EFFECTS OF VITAMIN SUPPLEMENTATION ON PLASMA HOMOCYSTEINE AND APOLIPOPROTEIN A1 LEVELS IN CHILDREN WITH NEPHROTIC SYNDROME IN IBADAN, NIGERIA

BY

BOSE ETANIAMHE, ORIMADEGUN
B.Sc. Biochemistry, M.Sc. Chemical Pathology (Ibadan)
Matric No: 65477

APRIL 2013

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

EFFECTS OF VITAMIN SUPPLEMENTATION ON PLASMA HOMOCYSTEINE AND APOLIPOPROTEIN A1 LEVELS IN CHILDREN WITH NEPHROTIC SYNDROME IN IBADAN, NIGERIA

BY

BOSE ETANIAMHE, ORIMADEGUN
B.Sc. Biochemistry, M.Sc. Chemical Pathology (Ibadan)
Matric No: 65477

A Thesis in the Department of Chemical Pathology, Faculty of Basic Medical Sciences, College of Medicine Submitted in Partial Fulfilment of the Requirements for the Award of the Degree of

DOCTOR OF PHILOSOPHY

of the

UNIVERSITY OF IBADAN

APRIL 2013

UNIVERSITY OF IBADAN LIBRARY
AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

CERTIFICATION

I certified that this work was carried out by Bose Etaniamhe ORIMADEGUN in the Department of Chemical Pathology, Faculty of Basic Medical Sciences, University of Ibadan, Nigeria.

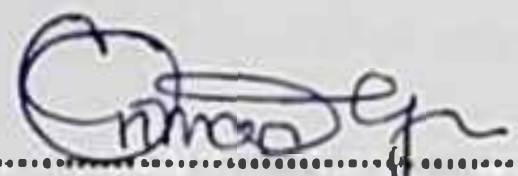
SUPERVISOR 30/4/13

E.O. AGBEDANA, Ph.D.
Professor of Chemical Pathology
Department of Chemical Pathology
University of Ibadan
Ibadan, Nigeria.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

EFFECTS OF VITAMIN SUPPLEMENTATION ON PLASMA HOMOCYSTEINE AND APOLIPOPROTEIN A1 LEVELS IN CHILDREN WITH NEPHROTIC SYNDROME IN IBADAN, NIGERIA



20/4/13

BOSE ETANIAMHE, ORIMADEGUN
Chemical Pathology Department
University of Ibadan
Ibadan, Nigeria

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

DEDICATION

This thesis is dedicated to:

God Almighty,

The three men in my life (Adebola, Isaac and Israel)

and all Nigerian children whose healthcare needs are scarcely met.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

ACKNOWLEDGEMENT

I thank God Almighty, the Ancient of Days for giving the grace and wisdom to complete this research work successfully. He is the solid rock on which I stand.

I wish to express my profound gratitude to my supervisor who is my father and mentor, Prof. E.O. Agbedana for his immense contribution to my academic development. He was very understanding, encouraging, patient and critical during the course of this project. The Lord will continue to bless and strengthen you.

My gratitude also goes to my lecturers in the Department of Chemical Pathology, who have contributed to my successful performance during my study. Prof. Emeritus G. Oladunni Taylor, Prof. O.G. Arinola, Prof. J.I. Anetor for his constructive criticism, Dr. F.M. Abbiyesuku, Dr. K.S. Akinlade, Dr. M.O. Akinosun and Dr. K.S. Adedapo, I sincerely thank the postgraduate co-ordinator Dr. (Mrs.) M. A. Charles-Davies for her motherly role and advise at all times.

I wish to express my gratitude to the Head of Department of Paediatrics at the time of commencing this research in person of Professor A. G. Falade. I am grateful to Dr. A.D. Ademola (Consultant Paediatric Nephrologist), all the Resident Doctors and nurses of the Department of Paediatrics, University College Hospital, Ibadan for their assistance in caring for patients who participated in the study. I also want to thank Mr. N.K. Afolabi for his help during the selection of volunteers for control subjects.

My special thanks to Mr. T.J. Oyewunmi for his assistance in the laboratory work. I want to thank the non-academic staff of the Department of Chemical Pathology, Mrs. G.O. Popoola, Mr. Dele, Mrs. A. Adeyemo, Mrs. M.E. Taiwo, Mr. T.S. Ariwoola for their support. I am also grateful to Mr Emmanuel Oni, Mr. Olabiyi formerly of the Department of Chemical pathology, University College Hospital, Ibadan; Miss.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Olajumoke Oni, Dr. Subulade Olaniyan both of the Institute of Child Health, College of Medicine, University of Ibadan for the laboratory assistance rendered during the project work.

I owe so much to my very close friend and classmate Dr. E. B. Bolajoko for her ever ready academic discussions and helpful criticism. To the other students in the Department of Chemical Pathology who have one way or the other contributed to the success of this work I say thank you: Dr. (Mrs.) O. Ayelagbe, Dr. (Mrs.) A. Nsonwu, and Mrs. U. Fabian.

My sincere appreciation to my other friends, Mrs. Olajumoke Abu-Okolo, Mrs. Rachael Agbaje, Mrs. Adedayo Aleshin (who attended two of my seminar presentations on this work), and Mrs. Yetunde Oiku, also Elder & Elder (Mrs.) Oyeniyi for their moral support and prayers.

To my family, I say you are the best. I am most indebted to my beloved husband, Dr. Adebola E. Orimadegun, who never gave up on me but was always there and had to stay in the laboratory with me overnight at some point during the laboratory work. Thank you for your moral, financial and spiritual support, the Lord will continue to bless you. My sons (Isaac and Israel) have been very understanding. Lastly, I thank my mother for taking good care of the children during my absence from home.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

ABSTRACT

Nephrotic Syndrome (NS), a condition characterised by proteinuria, hypoproteinaemia, and hypercholesterolaemia resulting from damage to the kidneys' glomeruli blood vessel, is a common childhood disease worldwide. Elevated Total Homocysteine (tHcy) has been proposed as a major contributor to morbidity and mortality in children with NS and lowering tHcy level may improve survival in children. Though childhood NS is prevalent in Nigeria, evidence of hyperhomocysteinaemia and the effects of vitamin supplementation on tHcy and lipids are scanty. Therefore, this study was conducted to evaluate plasma tHcy, lipids and apolipoprotein-A1 in NS before and after vitamin supplementation.

Using a pre-post, quasi-experimental design, 84 children (42 with NS and 42 age-sex and matched controls) were consecutively recruited at the University College Hospital, Ibadan. A structured questionnaire was used to collect clinical and anthropometric data. Weight and height were measured using a weighing scale and stadiometer. All children with NS were given 5 mg folate and one tablet of vitamin-B-complex daily. Blood samples collected at baseline and 3-month post supplementation, were analysed for tHcy using enzyme immuno-assay; Total Cholesterol (TC) and triglycerides using enzymatic colorimetric method; High Density Lipoprotein Cholesterol (HDL-C) with phosphotungstate magnesium precipitation; and Apolipoprotein-A I (Apo-A1) by turbidimetric method. Serum folate, pyridoxine (B_6) and cyanocobalamin (B_{12}) were determined using high performance liquid chromatography and Serum Creatinine (SC) with fixedtime Jaffe method. Low Density Lipoprotein Cholesterol (LDL-C) was estimated by means of Friedewald formula. Data were analysed using descriptive statistics, Student's t-test and Mann-Whitney U test at $p = 0.05$.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

The mean age for NS and control were 103.5 ± 32.7 and 100.9 ± 29.4 months respectively. The mean BMI of NS (17.3 ± 1.6 kg/m^2) was significantly higher than that of control (15.9 ± 1.2 kg/m^2). At baseline, NS had significantly higher value than control with respect to tHcy (11.3 ± 2.6 µmol/L versus 5.5 ± 2.3 µmol/L), TC (238.8 ± 93.9 mg/dL versus 155.0 ± 70.6 mg/dL), triglycerides (161.5 ± 114.4 mg/dL versus 108.4 ± 52.6 mg/dL), LDL-C (191.9 ± 91.2 mg/dL versus 122.5 ± 70.2 mg/dL) and Apo-A1 (173.8 ± 43.4 mg/dL versus 136.7 ± 56.9 mg/dL) but no significant difference in HDL-C. In addition, NS had significantly lower values than control for folate (9.1 ± 3.9 ng/mL versus 11.2 ± 3.1 ng/dL) and cyanocobalamin (268.5 ± 95.7 pg/mL versus 316 ± 117.2 pg/mL). The concentrations of pyridoxine (NS: 72.4 ± 13.1 nmol/L; control: 75.8 ± 15.2 nmol/L) and creatinine (NS: 0.7 ± 0.4 mg/dL; control: 0.5 ± 0.2 mg/dL) were not significantly different. Vitamin supplementation significantly lowered tHcy by 52.6%, TC by 21.1%, triglycerides by 26.1%, LDL-C by 21.2%, Apo-A1 by 12.5%, creatinine by 28.6%; but increased folate by 26.4%, cyanocobalamin by 15.4% and pyridoxine by 15.3% when compared with the respective baseline values.

Elevated plasma homocysteine and atherogenic lipids were associated with low serum folate and cyanocobalamin in children with nephrotic syndrome. These abnormalities were improved by supplementation with oral vitamins.

Keywords: Nephrotic syndrome, Vitamin supplementation, Plasma homocysteine

Word counts: 483

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

TABLE OF CONTENTS

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

LIST OF TABLES

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

LIST OF TABLES (Continued)

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

LIST OF FIGURES

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

LIST OF ABBREVIATIONS

Acronyms/Abbreviations	Full meanings
95% CI	95% Confidence Interval
A:	Absorbance
Apo-AI:	Apolipoprotein A1
ARIC	Atherosclerosis Risk in Communities Study
ATP:	Adenosine Tri Phosphate
BHMT:	Betaine Homocysteine Methionine Transferase
BMI:	Body Mass Index
CBS:	Cystathionine β Synthase
CHD:	Coronary Heart Disease
CRI:	Chronic Renal Insufficiency
CSF	Cerebrospinal Fluid
Cu:	Copper
CVD:	Cardiovascular Disease
DHFR:	Dihydrofolate reductase
dTMP:	Deoxythymidine monophosphate
dUMP:	Deoxyuridine monophosphate
ESRD	End Stage Renal Failure
fHcy:	Free Homocysteine
FSGS:	Focal and Segmental Glomerulosclerosis
GFR:	Glomerular Filtration Rate
H_2O_2:	Hydrogen peroxide
H_2S:	Hydrogen Sulphide
HCL:	Hydrogen Chloride
Hcy:	Homocysteine
HDL-C:	High Density Lipoprotein Cholesterol
HPLC:	High Performance Liquid Chromatography
$KMnO_4$:	Potassium permanganate
LDL-C:	Low Density Lipoprotein Cholesterol
MCNS:	Minimal Change Nephrotic Syndrome
MS:	Methionine Synthase

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

MTHFR:	Methylenetetrahydrofolate reductase
NaOH:	Sodium Hydroxide
NS:	Nephrotic Syndrome
O_2	Oxygen
OD:	Optical Density
OR	Odds Ratio
SAH:	S-adenosylhomocysteine
SAM:	S-adenosylmethionine
SC:	Serum Creatinine
SD:	Standard Deviation
TAS:	Total Antioxidant Status
TC:	Total Cholesterol
TG:	Triglycerides
tHcy:	Total Homocysteine
THF:	Tetrahydrofolate
UK	United Kingdom
USA	United State of America
VLDL-C:	Very Low Density Lipoprotein Cholesterol

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

CHAPTER ONE

INTRODUCTION

1.1. Background

Nephrotic syndrome (NS) is a disease of the kidneys characterised by the presence of hypercholesterolemia, massive proteinuria, and hypoalbuminaemia with or without oedema (2000). The causes of NS may be primary or secondary. The term "primary" indicates that the disease is limited to the kidneys and it is of unknown aetiology. Secondary nephrotic syndrome is present when the disease has extra-renal cause not dependent on the renal abnormality or has a specific aetiology. Secondary nephrotic syndrome may result from complications from systemic diseases such as diabetes, infections and drug or heavy metal poisoning (Bergstain, 2000). Nephrotic syndrome is primarily a paediatric disorder and it is 15 times more common in children than adults (Bergstain, 2000). Worldwide, the estimated annual incidence of nephrotic syndrome is 20 to 70 cases per 1 million children and 3 cases per 1 million adults (Bergstain, 2000). According to Bergstain (2000), approximately 90% of children with nephrotic syndrome have idiopathic nephrotic syndrome. In Nigeria, the prevalence of nephrotic syndrome is about 14.6% among hospitalised children (Eke and Eke, 1994) and the incidence is highest in children aged 1 to 6 years. NS is twice more common in boys than in girls in a typical Nigerian hospital (Ibadin and Abiodun, 1998).

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Homocysteine is a thiol-containing amino acid produced by intracellular demethylation of dietary methionine (Refsum et al., 2004). Homocysteine is remethylated forming methionine or catabolised to form cystathionine and cysteine. The remethylation reaction is dependent on the cofactor activity of folate and vitamin B_{12} (Klee, 2000). Thus the deficiencies of folate, vitamin B_{12} and sometimes vitamin B_6 have been shown to result in hyperhomocysteinaemia (Verhoef et al., 1996). Reduced and oxidized forms of homocysteine are present in plasma, and their fasting plasma concentrations, denoted total homocysteinaemia (tHcy), are thought to be a reflection of intracellular metabolism and cellular export of homocysteine (Refsum et al., 2004). Hyperhomocysteinaemia has been reported to be an independent risk factor in the actiopathogenesis of coronary, cerebrovascular, and peripheral vascular diseases in population-based studies (Malinow, 1994, Boushey et al., 1995, Hoogeveen et al., 1998).

Cardiovascular disease (CVD) is an important cause of death in patients with chronic renal diseases including nephrotic syndrome. It is recognised that nephrotic syndrome patients are at higher risk of arterial and venous thrombosis. There is evidence of accelerated atherosclerosis in these patients (Ordonez et al., 1993). The atherothrombotic risk pattern of the NS closely resembles those reported in other diseases associated with hyperhomocysteinaemia (Boushey et al., 1995, den Heijer et al., 1996, Piolot et al., 1996). The markedly increased level of plasma total homocysteine (tHcy) in chronic renal diseases has been suggested as an independent risk factor for the development of premature coronary heart disease (CHD) in such conditions (Casanueva et al., 2003, Chamberlain, 2005). Moreover, patients with the nephrotic syndrome frequently have low circulating levels of vitamin B_6 and folate.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

which are associated with a heightened risk for venous and arterial thrombosis (Monnerat and Ilayoz, 1997, Podda et al., 2007).

Hyperlipidaemia is a major feature in Nigerian children with Nephrotic syndrome. In a study of forty-eight Nigerian adults consisting of 28 patients with nephrotic syndrome and 20 control subjects, the plasma levels of low density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL) cholesterol were all reported to be significantly elevated in patients with nephrotic syndrome when compared with healthy controls (Adigun et al., 1999). The significant increase in LDL cholesterol and the reduction in the ratio of high density lipoprotein (HDL) cholesterol to total cholesterol, despite the high HDL cholesterol, probably suggested an increased risk for developing coronary heart disease in Nigerian adults suffering from nephrotic syndrome (Adigun *et al.*, 1999). However, the links among plasma homocysteine, lipids and related metabolites have not been explored among Nigerian children with nephrotic syndrome.

