560	0.007473	0.004473	0.004173	0.004103	0.004983	0.002213	0.003853	0.005953	0.002723	0.009123	0.002793	0.004633	0.006683
120 minutes	0.006417	0.006730	0.008108	0.003726	0.004336	0.003846	0.004036	0.001866	0.003436	0.005746	0.002496	0.008366	0.007336	0.004346	0.006356

Appendix X: Raw ^{13}C-enrichment (APE) data for children with kwashiorkor

Raw data for children with kwashiorkor before nutritional rehabilitation

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
15 minutes	0.001280	0.001830	0.000024	0.002680	0.002220	0.001530	0.001850	0.002720	0.002280	0.003120	0.001240	0.000720	0.003260	0.002150	0.000940
30 minutes	0.004470	0.004080	0.008480	0.004340	0.004220	0.002220	0.003440	0.003660	0.003740	0.004820	0.003300	0.002130	0.005040	0.004040	0.001440
45 minutes	0.004902	0.002832	0.002112	0.005932	0.004932	0.003462	0.004942	0.004892	0.004222	0.005232	0.003932	0.002882	0.006772	0.005026	0.002092
60 minutes	0.004860	0.002130	0.001630	0.005350	0.005550	0.002850	0.003900	0.004820	0.004230	0.005530	0.005210	0.003890	0.005790	0.004510	0.001510
75 minutes	0.004570	0.002330	0.002290	0.005610	0.005990	0.003160	0.004410	0.004900	0.004380	0.005880	0.004100	0.004300	0.005440	0.004670	0.002030
90 minutes	0.004590	0.002650	0.002710	0.005590	0.006230	0.003040	0.004820	0.004800	0.004290	0.006240	0.004320	0.004570	0.004710	0.004710	0.002030
105 minutes	0.005430	0.002810	0.002670	0.005690	0.006380	0.002540	0.004770	0.004980	0.004210	0.006240	0.004960	0.004510	0.006390	0.004670	0.002150
120 minutes	0.007080	0.002900	0.003120	0.006470	0.005900	0.002980	0.005620	0.005300	0.004710	0.006000	0.005000	0.004180	0.005610	0.004520	0.002180

Raw data for children with kwashiorkor after nutritional rehabilitation

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
15 minutes	0.005660	0.001260	0.001340	0.002680	0.001670	0.001340	0.002870	0.003620	0.002870	0.004970	0.002940	0.001900	0.005060	0.001913	0.002030
30 minutes	0.007300	0.002070	0.002960	0.003980	0.003490	0.003020	0.005480	0.005930	0.004730	0.007450	0.005670	0.003030	0.008800	0.004831	0.001060
45 minutes	0.007012	0.002302	0.003182	0.004722	0.004302	0.002692	0.005152	0.005782	0.004832	0.006412	0.005502	0.003342	0.007362	0.007566	0.009332
60 minutes	0.006570	0.002910	0.003880	0.005880	0.005680	0.004310	0.006160	0.006400	0.005690	0.007040	0.006580	0.004300	0.007300	0.005704	0.001340
75 minutes	0.007460	0.003540	0.004160	0.005930	0.006620	0.004500	0.006000	0.006360	0.006100	0.007110	0.006390	0.004400	0.007260	0.005638	0.001580
90 minutes	0.007950	0.003510	0.003830	0.006500	0.006050	0.004500	0.005210	0.006530	0.005520	0.007230	0.006380	0.004090	0.008320	0.005884	0.001250
105 minutes	0.007950	0.003500	0.004000	0.006030	0.006610	0.004250	0.005820	0.005870	0.005130	0.007290	0.005500	0.004840	0.007770	0.005859	0.001670
120 minutes	0.007710	0.003500	0.004230	0.006270	0.006210	0.004850	0.004780	0.005950	0.004910	0.006730	0.004300	0.004710	0.007610	0.006196	0.002140