In many developed and developing countries, studies have demonstrated elevated fasting serum tHcy concentrations in patients with chronic renal insufficiency with nephrotic syndrome and low levels of proteinuria (Chauveau et al., 1992, Hultberg et al., 1995, Arnadottir et al., 1996, Hong et al., 1998). This elevation is present in the early stage of Chronic Renal Insufficiency (CRI) and increases in parallel with the degree of reduction in renal function (Chauveau et al., 1992, Hultberg et al., 1995, Arnadottir et al., 1996, Hong et al., 1998). The increase in fasting tHcy concentrations is mainly due to the reduction in plasma homocysteine clearance, although the cause of this decrease is still unknown (Guttormsen et al., 1997). However, studies which examined the relationship between circulating tHcy and risk of cardiovascular diseases in nephrotic syndrome among children are scarce.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

It is evident from literature that the roles of homocysteine in the pathophysiology, pathogenesis and manifestations of nephrotic syndrome are still largely under investigations. It is not clear from literature what interactions exist between homocysteine and lipid metabolism in nephrotic syndrome patients. Hyperhomocysteinaemia may be an independent risk factor for cardiovascular diseases including atherothrombosis in several clinical settings with renal dysfunction, but its prevalence and correlation with any of the biochemical components of the nephrotic syndrome especially lipids have not been adequately investigated. This study was carried out to determine the plasma level of homocysteine, examine the correlations of plasma homocysteine with albumin, creatinine and lipids and evaluate the effects of folate, vitamin B_6 and vitamin B_{12} supplementation on plasma homocysteine in patients with nephrotic syndrome.

1.2. RATIONALE FOR THE STUDY

The fact that nephrotic syndrome contributes significantly to childhood morbidity and monality in Nigeria is well known. With the transition in the pattern of diseases in Nigeria from mainly infectious diseases to non-infectious diseases it is anticipated that childhood morbidity will be largely dominated by non-communicable diseases like nephrotic syndrome and cardiovascular diseases in the near future. However, despite the abundance of data on homocysteine metabolism, mainly among the Caucasians, the pathogenesis of hyperhomocysteinemia and its relationship with other metabolites in renal disease remain unclear. There is considerable controversy surrounding the extent and mechanisms of the role of the kidney in homocysteine metabolism. It is not clear from existing literature what interactions are present among plasma homocysteine, lipids and other metabolites such as apolipoprotein A, creatinine and albumin in children with nephrotic syndrome.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Elevation of plasma homocysteine (Hey) is independently associated with adverse cardiovascular and non-cardiovascular outcomes (Vollset et al., 2001, Ferechide and Radulescu, 2009, Bao et al., 2010, Ciaccio and Bellia, 2010, Gokkusu et al., 2010) but reports on its prevalence and correlation with other biochemical components of the nephrotic syndrome are still controversial. A better understanding of this deranged state would advance current knowledge of renal physiologic processes, as well as efforts to find an effective therapy. Also by understanding the effects of folate, vitamin B_6 and B_{12} supplementation on plasma homocysteine in children suffering from nephrotic syndrome may place physicians in better position to make appropriate treatment policy.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

1.3. OBJECTIVES OF THE STUDY

1.3.1. General Objectives

The general objectives of this study were to compare the concentration of plasma homocysteine in children suffering from nephrotic syndrome with those who are apparently healthy as well as assess the effects of giving daily folic acid, vitamin and B_6 on plasma homocysteine in childhood nephrotic syndrome.

1.3.2. Specific Objectives

The specific objectives of this study were to:

1. Determine the prevalence of hyperhomocysteinaemia (plasma homocysteine concentration >10μmol/L) among children with nephrotic syndrome.
2. Compare the prevalence of low serum folate (<3.4ng/mL), low vitamin B_{12} (<133pg/mL) and low vitamin B_6 (<20.0nmol/L) among children with nephrotic syndrome and control.
3. Compare mean plasma homocysteine in children suffering from nephrotic syndrome with control.
4. Determine the effects of oral vitamins (folate, B_{12} and B_6) supplementation on plasma homocysteine in children with nephrotic syndrome.
5. Compare some biophysical parameters (anthropometry and skinfold thickness) between children with high and normal homocysteine levels in NS and control groups.
6. Compare some biochemical parameters (lipids, albumin, creatinine and vitamins) between children with high and normal homocysteine levels in NS and control groups.
7. Find out the correlation between plasma homocysteine and any of the plasma lipids, apolipoprotein A1, albumin, creatinine, and estimated glomerular filtration rate (GFR) in nephrotic syndrome as well as control.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

1.4. Study hypotheses

This study was carried out to test the following hypothesis;

1. That the mean total plasma homocysteine will be higher in children with nephrotic syndrome than those who are apparently healthy.
2. That giving daily folic acid, vitamin B_{12} and Vitamin B_6 will significantly lower the plasma level of homocysteine in nephrotic syndrome patients.
3. That the plasma level of homocysteine in nephrotic syndrome patients correlates significantly with plasma lipids, apolipoprotein A1, albumin and eGFR.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

CHAPTER TWO

LITERATURE REVIEW

2.1. Brief Historical Perspective

Abnormalities in the homocysteine metabolism with homocystinuria were first described in 1962 by Carson and Neill on two siblings from Northern Ireland, both with mental retardation (Carson and Neill, 1962). Nearly simultaneously, Gerritsen et al., (1962) discovered an infant with congenital anomalies, mental retardation and failure to thrive, and provided definitive proof of excessive homocysteine excretion in the urine. Two years later, another study showed that the cystathionine-β-synthase was lacking in a liver biopsy specimen from another patient with homocystinuria (Mudd et al., 1964). Thereafter, the relationship between folate and homocysteine became a subject of discussion among scientists and low circulating folate levels were reported in patients with homocystinuria and mental retardation (Carey et al., 1966). Two years later, Carey and co-workers also demonstrated a decrease in urinary homocysteine excretion by high-dose folate supplementation (Carey et al., 1968).

The first study suggesting that elevated homocysteine might be a general cardiovascular disease risk factor was published in 1976 (Wilcken and Wilcken, 1976). During the next 15 years, there were few isolated reports on the possibility of hyperhomocysteinaemia being a cardiovascular disease risk factor. In 1991, an abnormally high level of total plasma homocysteine concentration became recognized as an independent cardiovascular disease risk factor (Clarke et al., 1991). Besides the

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

role of hyperhomocysteinaemia as risk factor for atherosclerotic vascular disease, elevated homocysteine level was also recognised as a risk factor for venous thrombosis by the same scientists.

Historically, high blood cholesterol and hypoalbuminaemia have been the most discussed biochemical abnormalities characterising nephrotic syndrome and they are thought to be responsible for many of the other pathophysiological changes documented in people suffering from this condition with little or no attention paid to homocysteine levels. Just recently, some authors drew attention to the fact that hyperhomocysteinaemia may in fact be an independent risk factor for atherothrombosis seen in several clinical settings with renal function impairment including nephrotic syndrome (Nath. 1998. Cattanco. 2000).

2.2. Homocysteine metabolism in health

Homocysteine (Hcy) is a non-protein sulfur-containing amino acid that consists of three main forms: a protein-bound fraction, a free oxidized form and a free reduced form as shown in Figure 2.1 (Mudd et al., 2000). In healthy individuals, approximately 70–80% of total plasma Hcy is bound via a disulfide bond, to protein, primarily albumin, [bound Hcy (bHcy)], while the remaining 20–30% exists in a free unbound form [free Hcy (fHcy)] with <1% in free reduced form (Refsum et al., 1985, Mudd et al., 2000). Selhub and Miller (1992) proposed that the estimated average fasting plasma total Hcy levels for healthy adult human subjects range from 6 to 12 *μmol/L*, with "moderate" hyperhomocysteinemia occurring when levels are between 12 and 30 *μmol/L*, "intermediate" hyperhomocysteinaemia occurring when levels are between 31 and 100 *μmol/L*, and "severe" hyperhomocysteinemia occurring when levels are greater than 100 *μmol/L*. Free Hcy is composed almost entirely of

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

oxidized, disulfide-linked heterodimers (Hcy-cysteine) or homodimers (Hcy-Hcy, or homocysteine); with perhaps 1 to 2% existing in a reduced sulfhydryl state (Ueland, 1995). Free Hcy is inherently unstable, and accurate levels may be difficult to measure. Of importance, only the fHcy fraction is thought to be freely filtered at the glomerulus.

Homocysteine production occurs in all cells as a consequence of the normal methylation process. The volume of homocysteine distribution in healthy subjects was observed to be approximately 0.4L/kg, similar to that in subjects with severe renal insufficiency (Guttormsen *et al.*, 1997). Intracellular Hcy levels rise with enhanced intracellular Hcy production and/or inhibition of intracellular metabolism. To maintain low intracellular levels of this putatively cytotoxic substance, Hcy that is not metabolized within the cell is exported to the plasma compartment (Christensen et al., 1991, Selhub, 1999). Calculations based on steady-state kinetics in healthy adult humans showed that 1.2 mmol of Hcy, or approximately 5 to 10% of the total daily cellular production, is delivered daily to the plasma compartment (Mudd and Poole, 1975, Refsum et al., 1998a). However, since Hcy is constantly produced and exported by cells, it must also be constantly cleared for plasma levels to remain within 10% of baseline values, as they do in healthy human subjects but plasma fHcy levels are not known to be actively regulated (Guttormsen et al., 1994).

Hyperhomocysteinaemia occurs when the regulation of intracellular Hcy levels is disrupted and fHcy export to the plasma compartment is accelerated and/or normal Hcy plasma clearance is decreased (Guttormsen *et al.*, 1994). Reduced and oxidized forms of homocysteine are present in plasma, and their fasting plasma concentrations, denoted total homocysteinaemia, are thought to be a reflection of intracellular metabolism and cellular export of homocysteine (Refsum et al., 2004).

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

S-adenosylmethionine and S-adenosylhomocysteine are the intermediates in the transmethylation pathway. In the remethylation pathway, homocysteine is reconverted to methionine by receiving a methyl group from 5-methyltetrahydrofolate, the active form of folic acid, or betaine. Irreversible disposal of homocysteine occurs through the transulfuration pathway, in which homocysteine condenses with serine to form cystathionine, which is split into cysteine and alphaketobutyrate. There are several metabolic fates of cysteine, such as incorporation into proteins and conversion to metabolites such as 3-mercaptopyruvate, cysteinesulphinate, gamma-glutamylcysteine or cystine. The sulphur end product of cysteine metabolism is sulphate, which is excreted by the kidneys.

Homocysteine has three metabolic fates as shown in Figuure 2.2. Homocysteine could:

1. be remethylated to methionine (activated methyl cycle)
2. enter the cysteine biosynthetic pathway (transulfuration pathway)
3. be released into the extracellular medium – the plasma (activation to homocysteinyl tRNA)

Methionine is activated by ATP to S-adenosylmethionine (SAM), which serves as a universal donor for methyl transfer reactions S-adenosylhomocysteine (SAH) is produced as a product of methyl transfer reactions that utilize S-adenosyl methionine (SAM) as a methyl donor. L-Homocysteine is formed from the reversible hydrolysis of S-adenosyl homocysteine (SAH). Levels of homocysteine are regulated by remethylation of homocysteine to methionine by the enzyme methionine synthase (MS) and transulfuration of homocysteine to cystathionine by the enzyme cystathionine β-synthase (CBS). Homocysteine remethylation requires vitamin B_{12} and 5, 10-methyltetrahydrofolate (methyl THF), which is generated by 5, 10-

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

methylene tetrahydrofolate reductase (MTHFR). Homocysteine transulfuration requires vitamin B_6. In the liver and kidney, some proportion of homocysteine is remethylated to methionine through an alternative pathway catalysed by betaine: homocysteine methyltransferase (BHMT). The third metabolic fate of homocysteine is the direct cause of increased concentrations of total homocysteine in the extracellular fluids like urine and plasma.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

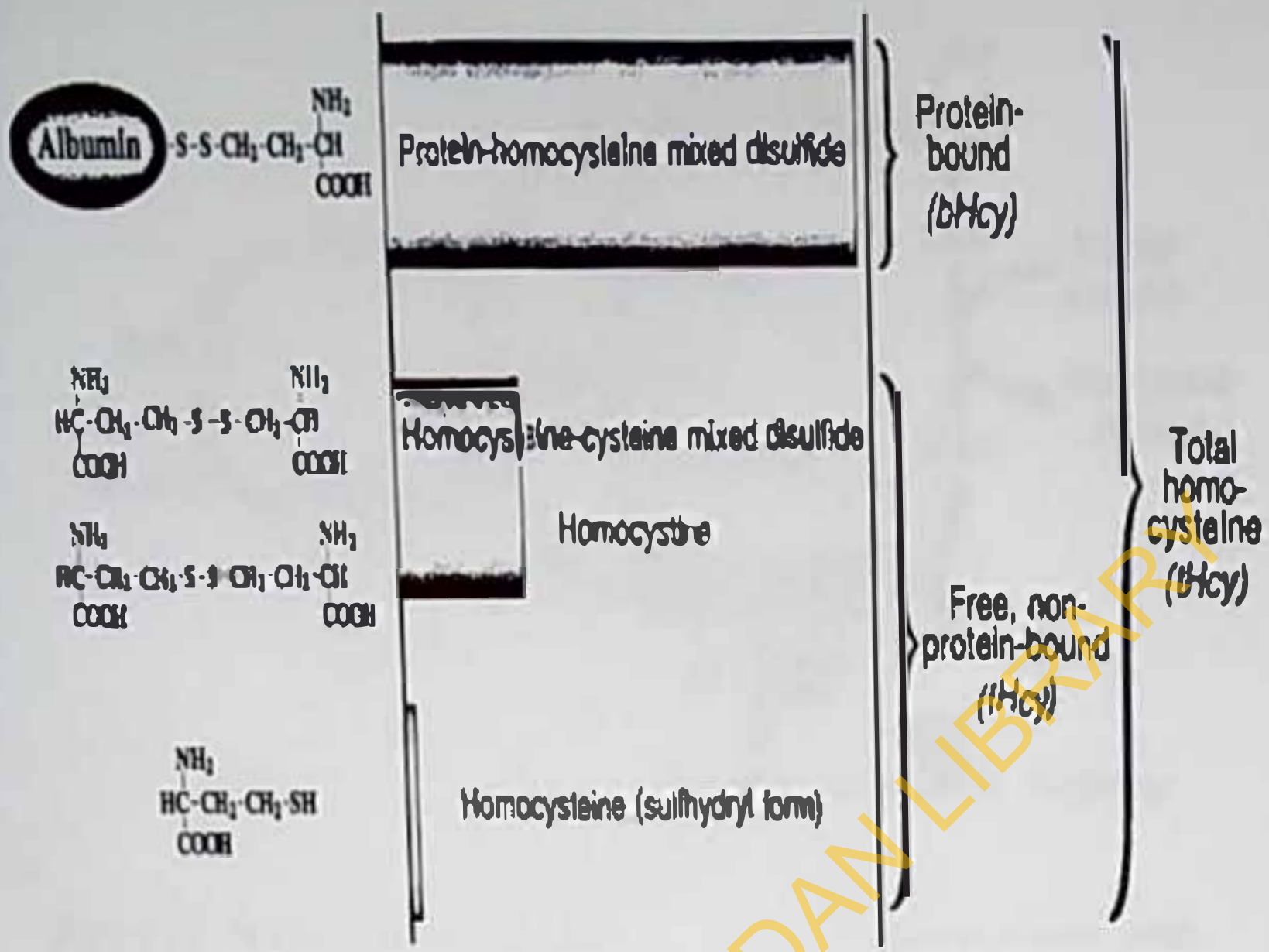


Figure 2.1: Homocysteine (Hcy) and the major related disulfides in normal human plasma

Adapted from Mudd et al., (2000)

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

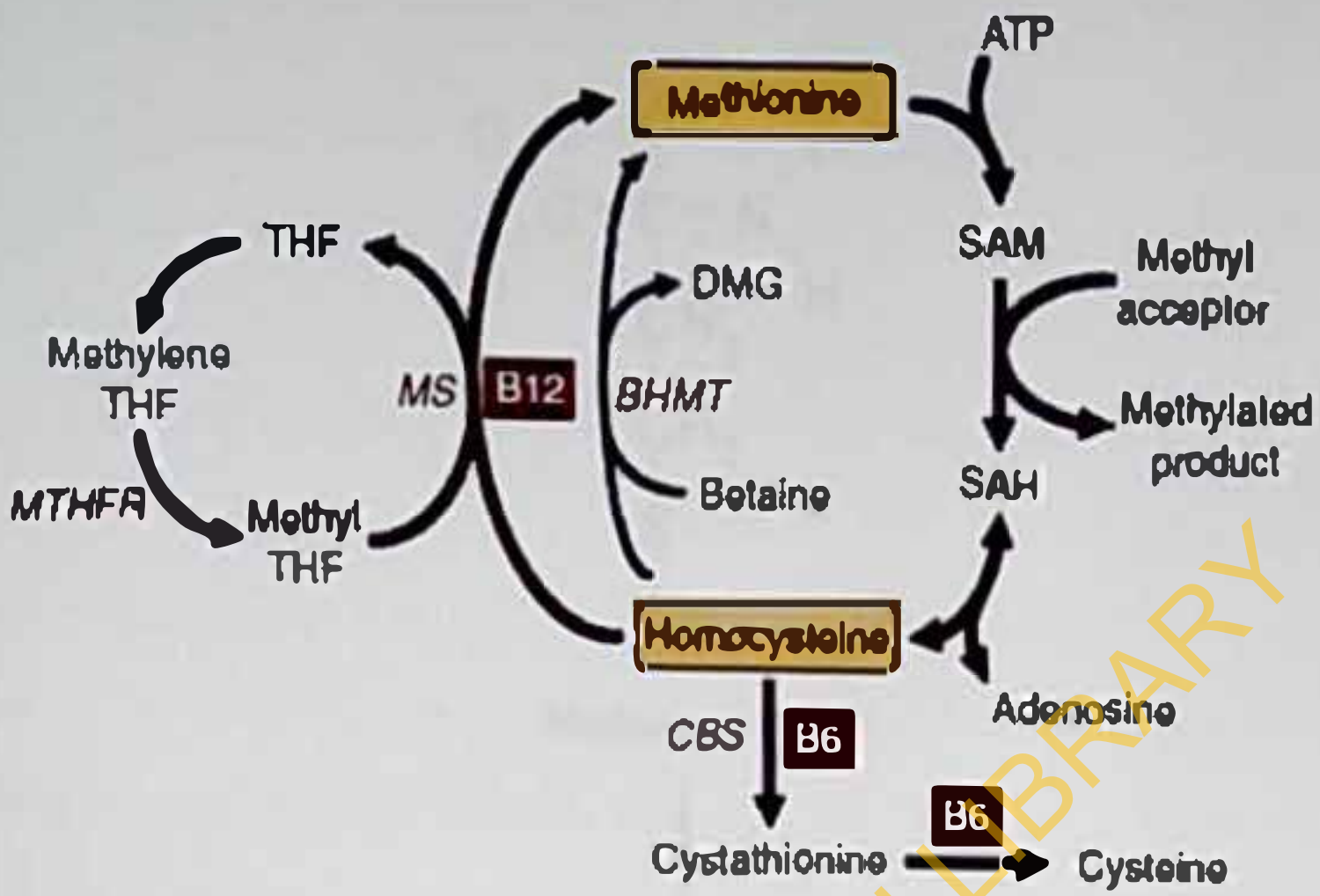


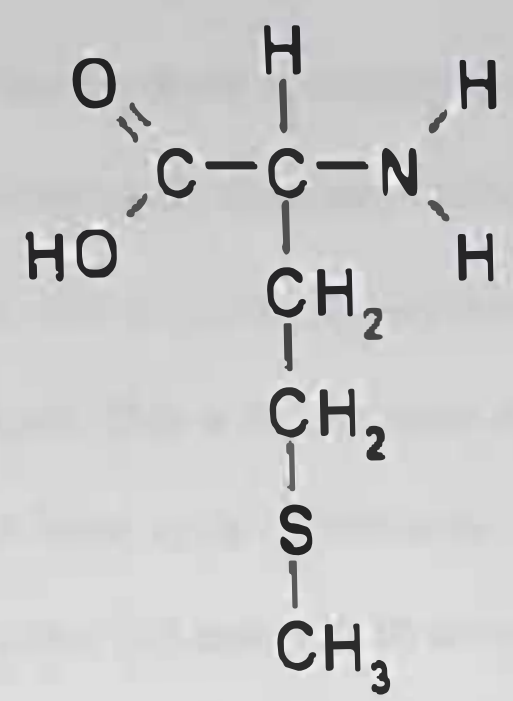
Figure 2.2: Homocysteine metabolism

Source: (Lentz, 2005)

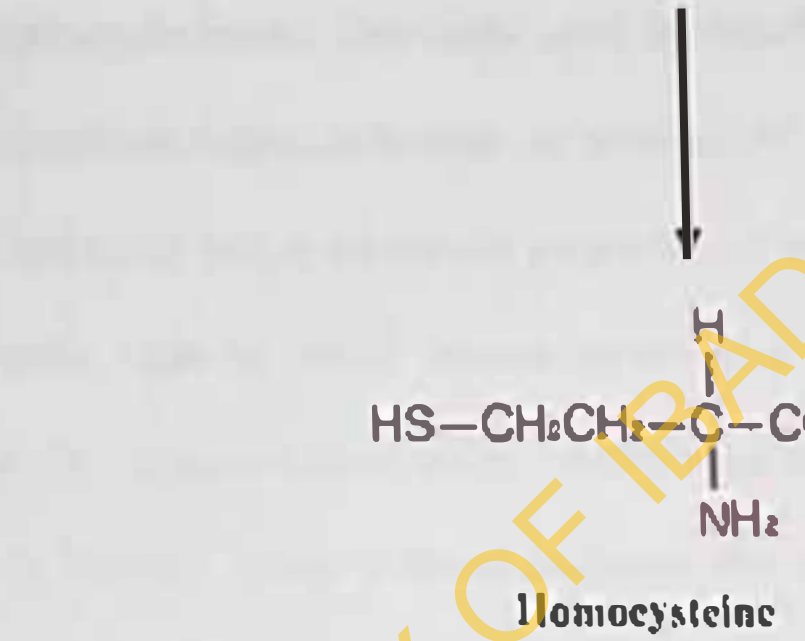
RM: remethylation, TM: transmethylation, TS: transulphuration, AdoMet: S-adenosylmethionine, AdoHcy: S-adenosylhomocysteine, 5-methyl THF: 5-methyl tetrahydrofolate (i.e. the active form of folic acid)

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT



Methionine



Homocysteine

Figure 2.3: Demethylation of dietary methionine to homocysteine

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

2.3.1. The Activated Methyl Cycle or Remethylation Pathway

In the activated methyl cycle, homocysteine is remethylated to methionine by transfer of a methyl group from N-5-methyltetrahydrofolate, catalyzed by methionine synthase or N-5-methyltetrahydrofolate-homocysteine methyltransferase, an enzyme that requires vitamin B_{12} as a cofactor. This is the last point of the activated methyl cycle and the point shared with the folate cycle (Finkelstein, 1990). Tetrahydrofolate can accept one-carbon groups in either N-5 and/or N-10 nitrogen atoms. For instance, the transfer of a one-carbon group from serine produces glycine and N5-N10-methylenetetrahydrofolate. This folate cycle is closed with the reaction catalyzed by methylenetetrahydrofolate reductase, to produce N5-methyltetrahydrofolate. In the liver and kidney of rats, a substantial proportion of homocysteine is remethylated by an alternative route in which betaine serves as a methyl donor for the reaction catalyzed by betaine-homocysteine methyltransferase (Finkelstein and Martin, 1984a). In humans, some evidence indicates that a significant amount of dietary choline can be utilized for betaine-dependent methyl transfer (Selhub and Miller, 1992).

2.3.2. The Transulfuration Pathway

Cysteine is biosynthesized from methionine through the transulfuration pathway. The first three steps of this pathway are shared with the activated methyl cycle and lead to formation of homocysteine from methionine. In the transulfuration pathway, homocysteine is the substrate of the vitamin B_6-dependent enzyme cystathionine β-synthase, which catalyses its condensation with serine to form cystathionine. This is the critical step in the pathway because it is irreversible under physiological conditions; from this point on, homocysteine is committed to follow this pathway. In the last step of the transulfuration pathway, cystathionine is cleaved by γ-

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

cystathionase, another vitamin B_6-dependent enzyme, to form 2-oxoglutarate and cysteine. Excess cysteine is oxidized to taurine and eventually to inorganic sulfates. Thus, in addition to the synthesis of cysteine, this pathway can catabolize effectively potentially toxic excess homocysteine that is not required for methyl transfer.

2.3.3. Homocysteine Thiolactone Formation

A third metabolic role for homocysteine is its activation to homocysteinyl tRNA, with the potential production of the highly reactive derivative homocysteine thiolactone (Jakubowski, 1999). Homocysteine thiolactone formation is a sign of inadequate methylation of homocysteine-tRNA to methionine-tRNA because in many cases N-terminal methionine is released from the polypeptide after chain maturation, homocysteine converted by this pathway can enter the cellular methionine pool (Medina et al., 2001).

2.4. Regulation of plasma homocysteine

Studies with isolated cells show that homocysteine export into the extracellular media reflects an imbalance between homocysteine production and metabolism, either at low or high homocysteine concentrations (Ueland et al., 1993). At low homocysteine concentrations, homocysteine export rates are higher because methionine synthase activity is lower (Christensen and Ueland, 1993); for this reason, plasma total homocysteine is markedly increased with folate or vitamin B_{12} deficiency (Allen et al., 1990). On the other hand, at high homocysteine concentrations, homocysteine export increases with decreased cystathionine β-synthase activity (Miller et al., 1992). In spite of the relevance of increased export in explaining increased homocysteinaemia, to date there is no comprehensive study of homocysteine transport across the plasma membrane, or identification of the carriers involved, although the

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

involvement of a general, nonspecific transporter for neutral amino acid efflux (such as the L system) may be suspected.

Human plasma contains both reduced and oxidized species of homocysteine. The thiol group of homocysteine allows it to form a disulfide bond with other homocysteine molecules (leading to formation of homocystine), with free cysteine, or with thiol groups of plasma proteins, such as albumin. It is remarkable that the oxidized forms are overwhelmingly in the majority (up to 99%) and reduced homocysteine represents no more than 1% of total plasma homocysteine (Jacobsen, 1998). The sum of all the forms of homocysteine existing in plasma is usually called total homocysteine. Protein-bound homocysteine represents up to 75% of total homocysteine. Both experimental and clinical studies demonstrate the presence in plasma of binding sites for aminothiols, which interact preferentially with homocysteine. A rapid equilibrium exists between free and protein-bound homocysteine fractions in vivo. Thus, transitorily increased free homocysteine, induced by an increase in homocysteine export or by methionine loading, becomes progressively bound to plasma protein in a redistribution which takes place in less than 24 h (Ueland and Refsum, 1989). There is a tight regulation of homocysteine metabolism based upon the very different affinities of methionine synthase and cystathionine β-synthase for homocysteine: the first enzyme shows low Km values for homocysteine (below 0.1 mm), and the second one has high Km values for homocysteine (over 1 mm). Thus, at low homocysteine concentrations, methionine conservation is favoured; and at high homocysteine concentrations, immediate and long-term drainage of homocysteine via the transulfuration pathway is ensured (Finkelstein, 1990). Abnormal elevation of homocysteine in plasma and urine are the result of increased levels of homocysteine export, and this reflects an imbalance between homocysteine production and

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

metabolism. Several congenital and nutritional disorders, as well as renal failure can induce this situation.

2.5. Factors that may affect homocysteine metabolism

Factors influencing plasma homocysteine levels can be classified into demographic, genetic and physiological factors such as the metabolites of homocysteine, as well as acquired determinants including habits, nutrition and diseases (Medina and Amores-Sanchez, 2000).

2.5.1. Demographic factors: Age, Gender and Ethnicity

Total homocysteine concentrations increase throughout life and approximately double from childhood to old age (Refsum et al., 2004). After puberty, males have higher mean tHcy concentrations than females. The approximate gender difference in mean tHcy is 2μmol/L, but it becomes less with increasing age (Must et al., 2003). Adebayo et al., (2008) also showed that tHcy concentration increased as a function of age in both sexes in a study of children aged 10 to 19 years drawn from secondary schools in Jos, Nigeria.

Differences in the prevalence of hyperhomocysteinaemia have been reported in various ethnic groups. Previous studies have shown that plasma tHcy concentrations differ among ethnic groups (Jacques et al., 1999), but the effect on the upper reference limit is relatively small between groups living in the same area and eating a similar diet (Jacques et al., 1999). In some regions of the world, in particular in developing countries, tHcy concentrations may be very high in the general population. For example, in a group of presumed healthy Asian Indians, the 95th percentile was 50μmol/L, a finding that was only partly explained by low cobalamin status among the study population (Nurk et al., 2001).

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Asian Americans have significantly lower total homocysteine (tHcy) concentrations than whites whereas Hispanic Americans have intermediate values (Carmel et al., 1999). Another study reported higher concentrations of tHcy in African Americans than in European Americans, which may be related to the prevalence of nutrient deficiencies (Stabler et al., 1999). In South Africa, plasma homocysteine concentrations were reported to be significantly lower in traditionally living adult black men than in whites (Ubbink et al., 1996). The researchers explained that blacks metabolized homocysteine more efficiently than whites, even after vitamin supplementation and suggested the influence of genetic factors.

2.5.2. Genetic factors

Polymorphism in the genes coding for methylenetetrahydrofolate reductase, methionine synthase and cystathionine β-synthase have been implicated in hyperhomocysteinaemia (Frosst et al., 1995). The most common genetic defect associated with mild hyperhomocysteinemia is a point mutation, namely, a C to T substitution at nucleotide 677 (677C→T) in the open reading frame of the gene for methylene tetrahydrofolate reductase. This point mutation causes a substitution of valine for alanine in the functional enzyme (Frosst *et al.*, 1995), giving rise to a thermo-labile variant of the enzyme with decreased total activity (Brattstrom et al., 1998, Bailey and Gregory, 1999). This is an autosomal recessive mutation, and the frequency of the 677C→T polymorphism varies among racial and ethnic groups, with 10 – 13% of T/T homozygous and 50% C/T heterozygous among Caucasian and Asian populations and very low incidence among African-Americans (Brattstrom et al., 1998, Bailey and Gregory, 1999).

Bailey and Gregory (1999) suggested that the widely documented elevations in plasma total homocysteine levels associated with the homozygous T/T genotype

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

might lead to a higher expected incidence of cardiovascular disease in the T/T population. However, little or no evidence has been found so far linking the T/T genotype with increased rates of cardiovascular disease, although some reports seem to link the T/T genotype with increased incidence of certain forms of vascular disease in selected populations (Refsum et al., 2004). It was suggested that an elevated plasma homocysteine level may not necessarily be deleterious, but it could promote vascular blockage under conditions predisposing to vascular disease (Refsum et al., 2004).

Three single-nucleotide polymorphisms, 677C→T, 1298A→C, and 1317T→C (a silent mutation), have been identified in the *MTHFR* gene (Weisberg et al., 1998). The 677C→T mutation leads to moderate hyperhomocysteinemia when associated with low plasma folate (Arruda et al., 1997). The 1298A→C mutation was shown to be related to hyperhomocysteinemia in association with the *MTHFR* 677C→T genotype. The percentage of individuals homozygous for the 677C→T mutation ranges between 14% and 18% among whites but is considerably lower in African Americans, of the order of 0–2% (Stevenson et al., 1997).

2.5.3. Physiological factors: Role of S-adenosylmethionine

S-adenosylmethionine plays a central role in the coordinated control of homocysteine metabolism (Tehlivets et al., 2013). S-Adenosylmethionine is an allosteric inhibitor of methylenetetrahydrofolate reductase, an in vitro inhibitor of betaine-homocysteine methyltransferase and an activator of cystathionine β-synthase (Finkelstein and Martin, 1984b). The ability of S-adenosylmethionine to act as an enzymatic effector of homocysteine metabolism provides a means by which remethylation and transulfuration pathways can be coordinated (Selhub, 1999). When cellular S-adenosylmethionine concentration is low, the synthesis of 5-methyltetrahydrofolate will proceed uninhibited whereas cystathionine synthesis will be suppressed, resulting

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

in the conservation of homocysteine for methionine synthesis. Conversely, when S-adenosylmethionine concentration is high, homocysteine is diverted through the transulfuration pathway because of inhibition of 5-methyltetrahydrofolate synthesis and stimulation of cystathionine synthesis. Thus, although the primary effect of this coordinated control is the regulation of cellular S-adenosylmethionine concentrations, it also contributes to the maintenance of a homocysteine concentration compatible with the need for de novo methyl groups.

2.5.4. Nutritional factor

Plasma homocysteine level is known to increase after meal in human. However, a small meal may not influence tHcy concentrations in healthy people (Ubbink et al., 1992), whereas intake of a large, protein-rich meal may increase the plasma tHcy concentration by approximately 10–15% after 6–8 hours (Nurk *et al.*, 2001). Some nutritional disorders potentially lead to an impairment of homocysteine metabolism. These include deficiencies of vitamin B_{12}, folate and vitamin B_6, as the de novo synthesis of methionine methyl groups requires both vitamin B_{12} and folate cofactors whereas the synthesis of cystathionine requires pyridoxal 5′-phosphate (vitamin B_6); (Refsum et al., 1998b, Medina and Amores-Sanchez, 2000). Although it has been shown that deficiencies of vitamin B_{12} and folate are related to increased plasma concentrations of homocysteine (Refsum et al., 1985, Medina and Amores-Sanchez, 2000), the relationship of homocysteine levels to vitamin B_6 status is less clear (Selhub and Miller, 1992).

2.5.5. Diurnal and Seasonal Variations.

Homocysteine is subject to diurnal variation, with tHcy concentrations being lowest in the early part of the day and highest in the evening (Bremner et al., 2000). Plasma tHcy is probably not subject to seasonal variation (McKinley et al., 2001).

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

2.6. Plasma Homocysteine Normal Reference Values

According to experts opinion (Refsum et al., 2004), reference ranges, normally defined as the central 95% confidence interval of tHcy in presumably healthy population vary with age, gender and ethnicity of the specific population studied. Therefore, the reference range for any population needs to be determined by each laboratory to conform to the characteristics of the population being tested. Furthermore, lower and upper reference ranges have to be established in countries with mandatory food folic acid fortification, like in the U.S.A and Canada. In these countries, vitamin supplementation has resulted in a considerable reduction of tHcy values in the general population.

Table 2.1 shows plasma total homocysteine reference ranges in healthy population as presented by Vilaseca et al., (1997), and Faure-Delanef et al., (1997). In most of the U.S.A clinical laboratories, the cut-off value for adults (<65 years old) is 15 µmol/L. However, in the European countries, a plasma tHcy concentration of 10 µmol/L has been used as the upper limit of the "normal" range.

However, since total homocysteine is dependent on renal function and creatinine synthesis (van Guldener et al., 2001), the reference limits for tHcy are usually calculated after excluding persons with increased creatinine or impaired renal function. Another possibility is to establish different reference limits for different creatinine concentrations, for example, by use of a nomogram. Such data are currently not available. The marked effect of vitamin status on the reference intervals highlights the problem of defining "presumed healthy individuals". In most adults who do not eat food fortified with folic acid, the upper reference limit is 15–20 µmol/L, or even higher (Nygard et al., 1998). Many of the studies conducted among children put the lower limit of plasma tHcy at 10 µmol/L (Jacques et al., 1999, Joven et al., 2000).

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Table 2.1: Plasma Total Homocysteine Reference Ranges in Healthy Population

Age	tHcy (μmol/L)
Newborns (birth – 28 days)	3.0 – 6.0
Child – Adolescence (29days – 15years)	5.0 – 10.0
Adults (15-65 years old):	
Male	6.0 – 15.0
Female	3.0 – 12.0
Elderly (>65 years old)	15.0 – 20.0
Centenarians	25.0 – 27.0

Source: Refsum et al. (2004)

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

2.7. Homocysteine metabolism and Kidney functions

Kidney has been identified as the major site for the removal and metabolism of homocysteine in mammals (House et al., 1997). It has been hypothesised that a metabolic channelling occurs leading homocysteine, removed from the blood by the kidney, to be metabolised primarily through the transulfumtion pathway. In human, there are contradictory results. Some data support this hypothesis (Hankey and Eikelboom, 1999, Medina and Amores-Sanchez, 2000) but van Guldener (2006) showed data at variance with the hypothesis. This controversial issue requires further research efforts. Renal impairment commonly causes hyperhomocysteinemia, reflecting the key role of kidney in homocysteine clearance from plasma (Bostom and Lathrop, 1997); this fact may contribute to the high incidence of vascular complications in patients with chronic renal diseases (Hankey and Eikelboom, 1999). In general, patients with renal diseases could have normal plasma levels of methionine, betaine and B-vitamins, elevated levels of S-adenosylmethionine, S-adenosylhomocysteine, cystathionine, cysteine and sulphate and low serine levels (Loehrer et al., 1998, Hermann et al., 2001). Some studies suggest that hyperhomocysteinaemia in those with vitamin B_{12} or folate deficiency could be as a result of enhanced tissue export of Hcy (Guttormsen et al., 1998).

In contrast, hyperhomocysteinaemia in renal disease is often related to reduced plasma Hcy clearance (Guttormsen *et al.*, 1997). Though the underlying cause of this reduction remains unknown but involves a defect in renal and/or extra-renal clearance. The implication of above information is that the kidneys play important roles in Hcy clearance and metabolism.

Moreover, plasma homocysteine strongly correlates with estimated glomerular filtration rate (GFR) in healthy individuals. Hyperhomocysteinaemia was shown to

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

occur at a GFR of about 60mL/min and when end-stage renal disease (ESRD) has been reached (van Guldener, 2006). The prevalence of hyperhomocysteinaemia among individuals with this condition could be as high as 85 – 100%. Patients with End-Stage Renal Diseases (ESRD) usually have 2–3 times higher level of tHcy, the prevalence of hyperhomocysteinaemia in this group of patient is usually above 90% (Suliman et al., 2000, Suliman et al., 2002).

Although several processes may explain the close correlation between kidney function and the plasma tHcy concentration, the exact mechanism(s) by which renal failure leads to hyperhomocysteinaemia are not completely understood. Patients with chronic kidney disease, especially ESRD, exhibit many abnormalities in protein and amino acid metabolism. One of these alterations involves an increased plasma concentration of the sulphur-containing amino acid homocysteine. However, it has been reported that the fractional extraction of tHcy across the kidney may be related to renal plasma flow (Garibotto et al., 2003).

Hyperhomocysteinaemia has attracted a lot of attention in renal patients, not only because of its close relationship with renal function, but also because some researchers have implicated hyperhomocysteinaemia as an independent cardiovascular risk factor (Winkelmayer et al., 2005). On the contrary, two recent studies have found no significant or even an inverse association between plasma homocysteine level and cardiovascular events and mortality in ESRD patients (Fuiano et al., 2000, Suliman et al., 2000). These discordant findings may have been caused by strong confounders which are associated with low homocysteine levels and increased mortality, such as protein energy malnutrition and/or inflammation (Suliman et al., 2005). Nephrotic syndrome is one of the common renal diseases that may alter

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

homocysteine metabolism in children but data on this relationship are scarce among Nigerians.

2.8. Nephrotic Syndrome

Nephrotic Syndrome (NS) is a disease of the kidneys characterised by heavy proteinuria (urine total protein excretion greater than 3.5 g/d or total protein/creatinine ratio greater than 3.5 g/g) due to abnormal increase of glomerular permeability and hypoalbuminaemia, hyperlipidaemia and/or oedema (Stoycheff et al., 2009). Nephrotic Syndrome occurs because the tiny blood vessels (the glomeruli) in the kidney become leaky (Deschenes, 2004). Disorders of size selective barrier, charge selective barrier, slit diaphragm and circulating permeability factors are thought to be the causes of proteinuria in patients with nephrotic syndrome. Most of the patients with oedema have primary salt retention. Overproduction and impaired catabolism of lipoproteins were proposed to be the causes of hyperlipidaemia (Togawa et al., 2004).

The term 'idiopathic' nephrotic syndrome (NS) is often used to describe a heterogeneous group of proteinuric glomerulopathies that occur predominantly in children. The idiopathic NS is associated with complex disturbances in coagulation system and numerous abnormalities of lipid and homocysteine metabolism which may increase the risk of thromboembolic complications and atherosclerosis. Over the last decade it has become recognized that some forms of nephrotic syndrome formerly assigned as 'idiopathic' are caused by mutations in genes that encode structural components of the glomerular filter as stated in a review by Gubler (2003). Idiopathic NS can be familial or non-familial.

Familial, sporadic cases of these diseases have been described and they are clinically characterized by steroid-resistance and eventual progression to end-stage renal failure

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

(Caridi et al., 2001, Karle et al., 2002). Non-familial forms of nephrotic syndrome are more common. Based on the renal biopsy findings, non-familial idiopathic nephrotic syndrome can be grossly subdivided into minimal change NS (MCNS) and focal segmental glomerulosclerosis (FSGS). As indicated by its name, renal tissue from MCNS patients shows no changes under light microscopy. More explicitly, there are no signs of inflammation, immune complex deposition or sclerosis. FSGS is characterized by collapse of the glomerular capillaries with sclerosis and hyalinosis and the formation of adhesions of the glomerular tuft.

2.8.1. Nephrotic syndrome and homocysteine metabolism

Nephrotic Syndrome (NS) may affect the metabolism of tHcy in two major ways. First, NS may result in significant reduction in plasma total Hcy concentration as a result of the reduction in albumin-bound Hcy as opposed to the free Hcy fraction. This is coupled with increased urinary excretion of albumin-bound Hcy. Many studies have sought to determine plasma Hcy concentration in patients with NS. However, the results of these studies have been contradictory. While some studies have reported elevated plasma Hcy levels in NS patients (Joven et al., 2000. Podda et al., 2007), others have shown no difference in the mean total plasma homocysteine concentration between nephrotic and non-nephrotic syndrome patients despite a significant difference between groups in urinary protein to creatinine ratio and serum albumin (Dogra et al., 2001). More recently two publications demonstrated significantly lower plasma homocysteine levels in nephrotic patients compared with non-nephrotic patients matched for renal function (Arnadottir et al., 2001, Thaczyk et al., 2009). The reason for the differences in the results of the reported studies is uncertain. However, it may be due to differences in the magnitude and the underlying causes of proteinuria, severity of hypoalbuminaemia, or concomitant renal

UNIVERSITY OF IBADAN LIBRARY

insufficiency (Friedman et al., 2002, Perechide and Radulescu, 2009, Perna et al., 2009) which can potentially impact Hcy metabolism and its plasma concentration. In the plasma, the majority of Hcy is bound to albumin, and only a small fraction is present in free form (Hortin et al., 2006). Moreover, under normal conditions, 99% of filtered free Hcy is reabsorbed in the proximal tubules (van Guldener, 2006). Thus, under physiological conditions, disposal of Hcy primarily depends on its intracellular metabolism (Farrell et al., 1995), and contribution of the kidney to this process is relatively small. However, heavy losses of albumin in the urine and the consequent fall of the albumin concentration in the plasma as in the case of NS can profoundly affect plasma concentration and metabolism of Hcy. Friedman *et al.* (2002), found a weak association between serum Hcy and albumin concentrations but not with urine protein excretion. However, the mean serum albumin concentration was nearly normal, urinary albumin excretion was relatively mild and renal function was significantly impaired in the study population. A positive correlation between serum creatinine, glomerular filtration rate, and homocysteine concentration has been documented in previous studies (Arnadottir et al., 1996, Bostom and Lathrop, 1997).

Second, NS may cause down-regulation of cystathionine-β-synthase (CBS) which can curtail conversion of Hcy to cysteine and reduce production of hydrogen sulphide (H_2S) which is an important endogenous signalling molecule. Hcy is converted to methionine by re-methylation and to cysteine via trans-sulfuration (Aminzadeh et al., 2010). These reactions, which represent key pathways for disposal of Hcy, are catalysed by MTHFR and CBS, respectively (as shown earlier in Figure 2.2). Recently, Aminzadeh et al., (2010) showed that animals with NS used in their study exhibited significant down regulation of CBS expression. This observation pointed to diminished Hcy metabolism through trans-sulfuration pathway and consequent

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

impairment of Hcy to cysteine conversion. It is important to restate that CBS is one of the enzymes that catalyse biosynthesis of endogenous hydrogen sulphide (H_2S). Endogenous H_2S is a recognized gaseous mediator with diverse biological effects which include regulation of cardiovascular and neurological functions, modulation of inflammatory response and anti-atherogenic and antioxidant actions (Beltowski et al., 2010). Thus, in addition to its impact on Hcy metabolism, down-regulation of CBS may contribute to adverse cardiovascular and other complications of NS by limiting endogenous production of H_2S. Further studies are needed to explore this possibility.

2.8.2. Homocysteine and plasma protein in nephrotic syndrome

High level of Hcy has been shown to be independently related to microalbuminuria in a population-based study (Hoogeveen *et al.*, 1998). Generally, only two studies have explored the relationship between homocysteine and plasma proteins in accessible literature. Amadottir *et al.*, (2001). in a study demonstrated that total plasma homocysteine was negatively correlated with serum albumin and positively correlated with urinary albumin excretion. Dogra and co-workers (2001), reported that within the nephrotic group, homocysteine was significantly correlated with serum creatinine and calculated GFR, but not with urinary protein or serum albumin. There was also no change found in the plasma homocysteine level despite significant improvements in serum albumin and marked reduction in proteinuria consistent with remission of nephrosis (Dogra *et al.*, 2001). The study populations in these two studies were adults with nephrotic syndrome. Data on the relationship between homocysteine and plasma albumin in children with nephrotic syndrome were scarce.

2.8.3. Lipid metabolism in nephrotic syndrome patients

Nephrotic syndrome is universally characterised by abnormalities of lipids. These disturbances of lipid metabolism are invariable features. In patients with NS,

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

dyslipidaemia constitutes significant risk factors for atherosclerosis, Cardiovascular Heart Diseases (CHD) and progression of renal insufficiency (Doucet et al., 2000, Rifai, 2000). Patients with nephrotic syndrome have one of the most pronounced secondary changes in lipoprotein metabolism and the magnitude of the changes correlates with the severity of the diseases (Moulinc et al., 1992). Lipoprotein abnormalities of the nephrotic syndrome are also related to proteinuria (Rifai, 2000). On the other hand, hyperhomocysteinaemia is a cardiovascular risk factor present in patients with nephrotic syndrome and renal failure but it is not directly associated with proteinuria (Joven *et al.*, 2000).

Studies have shown that significantly high levels of plasma lipoprotein (a) [Lp(a)], total cholesterol, LDL-cholesterol apolipoprotein-B (apo-B), and apolipoprotein A-I occur in association with elevated Hcy level among NS patients (Kuzma and Roszkowska, 2006, Kniazewska et al., 2009, Dwivedi and Sarkar, 2010). Abnormalities of lipids are thought to frequently result from abnormal apolipoprotein B-100 (apoB) transport (Kaysen and de Sain-van der Velden, 1999). In addition, qualitative changes in HDL in conjunction with an Lp(a), may also occur (Kaysen and de Sain-van der Velden, 1999). Overall, a combination of dyslipoproteinemia, hypoalbuminaemia, and hyperfibrinogenaemia are recognized risk factors for cardiovascular diseases reported in nephrotic syndrome patients and may collectively account for the increased incidence of cardiovascular disease even in the absence of renal failure (Kannel et al., 1987, Phillips et al., 1989, Ordonez et al., 1993). However, the mechanisms by which the interactions of abnormalities of lipids, protein and homocysteine result in cardiovascular complication are still under investigation but lipid peroxidation is being suspected.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

2.8.4. Homocysteine and Lipid peroxidation in nephrotic syndrome

Experimental and clinical evidence suggest that moderate hyperhomocysteinaemia may predispose individuals to endothelial dysfunction through a process that involves generation of reactive oxygen species (Kanani et al., 1999). Potential mechanisms for predisposition of individuals to endothelial dysfunction include oxidant actions, effects on cofactors, availability and/ or direct interactions with the enzyme endothelial nitric oxide synthase (Bayes et al., 2003). Homocysteine contains a reactive thiol group that can undergo disulfide exchange reactions (Sengupta et al., 2001) and disrupt the folding and processing of newly synthesized proteins in the endoplasmic reticulum (ER) (Lentz and Sadler, 1991, Lentz and Sadler, 1993). The cellular consequences of this condition, known as ER stress, include dysregulation of lipid metabolism, activation of inflammatory pathways, and impaired insulin signalling (Austin et al., 2004). These processes are as depicted in Figure 2.3.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

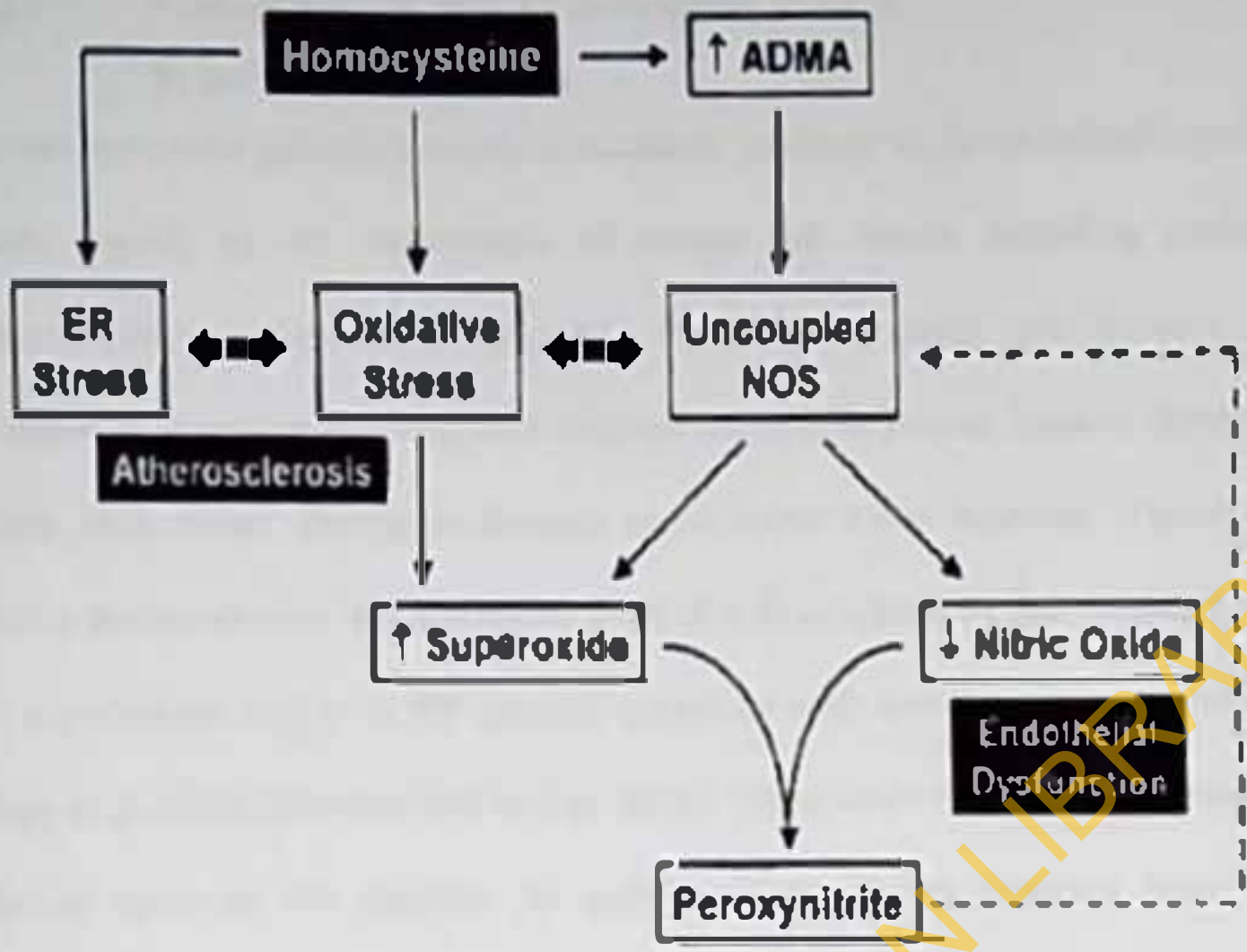


Figure 2.4: Possible mechanisms of endothelial dysfunction, atherosclerosis, and thrombosis in hyperhomocysteinaemia
Source: Lentz (2005)

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

2.8.4.1. Pathogenesis of damage of vascular walls in hyperhomocysteinaemia

Nephrotic syndrome patients showed an increased tendency to develop cardiovascular diseases, mainly as the consequence of several risk factors including increased oxidative stress, inflammation, physical inactivity, vascular calcification, and endothelial dysfunction. Existing data indicate that homocysteine induces damage of vascular walls either directly or through an oxidative stress response (Figure 2.4). Previous studies showed that the serum level of malondialdehyde and oxidized lipids were significantly higher in NS patients compared with non-nephrotic subjects (El-Melegy et al., 2008, Dwivedi and Sarkar, 2010). These results suggest the presence of oxidative stress in NS patients. In addition, these studies reported lower total antioxidant status (TAS) level in NS compared with non-nephrotic subjects.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

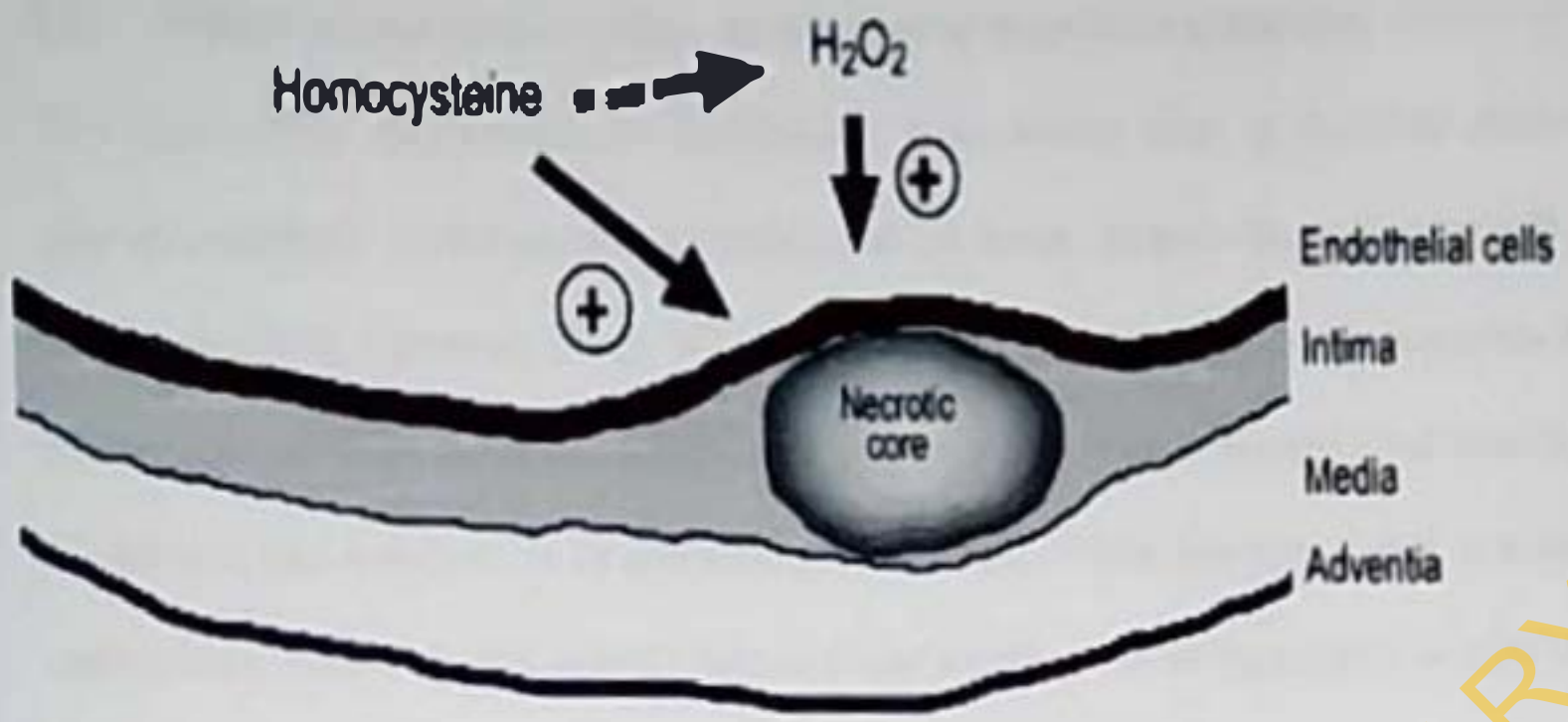


Figure 2.5: **Homocysteine can cause vascular damage either directly or through the generation of reactive oxygen species**
Source: Medina *et al.*, (2001)

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

2.9. Vitamins and homocysteine metabolism in nephrotic syndrome

The intracellular metabolism of methionine, from which Hcy is derived, occurs through enzymatic pathways that are dependent on folate, vitamin and vitamin (Cattaneo, 1999, Cattaneo, 2000). High plasma levels of tHcy are often consequent to deficiencies of these vitamins, which may be compounded by inherited abnormalities of the enzymes involved in its metabolism (Cattaneo, 1999). Homocysteine, a sulfur amino acid, is an essential intermediate in folate metabolism, being central to both the DNA and methylation cycles. As a result of the toxic nature of homocysteine, the concentration of homocysteine within cells and in the plasma is kept within very narrow limits by the efficient functioning of three enzymes, methionine synthase, MTHFR, and cystathionine synthase. However, all of these enzymes are dependent on folate, vitamin , and vitamin B_6. Even a modest reduction in the status of any of these three vitamins can disrupt the balance, causing an accumulation of intracellular S-adenosyl homocysteine (SAH) as well as increased export of homocysteine into the plasma (Ueland et al., 1993).

The sole circulating form of folate is 5-methyl-THF, and this is the form presented to cells in vivo. However, 5-methylTHF is a poor substrate for the enzyme that adds polyglutamate residues and ensures its retention in the cell. Thus, when 5-methylTHF enters a cell, it must lose its methyl group to ensure retention within a cell. Essentially, 5-methylTHF combines with homocysteine to form methionine and THF. This is a complex reaction, catalyzed by the enzyme methionine synthase, and requires folate as a co-substrate carrying the methyl group to be transferred and vitamin B_{12} as a cofactor for the enzyme.

Once inside the cell, folate participates in two metabolic cycles: one involving the synthesis of DNA, and the other involving the supply of methyl groups for

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

methylation reactions. Overall, they function as acceptors and donors of one-carbon units. The one-carbon units are obtained from serine, glycine or formate. In DNA synthesis, folate-linked one-carbon units are incorporated into the C2 and C8 of purines via 10-formylTHF and into pyrimidines via the 5,10-methyleneTHF-dependent conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP). During the synthesis of dTMP from dUMP, the tetrahydroform of folate also is oxidized to the dihydro-form. The enzyme dihydrofolate reductase (DHFR) converts this form back to THF.

In health and disease states, homocysteine levels in plasma are maintained within narrow limits by the efficient functioning of methionine synthase, methylenetetrahydrofolate reductase (MTHFR), cystathionine β-synthase, and (in liver only) betaine methyltransferase. The first three of these enzymes require vitamins either as cofactor or substrate. Folate is a substrate for methionine synthase and MTHFR. Methionine synthase also requires vitamin B_{12} as cofactor, and cystathionine requires vitamin B_6 (pyridoxine). Thus, optimally low plasma homocysteine depends not only on the efficient functioning of these enzymes but also on adequate vitamin status.

Homocysteine metabolism is also affected by polymorphisms in the genes encoding the enzymes involved, of which the thermo-labile (C677T) variant of MTHFR is the best characterized polymorphism (Frossi *et al.*, 1995). People who are homozygous for the mutation tend to have higher fasting plasma homocysteine concentrations and lower folate concentrations than heterozygous or wild-type groups (Molloy et al., 1997). 5MeTHF has been reported to reduce oxidative damage to human LDL lipid through its free-radical scavenging activity (Nakano et al., 2001)

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

2.9.1. Abnormalities of homocysteine and B vitamins in the nephrotic syndrome

Low levels of vitamin B_6 have been reported in a number of previous studies in patients with NS (van Buuren et al., 1985). van Buuren et al., (1985), studied a group of 35 children with NS and showed that 88% had low plasma vitamin B_6 regardless of the actiology of NS. van Buuren and colleagues (1985), explained that this might be due to enhanced urinary excretion of albumin-bound pyridoxal phosphate in view of the severe proteinuria which characterises nephrotic syndrome. Similarly, Podda *et al.*, (2007) studied vitamin B_6, B_{12} and folate in 84 patients with nephrotic syndrome and 84 sex- and age-matched controls, the study showed that the circulating levels of total homocysteine (tHcy) were higher, vitamin B_6 and vitamin B_{12} levels were lower in nephrotic patients than in controls. The association of low vitamin B_6 levels with the nephrotic syndrome was described as independent of any other alteration associated with the disease. It is possible that other features of the NS, for example the loss of vitamin B_6 protein carriers in the urine, might influence vitamin B_6 status, although, similar to vitamin B_{12}, most studies found no correlation between vitamin B_6 levels and the degree of proteinuria in NS patients.

In a multicentre case-control study in Europe, low B_6 levels were shown to be independently associated with an increased risk of vascular disease and atherosclerosis (Robinson et al., 1998). In the ARIC study, the higher quintiles of the vitamin B_6 distribution were associated with protection towards coronary heart disease compared with the lower quintile (Folsom et al., 1998). In the same fashion, hyperhomocysteinaemia observed in dialysis patients has been associated with various factors, including deficiencies of vitamin B_6 and B_{12} or folic acid apart from low renal clearance, altered metabolism and genetic defects (Bostom et al., 1999). It is

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

well known that vitamin supplements reduce the concentration of homocysteine in haemodialysis patients (Shemin et al., 2001).

2.9.2. Effects of folic acid supplements on homocysteine

The effects of folic acid supplements on plasma homocysteine levels in various disease conditions have been discussed in published literature. While many studies have explored the use of folic acid in lowering homocysteine in other renal diseases. data on its use among children with NS are scarce.

Many investigators have reported that folic acid administration in doses varying from 1 to 15 mg/day produces, on average, a 20-40% reduction in the tHcy levels in dialysis patients, even in the presence of normal or elevated folic acid concentration (Bostom *et al.*, 1999). Chauveau *et al.*, (1996), showed that supplementation of folic acid at a pharmacological dose has been shown to reduce fasting plasma tHcy concentrations in CRI patients. In a randomized and controlled trial, Malinow and colleagues showed that consuming breakfast cereal fortified with 499 or 665 μg of folic acid per 30 g of cereal for 5 weeks reduced fasting plasma tHcy concentrations (Malinow et al., 1998). In typical Western populations, supplementation with both 0.5–5 mg daily folic acid and about 0.5 mg daily vitamin B_{12} should reduce blood tHcy concentrations by about a quarter to a third (for example, from about 12 μM to 8–9 μM) (Homocysteine Lowering Trialists' Collaboration, 1998). Similarly, another study demonstrated that oral folic acid supplementation at 5 mg three times weekly (together with pyridoxine (250 mg twice weekly) and vitamin B_{12} (1 mg twice weekly) allows a substantial (40%) and sustained (≤6years) reduction in fasting plasma tHcy concentrations without any apparent side effects in 78 CRI patients (Jungers et al., 1999). In the same vein, Cafolla et al., (2002), showed that

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

supplementation with 5 mg/day of folic acid was able to increase significantly both red blood cell folate and serum folate levels and reduce tHcy level in Italian smoker-blood donors. Araki et al. (2006) studied the effects of folic acid supplementation in 32 healthy male Japanese volunteers aged 20-29 years. At the end of the 2 week supplementation period, the tHcy concentration decreased significantly and serum and red blood cell folate concentrations increased. Anderson et al., (2010), later showed in another study that the decrease in tHcy was not dose-dependent.

Apart from its use in patients with kidney diseases, the use of 5-methyltetrahydrofolate or folinic acid has led to a greater decrease in fasting plasma tHcy levels than that observed with folic acid supplementation in heart disease (HD) patients (Perna et al., 1997, Touam et al., 1999). Although the use of the active reduced forms of folic acid appears to be preferable in HD patients (Touam *et al.*, 1999), additional trials are needed to confirm their efficacy, define their optimal dose and evaluate their safety in such patients. Guttormsen et al., (1997), explained that the homocysteine-lowering effect of folic acid was probably due to an improvement in the re-methylation pathway of homocysteine to methionine.

Moreover, folate supplementation in haemodialysis patients had no harmful consequences (particularly gastrointestinal distress), although data concerning long-term evaluation in such patients are limited (van Guldener et al., 1998). Since this supplementation has no apparent side effects, and is inexpensive, and renal patients are considered to have increased cardiovascular risk status, folate therapy may be given. For haematological, neurological and immunological reasons the combination of folate therapy with vitamin B_{12} (1 mg/day) (Elia, 1998) and vitamin B_6 (50 mg/day) is recommended in such patients (Mydlik et al., 1997). A more recent study has observed that folic acid treatment reduces LDL oxidation and regulates the

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

inflammatory response of peripheral blood mononuclear cells in hyperhomocysteinaemic subjects (Holven et al., 2002).

2.9.4. Effects of vitamins B_{12} and B_6 supplementation on homocysteine

Most researchers often combine the use of vitamins B_{12} and B_6 in studies relating to homocysteine lowering effects. While substantial evidence have been generated on the effectiveness of folate and folinic acid, pyridoxine supplementation, on the other hand has shown no significant effect on fasting tHcy levels in patients with renal diseases in two previous studies (Arnadottir et al., 1993, Chauveau et al., 1996). However, itt has been shown that giving folic acid and vitamin B_6 supplements to healthy siblings of patients with premature atherothrombotic diseases decreased occurrence of abnormal electrocardiographic tests. This was consistent with decreased risk of atherosclerotic coronary event in the same cohort (Vermeulen et al., 2000).

Dierkes *et al.*, (1999), in another study reported that vitamin B_{12} supplementation effectively decreases both plasma tHcy in ESRD patients with low B_{12} levels and concluded that the findings illustrated the close interrelation between vitamin B_{12} and folate metabolism. Vitamin B_{12} supplements are useful in folate treated patients to prevent cobalamin deficiency and its neurological consequences (Billion et al., 2002). On the other hand, vitamin B_6 supplementation was shown to abolish the increased risk of thrombotic event both arterial and venous in patients with NS (Podda et al., 2007). In the Atherosclerosis Risk in Communities (ARIC) study, the higher quintiles of the vitamin B_6 distribution were associated with protection towards coronary heart disease compared with the lower quintile (Folsom et al., 1998).

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

2.10 Apolipoprotein A1

APO A1 is the main protein component of HDL. APO A1 activates lecithin cholesterol acyltransferase which catalyses the esterification of cholesterol. The resulting esterified cholesterol can then be transported to the liver, metabolised and excreted (Rifai, 2000). Persons with atherosclerotic vascular changes frequently exhibit decreased levels of APO A1. Even if the concentrations of apolipoprotein B are normal, a decreased APO A1 level may be a risk factor for atherosclerotic processes. Decreased levels of APO A1 also occur in dyslipoproteinaemia, acute hepatic cirrhosis and insulin-treated patients. Agbedana et al. (1990) found an elevated apolipoprotein A concentration in childhood nephrotic syndrome suggesting the presence of an Apolipoprotein A-rich high density lipoprotein in nephrotic children.

As a major component of the high-density lipoprotein complex ("good cholesterol"), ApoA-I helps to clear cholesterol from arteries. Dastani et al. (2006) reported that five out of nine French Canadaian men who were 35 years or more with a mutation (E164X) had developed premature coronary artery disease. Apo A1 Milano is a naturally occurring mutant of Apo A1. It was first described in 1980, as the first known molecular abnormality of apolipoproteins (Franceschini et al., 1981). Carriers of this mutation have very low HDL cholesterol levels. Biochemically, ApoA-I contains an extra cysteine bridge which causes it to exist as either homodimer or heterodimer with ApoA-II. However, the enhanced cardioprotective activity of this mutant, which likely depends on cholesterol efflux, cannot easily be replicated by other cysteine mutants (Zhu et al., 2005). ApoA-I Milano has also been shown in small clinical trials to have a statistically significant effect in reducing (reversing) plaque build-up on arterial walls (Nissen et al., 2003).

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Apo A1 also plays some roles in other diseases. It protects against Alzheimer disease by a synergistic interaction with alpha-tocopherol (Vollbach et al., 2005). Apo A1 binds to lipopolysaccharide or endotoxin, and has a major role in the anti-endotoxin function of HDL. In one study, a decrease in ApoA1 levels was detected in schizophrenia patients' CSF, brain and peripheral tissues (Huang et al., 2007).

2.10.1. Factors affecting Apo A1 activity

Apo A1 production is decreased by calcitriol, and increased by a drug that antagonizes it. Exercise or statin treatment may cause an increase in HDL-C levels by inducing Apo A1 production, but this depends on the G/A promoter polymorphism.

2.10.2. Significance of Apo A1 in renal diseases

Apolipoprotein A1 (apoA1) is a component of the high density lipoproteins (HDL) that regulates the transport of cholesterol between the liver and peripheral cells and modulates the removal of any excess of cholesterol from membranes. Any variation in apoA1 composition may modify the plasma lipid profile and be involved in atherogenesis. The concentration of plasma apolipoproteins in the nephrotic syndrome generally reflects the alterations in lipoprotein metabolism. Thus, there are elevated levels of apo B, C-II, and E, which are associated with VLDL and LDL; on the other hand, the levels of the major apolipoproteins associated with HDL, apo A-I and A-II, are usually normal (Radhakrishnan et al., 1993).

Recently, Santucci et al. (2011) investigated Apo A1 composition in plasma of 6 children with nephrotic syndrome. Using the non-denaturing two-dimensional electrophoresis (Nat/SDS-PAGE), mass spectrometry, western blot and pull down experiments, Santucci and colleagues (2011), characterised Apo A1 proteins and

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

defined putative interactions. The study showed that apoAI isoforms were much more present in plasma of nephrotic patients compared to normal individuals. These findings show that apoAI plasma in nephrotic syndrome is heterogeneous in terms of molecular weight. Overall, the findings suggest that fragmentation and transport of Apo AI may be involved in the general disorder of lipid metabolism that characterizes nephrotic syndrome.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site and population

This study was carried out at the Paediatric Nephrology Clinic and children's wards of the University College Hospital (UCH), Ibadan. The Hospital is located in Ibadan North Local Government Area, Ibadan; IBNLGA (Appendix I). IBNLGA is one of the five LGAs that constitute the main Ibadan. The University College Hospital, Ibadan serves as a referral centre for the residents of Ibadan and the people of the South-Western region of Nigeria. The people living in this region are mainly Yoruba ethnic group in Nigeria. Estimated 15-20 new cases of nephrotic syndrome are seen at the Paediatric Nephrology Clinic of the UCH, Ibadan yearly.

Children age less than 18 years are the main population of interest for this study. According to 2006 Census, they constitute about 15 – 20% of the population of Nigeria. University College Hospital is one of the main centres where children with renal diseases are treated in the south-west of Nigeria.

3.2 Study design and subjects

This research was pre and post quasi-experimental study in design. It involved age-sex-matched 42 cases and 42 controls. Cases were children who presented with symptoms, signs and laboratory features of nephrotic syndrome while the control

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

group comprised apparently healthy children. who came for routine check up or minor surgeries at the clinics without symptoms suggestive of renal diseases.

For cases:

- Inclusion criteria were:
  - Children aged 0 to 15 years
  - Diagnosis of NS based on the presence of
    - o High total cholesterol ->220mg/dl
    - o Massive proteinuria (3 or more pluses)
    - o Hypoalbuminaemia – serum albumin < 2.5g/dl
    - o With or without oedema
- Exclusion criteria:
  - Absence of one or more of the features stated under inclusion criteria.
  - Presence of liver disorders. severe protein energy malnutrition and patient who had paracetamol poisoning.

For the control group:

- o Inclusion criterion was the absence of symptoms, signs and laboratory findings (nonnal urinalysis) suggestive of renal diseases.
- o The main exclusion criterion was presence of symptoms. signs and laboratory findings (normal urinalysis) suggestive of renal diseases. liver disorders, severe protein energy malnutrition

3.3 Sample size calculation

Joven et al (2000), reported that 26.0% of the patients with nephrotic syndrome and 7.4% of healthy control subjects had hyperhomocysteinaemia. Substituting the above

UNIVERSITY OF IBADAN LIBRARY

proportions of subjects with hyperhomocysteinaemia in the formula for calculating sample size for comparisons of two proportions, the number of nephrotic patients and controls to be studied was calculated as below:

$$N = \frac{2[Z_\alpha \sqrt{2\bar{p}(1-\bar{p})} + Z_\beta \sqrt{P_1(1-P_1) + P_2(1-P_2)}]^2}{(P_1 - P_2)^2}$$

. Where,

N= number of subjects in each group

Z_α = type I error, at level of significance of 0.05 (95% confidence interval) = 1.96

Z_β =type II error, at Power of 80% (that is β=0.2) = 0.84

P_1 = Assumed proportion of patients with Hyperhomocysteinaemia, 0.26

P_2 = Assumed proportion of control with Hyperhomocysteinaemia, 0.074

$$\bar{p} = \frac{P_1 - P_2}{2} = 0.093$$

Therefore,

$$N = 2\frac{[1.96\sqrt{2(0.093)(0.907)} + 0.84\sqrt{0.26(0.74) + 0.074(0.926)}]^2}{0.186} = 16$$

A minimum of 16 cases and 16 controls were required for the study. In order to adjust for non-participation or drop-out rate of 20%, approximately 20 cases and 20 controls were the minimum number of subjects required for this study.

3.4 Sampling procedures

Consecutive patients with diagnosis of nephrotic syndrome seen at the Paediatric Nephrology Clinic who fulfilled the above stated inclusion criteria were enrolled as cases. The control group were selected among apparently healthy children who came for routine medical check-up (such as school entrance examination) and minor surgical procedures such as hernioraphy, cleft lips repair and lipoma excision.

UNIVERSITY OF IBADAN LIBRARY

3.5 Conduct of the study

Parents of all eligible children were approached for consent to participate in the study at first visit. Children whose parents gave consents were interviewed by the investigator using a structured questionnaire (appendix VIII) that contained items on demographic characteristics of each subject. Blood and urine samples were collected for estimation of plasma homocysteine, lipids, serum albumin, serum protein, serum creatinine, serum folate, vitamin B_{12}, vitamin B_6 and urinary protein. Those whose test results and clinical features met the inclusion criteria were given another appointment on which day he or she was asked to fast during the previous night. About 5mL of fasting blood sample was collected and dispensed into an anticoagulant container and plain bottle. After the results were reviewed, all patients who had high level of homocysteine were given 5mg of folic acid tablet (Dr Meyer's Folic acid by VITABIOTIC) and a tablet of vitamin B complex daily for a period of three months under the supervision of the Consultant Paediatric Nephrologists. Patients were followed up at intervals of 2 weeks during this period in order to replenish their stock of vitamin supplements. Repeated phone calls were made by the investigator at least once weekly to remind the caregivers or mothers of the need to adhere to the vitamin supplements.

At three months another 5mL fasting blood samples were taken to repeat all the biochemical parameters determined at baseline. Other clinical data relevant to this research were obtained at baseline and end of the three months follow up.

3.6 LABORATORY PROCEDURES

All laboratory procedures were carried out under Good Laboratory Practices. All blood samples were convened to the laboratory and centrifuged within 1 hour after collection.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

3.6.1 Determination of plasma total cholesterol

Plasma cholesterol was determined using Enzymatic Colorimetric End Point method, ChOD-PAP (Vital Diagnostics SPb Ltd. Engelsa prospect 27, bld 12B, 194156, St. Petersburg, Russia). This method was based on the method of Allain (1974).

Principle:

This involves enzymatic hydrolysis and oxidation. The indicator quinoncimine is formed from hydrogen peroxide and 4-aminophenazone in the presence of phenol and peroxidise as shown below:

$$\text{Cholesterol esters} \xrightarrow{\text{cholesterol esterase}} \text{Cholesterol} + \text{Fatty Acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{Cholesterol-3-one} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Red quinoncimine} + 2H_2O$$

The optical density of the colour of the formed quinoncimine was measured at a wavelength of 500nm using a spectrophotometer SP-850 (Metertek UK). Commercially available human quality control samples as well as standardized pooled plasma of known concentrations were included as quality control samples for each assay. Cholesterol concentration in a sample is proportional to absorbance increase measured at 500 nm due to the red quinoncimine production.

Assay procedure: content of test tubes T1, T2 and T3 were

	T1	T2	T3
Reagent 1	2.0mL	2.0mL	2.0mL
Sample	0.02mL	-	-
Calibrator (standard)	-	0.02mL	-
Bi-distilled water	-	-	0.02mL

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

- Reagent 1, Sample, calibrator (standard) and bi-distilled water were pipetted into test tubes as shown above.
- The mixtures were incubated for 5 minutes at working temperature (37°C).
- Absorbance of the sample (A_{sample}) and calibrator ($A_{calibrator}$) were read against reagent blank at 500nm spectrophotometer SP-850 (Meterlek UK).

Calculation:

$$\text{Conc of (test)} = \frac{\text{Optical density of sample}}{\text{Optical density of standard}} \times \text{Concentration of standard}$$

3.6.2 Determination of triglycerides

Plasma triglycerides levels were determined by enzymatic colorimetric method using test kit (Vital Diagnostics SPb Ltd. Engelsa prospect 27, bld 12B, 194156, St. Petersburg, Russia).

Principle:

This involves an enzymatic lipolysis of the triglyceride content of the sample with lipases. The indicator is quinoneimine formed from hydrogen peroxide, 4-aminoantipyrine and 4-chlorophenol under the catalytic influence of peroxidise as shown below:

Triglycerides $\xrightarrow{\text{Lipase}}$ glycerol + fatty acids

Glycerol + ATP $\xrightarrow{\text{Glycerokinase}}$ glycerol-3-phosphate + ADP

Glycerol-3-phosphate + O_2 $\xrightarrow{\text{Glycerol-3-phosphate oxidase}}$ dihydroxyacetone phosphate + $2H_2O_2$

$2H_2O_2$ + 4-Chlorphenol + 4-Aminoantipyrine $\xrightarrow{\text{Peroxidase}}$ Red quinoneimine + $4H_2O$

The optical density of the colour of the formed complex was measured at a wavelength of 500nm using a spectrophotometer SP-850 (Meterlek UK).

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Commercially available human quality control samples as well as standardized pooled plasma of known concentrations were included as quality control samples for each assay.

Assay procedure: content of test tubes T1, T2 and T3 were

	T1	T2	T3
Working reagent	2.0mL	2.0mL	2.0mL
Sample	0.02mL	-	-
Calibrator	-	0.02mL	-
Bi-distilled water	-	-	0.02mL

- Reagent 1, Sample, calibrator and bi-distilled water were pipetted into test tubes as shown above.
- The mixture was incubated for 5 minutes at 37°C.
- The absorbance of the sample (A_{sample}) and calibrator ($A_{calibrator}$) were read against reagent blank (T3).

Calculation:

Triglycerides concentration = $(A_{sample}/A_{calibrator} \times 250) - 10$ mg/dl

Where A_{sample} = absorbance of the sample

$A_{calibrator}$ = absorbance of the calibrator

250mg/dl – triglycerides concentration in the calibrator

10mg/dl – free plasma glycerol correction

3.6.3 Determination of HDL cholesterol

HDL cholesterol levels were determined by Phosphotungstate magnesium precipitation method using test kit (Vital Diagnostics SPb Ltd. Engelsa prospect 27, bld 12B, 194156, St. Petersburg, Russia).

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Principle:

Chylomicrons, very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) were precipitated by phosphosphotungstate and magnesium. After centrifugation supernatant contained only high-density lipoproteins (HDL). The mixture was allowed to stand for 15 minutes at room temperature before centrifugation with Centaur centrifuge (England) at 3000 revolution per minutes for 10 minutes. The HDL concentration was measured on the same method as the concentration of total cholesterol (Allain *et al*., 1974).

Commercially available human quality control samples as well as standardized pooled plasma of known concentrations were included as quality controls for each assay.

Assay procedure: content of test tubes T1, T2 and T3 were

1. Sample Precipitation

	T1	T2	T3
Sample	0.15mL	-	-
Distilled water	-	-	0.15mL
Reagent 1	0.3mL	0.3mL	0.3mL
Calibrator	-	0.15mL	-

- Sample, distilled water and reagent were pipetted into test tubes.
- The mixtures were incubated at working temperature for 10 minutes.
- The sample was centrifuged at 3000g for 10 minutes at room temperature (18-25°C).
- The solutions for calibrator and reagent blank do not require centrifugation.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

2. HDL Assay: content of test tubes T1, T2 and T3 were

	T1	T2	T3
Supernatant	0.2mL	-	-
Reagent Blank Solution	-	-	0.2mL
Total Cholesterol Reagent	2.0mL	2.0mL	2.0mL
Calibrator Solution	-	0.2mL	-

- The supernatant, reagent blank and total cholesterol reagent were pipetted into the test tubes as shown.
- The mixtures were incubated at 18-25°C for 10minutes.
- The Sample Optical Density (OD_{sample}) and the Standard Optical Density (OD_{std}) were read against reagent blank.

Calculation

In (plasma):

$C = OD_{sample}/OD_{std} \times 50$ (mg/dl);

Where:

OD_{sample} = Sample optical density

OD_{std} = Standard optical density

50mg/dl = Cholesterol concentration in Calibrator.

3.6.4 Determination of LDL cholesterol

The Friedewald formula was used to calculate the Low Density Lipoprotein (LDL) cholesterol concentration, except in subjects with serum triglyceride levels >8.0mmol/litre (Friedewald et al., 1972), expressed as:

LDL cholesterol = total cholesterol - (high-density lipoprotein cholesterol + [triglycerides ÷ 5]).

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

3.6.5 Determination of total protein

Total protein was determined using Biuret method described by Krohn (2002)

Principle:

Cu^{++} + Protein ⟶ Cu-Protein Complex

Polypeptide containing at least two peptide bonds reacts with Biuret reagent. In alkaline solution, cupric ion forms a violet coloured complex with protein nitrogen. Absorbance measured at 520-560nm is proportional to the total protein concentration in a sample.

Reagents:

Reagent 1 – Monoreagent

Sodium hydroxide	500mmol/L
Potassium-sodium tartrate	80mmol/L
Potassium iodide	75mmol/L
Copper sulphate	30mmol/L

Calibrator

Albumin	70g/L
Nacl	154mmol/L

Reagents preparation

1. Preparation of Working Reagent:

Dilute Reagent 1 was diluted with water at the proportion 1+1.

2. Calibrator was ready for use.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Assay procedure: content of test tubes T1, T2 and T3 were

	T1	T2	T3
Sample	0.1mL	-	-
Working reagent	5.0mL	5.0mL	5.0mL
Calibrator	-	0.1mL	-
Bi-distilled water	-	-	0.1mL

- The sample, working reagent and calibrator were pipetted into the test tubes as shown.
- The mixture was incubated for 30 minutes at 18-25°C.
- Absorbance of the Sample (A_{sample}) and Calibrator ($A_{calibrator}$) were read against reagent blank at 540nm.

Calculation:

Total protein concentration = $A_{sample}/A_{calibrator}$ x 70g/L

Where:

A_{sample} = Absorbance of the Sample

$A_{calibrator}$ = absorbance of the calibrator

70g/L – Total protein concenuation in the Calibrator

Unit conversion: 1g/L = 0.1g/dl.

3.6.6 Determination of albumin

Albumin was measured using Colorimetric method with Bromocresol Green described by Maruthamuthu and Kishore (1988)

Principle:

Albumin concentration is proportional to absorbance increase at 628nm due to the albumin bromocresol green complex formation.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Reagent 1 – Monoreagent

Acetate buffer, pH 4.2	50.0mmol/L
Bromocresol Green	0.10mmol/L
Surfactant	26g/L

Calibrator

Albumin	60g/L
NaCl	154mmol/L.

Assay procedure: content of test tubes T1, T2 and T3 were

	T1	T2	T3
Sample	0.01mL	-	-
Reagent 1	2.0mL	2.0mL	2.0mL
Calibrator	-	0.01mL	-
Bi-distilled water	-	-	0.01mL

- The sample, reagent 1 and calibrator were pipetted into the test tubes as shown.
- The mixture was incubated for 5 minutes at 18-25°C.
- Absorbance of the Sample (A_{sample}) and Calibrator ($A_{calibrator}$) was read against reagent blank at 590nm.

Calculation

Albumin concentration = $A_{sample}/A_{calibrator}$ x 60g/L

Where:

A_{sample} = Absorbance of the Sample

$A_{calibrator}$ = absorbance of the calibrator

60g/L – Albumin concentration in the Calibrator

Unit conversion: 1g/l = 0.1g/dl

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

3.6.7 Determination of creatinine

Creatinine was determined using Fixed-time Jaffe method without deproteinisation described by Lamb et al., (2006).

Principle:

In alkaline medium Creatinine forms a red coloured complex with picric acid. The rate of absorbance increase is proportional to the creatinine concentration in the sample.

Reagents:

Reagent 1

Picric acid	20mmol/L

Reagent 2

NaOH	260mmol/L
Surfactant	26g/L
Calibrator	17.7mmol/L (2g/L)

Reagent preparation:

Preparation of Working Reagent

- Equal volumes of Reagent 1 and Reagent 2 were mixed. The mixtures were incubated at 37°C for 30 minutes before the test execution.

Preparation of Calibrator

- The required quantity of the Calibrator was diluted with bi-distilled water in proportion 1 + 99

Calibrator concentration after dilution = 177µmol/L (2mg/dl)

Assay procedure: content of test tubes T1, T2 and T3 were

T1	T2	T3

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Working reagent	400µl	400µl	400µl
Sample	80µl	-	-
Diluted Calibrator	-	80µl	-
Bi-distilled water	-	-	80µl

- The working reagent, sample and calibrator were pipetted into the test tubes as shown
- The mixtures were incubated for 1 minute at 37°C.
- Absorbance (A_1) was read
- Absorbance (A_2) was read in 60 seconds.
- Absorbance for the sample and calibrator was calculated $\Delta A = A_2 - A_1$

Calculation

Creatinine concentration = $\Delta A_{sample} / \Delta A_{cal} \times 177 \mu mol/L$

Where:

ΔA_{sample} = Sample Absorbance change

ΔA_{cal} = Calibrator absorbance change

177µmol/L – creatinine concentration in Calibrator

3.6.8 Creatinine clearance

The creatinine clearance was calculated from serum creatinine, using the Cockcroft and Gault formula (Cockcroft and Gault, 1976). Normal renal function, mild and moderate-severe renal failure were defined as creatinine clearance; ≥90 mL/min/1.73 m^2, 60-89 mL/min/1.73 m^2, 15 - 59 mL/min/1.73 m^2: respectively.

CrCl = [(140 – age) x IBW] / (Scr x 72) (x 0.85 for females)

IBW = Ideal body weight

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

3.6.9 Determination of serum urea

Principle:

A rapid and reproducible method is described for measurement of urea in serum (without deproteinisation). Urea is colorimetrically determined with diacetyl monoxime and thiosemicarbazide in the presence of sulphuric acid, phosphoric acid and ferric chloride. The sensitivity of the colorimetric reaction and stability of the colour are enhanced over existing related procedures and the serum blank diminished, enabling urea to be precisely measured in micro amounts (1-5µl) of serum.

Reagents: Prepare

1. (Acid-ferric solution. To 300 ml of concentrated sulphuric acid and 600 ml distilled, 100 ml of concentrated phosphoric acid was added. In this solution 100 mg ferric chloride was dissolved.
2. Diacetylmonoxime (DAMO)-thiosemicarbazide (TSC) solution. In distilled water 500 mg DAMO and 10 mg TSC were dissolved and diluted to 100 ml
3. Chromogenic reagent. Two parts of Reagent 1 was mixed with one part of Reagent 2 immediately before use

Procedure:

To 5µl of serum, 3 ml of chromogenic reagent was added followed by 0.1 ml of Brij-35 solution (Sigma Chemical Co.). This was mixed vigorously and heated for 5 min in a boiling water bath. It was cooled and absorbance was measured at 525 nm against a blank composed of 5µl of distilled water plus 3 ml of reagent 3 and 0.1 ml of Brij-35 solution. A standard curve was prepared with varying concentrations of urea (0-150 nmol) in a final volume of 5µl of distilled water. Serum blanks was prepared for each

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

sample. 10µl of urease solution (Type VII. Sigma Chemical Co., 0.1 mg in 1 ml distilled water) was added to 10µl of serum. incubated for 5 min at 37°C.

The correct blank absorbance for use in calculation was obtained by multiplication with the dilution factor (X2).

For ordinary purposes correct for serum blank by subtracting 0.4 nmol (0.08 mmol/l when using 5µl serum) from the result read off from the standard curve.

3.6.10 Determination of plasma homocysteine

Plasma homocysteine level was determined using an enzyme immunoassay method described by Frantzen et al., (1998) using test kit from Axis-Shield Diagnostics Ltd. UK.

Assay principle:

Axis Homocysteine Enzyme Immunoassay (EIA) is an enzyme immunoassay for the determination of Homocysteine in blood (Frantzen et al., 1998). Protein-bound Homocysteine is reduced to free Homocysteine and enzymatically converted to S-adenosyl-L-homocysteine (SAH) in a separate procedure prior to the immunoassay (Sundrehagen E. Axis Biochemicals ASA. Enzymatic assay for homocysteine and a kit therefor. EP 623174/US5631127). The enzyme is specific for the L-form of homocysteine, which is the only form present in the blood.

Reduction

Homocysteine (Hcy), mixed disulfide and protein-bound forms of homocysteine in the sample are reduced to free homocysteine by use of dithiothreitol (DTT).

Prot-SS-Hcy

*R1-SS-Hcy $\xrightarrow{\text{DTT}}$ Hcy

Hcy-SS-Hcy

*R1 is any thiol-residue

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

Enzymatic conversion

Homocysteine in the test sample is converted to S-adenosyl-L-homocysteine (SAH) by the use of SAH hydrolase and excess adenosine (Ad).

Hcy + Ad $\xrightarrow{\text{SAH-hydrolase}}$ SAH + H_2O

The following solid-phase enzyme immunoassay is based on competition between SAH in the sample and immobilised SAH bound to the walls of the microtitre plate for binding sites on a monoclonal anti-SAH antibody. After removal of unbound anti-SAH antibody, a secondary rabbit anti-mouse antibody labelled with the enzyme horse radish peroxidise (HRP) is added. The peroxidise activity is measured spectrophotometrically after addition of substrate, and the absorbance is inversely related to the concentration of Hcy in the sample.

Procedure:

All solutions and microtitre strips are equilibrated to room temperature before use. The kit was left at room temperature overnight. Calibrators were run in duplicate and a new calibration curve was performed for each run to avoid run-to-run variations using coated microtitre plates.

Sample pre-treatment procedure

1. Sample pre-treatment solution was made up no more than 1 hour prior to the start of the assay as instructed by the manufacturer

   Volume needed per 10 samples.

   Reagent A – 4.5mL

   Reagent B – 0.255mL

   Reagent C – 0.25mL

   Mixed

2. Calibrators and samples/controls were diluted in glass tubes as follows

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Twenty-five microlitre calibrator/sample/control + 500µL sample pre-treatment solution. The mixture was mixed well. The mixture was incubated for 30minutes at 37°C (covered with parafilm during incubation).

3. Before the samples have cooled, 500µL Reagent D was added, mixed and incubated for 15minutes at 18-25°C.
4. Then 500µL Reagent E was added and mixed well. The mixture was incubated for 5 minutes at 18-25°C.

Micotitre plate procedure

5. Diluted 25µL of calibrator/sample/control was pipetted from step 4 into the wells of the SAH coated microtitre strips.
6. Two hundred microlitre of Reagent F was added to each well and incubated for 30min at 18-25°C. The plate was covered with the enclosed lid during incubation.
7. The plate was then washed with diluted wash buffer [(BUF/WASH) + purified water] using 3 x 400µL. The wells were emptied on paper towels after washing to remove excess buffer.
8. One hundred microlitre Reagent G was added to each well and incubated for 20min at 18-25°C.
9. This was washed with diluted wash buffer [(BUF/WASH) + purified water] using 3 x 400µL. The wells were emptied on paper towel.
10. One hundred microlitre Reagent H was added to each well and incubated for 10min at 18 – 25°C.
11. One hundred microlitre Reagent S was added to each well.
12. This was shaken and absorbance of each well read on a microplate reader at 450nm wavelength within 15minutes.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Preparation of Standard Curve (Appendix III): A serial dilution of a known concentration of standards were prepared as shown below

Standard Tubes	µmol/L	OD Set A	OD Set B
S1	2	2.438	2.366
S2	4	2.272	2.155
S3	8	1.972	1.775
S4	15	1.375	1.164
S5	30	0.870	0.740
S6	50	0.616	0.524

Calculation:

From the calibration curve of absorbance against concentration of the standards, the concentrations of the samples were calculated.

3.6.11 Determination of Vitamin B_{12} (cyanocobalamin)

Vitamin B_{12} concentration was determined using HPLC

Principle:

HPLC system uses a mobile-phase pump, a reagent pump, an auto-sampler, a detector and a data system for data processing and system control.

The system is a chromatography, in which the eluent is filtered and pumped through the column, then the sample is loaded and injected onto the column and the effluent is monitored using a detector, and the peaks are recorded. The pump of the system must be able to generate high pressure, performing a pulse-free output and deliver flow rates ranging from 0.1 to 10 ml/min.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Sample Preparation:

- Into a set of clean beakers, 1.0mL of the sample was pipetted.
- Twenty-five millilitre of 0.2HCL was added and warmed on a water bath for 30 minutes.
- Then cooled and the pH adjusted to 6.0 using NaOH.
- To this was added IN HCl to lower the pH to 4.5, then, transferred into a set of 50.0mL centrifuge tubes.
- Mixture was shaken for 30minutes.
- This was transferred and centrifuged for 20minutes at 2000rpm.
- The supernatant was collected and stored for HPLC determination of the analytes as follows
    - On the instrument and the pump the mobile phase was selected and the stationary phase fixed.
    - Mobile phase was a mixture of Acetic acid + $KMnO_4$ + H_2O_2).
    - Stationary phase (Zorbax Sil 222)
    - Flow rate (1.5mL/minutes)
    - Column temperature (35°C).
    - Wavelength (450 – 500nm)
    - All these were computed on the software.
- Before running on the instrument, 1.0mL of acetic acid (spectrosol) was added to each sample, mixed and then 0.5mL of 3% $KMnO_4$ solution.
- It was kept for 20minutes and then 0.5mL of 3% H_2O_2 and mixed.
- Then the samples of unknown concentration were run on the HPLC.
- The analytical data was automatically displayed on the readout of the computer for processing and printing.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

3.6.12 Determination of folate

Preparation of Reagents

- Ten gram of Ascorbic acid was dissolved in 1litre of ultra-pure water, then followed by 5.0mL of HCl (Spectrosol or HPLC grade).
- Five gram of methenyl tetrahydrofolic acid was dissolved in 250mL of ultra-pure water and covered properly.

Sample preparation

- Into a set of centrifuge tubes, 1.5mL of the sample was pipetted.
- Twenty mililitre of Ascorbic acid was added and 10.0mL of NaOH, mixed properly.
- Five mililitre of HCl was added and shaken for 30minutes.
- The samples were spin at 1500 rpm for 30minutes.
- The supernatant were transferred to a set of clean vials and stored for determination of the folate on HPLC as follows:
  - Folate working standard was prepared in Diglutamatetrihydrochloride solution in the following concentrations:_ 0.0, 2.0, 4.0, 6.0, 8.0 ppb.
  - The mobile phase (i.e. methanol solution) was prepared.
  - The stationary phase was selected (Zorbax SiL).
  - The reagents were circulated.
  - The necessary information pertaining to the identity of the samples, dilution factor of the samples, standards, flow rate (1.0mL/min), column temperature (40°C), detector, wavelength were entered (EM = 260nm, Ex 358nm).

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

- The working standards were run on the instrument to obtain R^2 or standard equation for the calculation of the concentration of the unknown using the interphased software.
- The concentration of the unknown sample was displayed on the computer read out for printing.

3.6.13 Determination of apolipoprotein A1

Apo A1 is based on turbidimetric measurement using test kits from DIALAB production und Vertrieb von chemischtechnischen Produkten und Laborinstrumenten Gesellschaft m.b.H. A – 2351 Wiener Neudof Austria.

Principle:

The assay of APO A1 is based on turbidometric. Turbidity is caused by the formation of antigen-antibody insoluble immune complexes. The formation of the complexes is accelerated and enhanced.

Assay Procedure

Physiological saline (0.9%) was used in the preparation of the different dilutions of the APO A1 standard, saline (0.9%) was used as zero point.

Calibration Curve (Appendix IV): Apo A1/A2/B Calibrator High was used to generate a calibration curve by making 1:10, 1:20, 1:40, 1:80 and 1:160 dilutions with 0.9% saline as diluents.

Tubes	1	2	3	4	5	6
Standard mg/dl	0	10	20	40	80	160
Stage I						
Dilute Calibrator N/S	1	1/160	1/80	1/40	1/20	1/10

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Stage II						
Buffer	900µl	900µl	900µl	900µl	900µl	900µl
Diluted Calibrator from Stage I	10µl	10µl	10µl	10µl	10µl	10µl

The above was mixed and absorbance A1 of calibrated standard was read at 340nm.

Absorbance	0	0.006	0.012	0.033	0.048	0.073
Antibody reagent added	60µl	60µl	60µl	60µl	60µl	60µl

This was mixed. incubated for 5mins at assay temperature (18 – 37°C).

The absorbance A2 was read at 340nm

Calculation: $\Delta A = (A2 - A1)$

The $\Delta A = (A2 - A1)$ of the calibrators versus assigned concentration values were plotted on a linear-linear graph paper. The ΔA optical densities of samples and control(s) were read in mg/dl on the reference curve.

Samples yielding absorbance above highest calibrator were retested after further dilution.

A1	0	0.006	0.012	0.033	0.048	0.073
A2	—	0.084	0.100	0.120	0.220	0.322
$\Delta A = (A2 - A1)$	—	0.078	0.088	0.157	0.172	0.249

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

3.7 ANTHROPOMETRIC MEASUREMENTS

3.7.1 Waist and Hip circumference

Each child's waist and hip circumferences were measured using a non-stretchable tape (Butterfly, China). Every child was made to stand upright. The waist circumference was taken at the level of the umbilicus (see Appendix VII), while the hip was taken at the level of the greater trochanter (see Appendix VII). All readings were taken to the nearest centimetre (cm).

3.7.2 Height and Weight

Height and weight of each child were measured according to standard World Health Organization (WHO) prescribed procedures. The height (cm) of each child was measured using a graded stadiometer (Creative Health Products, Plymouth, Mich, USA) made of a vertical calibrated rod and a perpendicular movable headpiece on the calibrated rod (Appendix VI). The child was asked to stand barefooted on the measuring board, with his/her back and heel to the instrument, arms at the sides, heels close together and the eye looking straight ahead, the perpendicular movable headpiece was then lowered snugly to the crown of the head (vertex).

The weight was measured using a battery powered digital scale (Seca, Inc, Columbia, MD, USA). Though this equipment is self-calibrating, zero error of the weighing scale was checked against a standard tare daily for the purpose of recalibration. The children were weighed wearing only under-wears and barefooted. They were asked to stand at the centre of the platform with hand to the sides and the weight was recorded to the nearest 0.1kg. Participants' weight measurements were carried out when all oedema had resolved, that is there was no more clinical evidence of oedema. These weights were assumed to be the actual weights of the patients (dry weight).

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

3.7.3 Body Mass Index (BMI)

Body mass index for each child was calculated as weight (kg) divided by height (m) squared (kg/m^2).

3.7.4 Skinfold Thickness Measurements

Skinfold thickness readings were measured on the right side of the body at triceps, biceps, deltoid, subscapular, abdominal, thigh and calf using the Lange Skinfold Caliper (1985 Beta Technology Inc. USA). At these sites the skinfold was pinched up firmly between the thumb and forefinger and pulled away slightly from the underlying tissues before applying the callipers for measurements (Appendix VII).

- Triceps: - mid way between the Olecranon and tip of the acromion with the upper arm hanging vertically.
- Biceps: - over the mid portion of the biceps muscles.
- Subscapular at just below the tip of the inferior angle of the subscapular at an angle of 45° vertical.

3.7.5 Body fat proportion

The children's body fat proportions were calculated from the formula of Deurenberg *et al.* (1991):

Child Body Fat % = (1.51×BMI) – (0.70×Age in years) – (3.6× gender) + 1.4 (where male gender = 1, female = 0). Lean body mass was estimated by subtracting the estimated body fat from the body weight (kg).

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

3.8 Determination of socioeconomic class

Socioeconomic index scores were awarded to each child, based on the occupations and educational attainment of the parents or their substitutes as described by Oyedeji (1985).

For occupation:-

Class 1 was allocated to senior public servants, professionals, managers, large scale traders, businessmen and contractors.

Class 2:- Intermediate grade public servants and senior school teachers.

Class 3:- Junior school teachers, drivers and artisans.

Class 4:- Petty traders, labourers, messengers and similar grades

Class 5:- Unemployed, full-time housewives, students and subsistence farmers.

For the educational scale:-

Class 1 was awarded to University graduates or equivalents;

Class 2:- School certificate (Ordinary Level GCE) holders who also had teaching or other professional training.

Class 3:- School certificate or grade II teachers' certificate holders or equivalents.

Class 4:- Modern three and primary six certificates

Class 5:- Those who could either just read and write or were illiterate.

The mean of four scores (two for the father and two for the mother) to the nearest whole number was the social class assigned to the child. For example, a father who was a university lecturer scored 1 for his occupation and 1 for his education as a graduate. His wife who was a business-woman with the secondary school certificate level of education scored 1 for her occupation and 3 for her education. The total of these four scores would be 6 with an average of 1.5; when taken to the nearest whole, the number was 2. Thus the social class assigned to this child was II.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

In this study, average score of 1 above was assigned class 1 (upper class), scores of 2 and 3 above were assigned class 2 (middle class) while scores of 4 and 5 nbove were assigned class 3 (lower class).

3.9 DATA ANALYSIS

Data were analysed using Statistical Package for Social Scientists (SPSS) 17.0 for Windows (SPSS Inc., IL, USA). All continuous variables were checked for normality using the Shapiro-Wilks test and those found to be non-parametric in distribution were identified. Unpaired two tailed Student *t* test was used to detect significant differences between cases and control (because they were independent groups). Paired *t* test was used to assess the differences in parameters measured before supplementation and repeats at 3months among the cases (nephrotic syndrome patients). Spearman Rho correlation analysis method was used to assess correlations between variables because of the non-parametric nature of most of the measured parameters.

The differences in prevalence of high or low plasma levels of homocysteine, folate, vitamins B_6 and B_{12} in the study groups were analysed using Chi square test. The distribution of low levels of vitamin B_6, vitamin B_{12} and high level of tHcy was assessed by dividing vitamin levels into quantiles and calculating the differences by the chi-square test. Correlation coefficients (r) were calculated to determine the relation between tHcy, vitamin B_6, vitamin B_{12}, folate, serum albumin, eGFR in patients with the nephrotic syndrome and control. Since many of the variables that could influence the circulating levels of homocysteine were found significantly different between the nephrotic syndrome and control groups by univariate analysis, the relationships of homocysteine with folate, vitamins and Apo A1 among the

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

nephrotic syndrome group was examined using multivariate analysis. P values lower than 0.05 were considered statistically significant.

3.10 Ethical consideration

Participation in the study was completely voluntary and based on informed consent. Participants were made to understand that they were free to withdraw their consent at any time and they would not be denied their due treatments according to the UCH protocol. Privacy of participants was ensured by using a serial number on each of the questionnaire used to collect information, rather than a name. Only the researcher knew the identification, and this information was kept secured. The data were not discussed with anyone, except the participant's doctor, when required. The study was not responsible for payment for the treatments of any illness detected at the time of the data collection. However, results of laboratory tests carried out as part of this research relevant to their treatments were made available at no cost to the participants. The research was risk free, as all that was required were anthropometric measurements, blood sampling and filling of a form.

Ethical approval for this study was obtained from University of Ibadan / University College Hospital Ibadan, Ethics Committee, IAMRAT College of Medicine, University of Ibadan, Ibadan (Appendix VIII). Written informed consent was obtained from the parents or caregivers of each child (Appendix IX). Verbal accent was obtained from all the children before blood sampling and measurements were taken.

UNIVERSITY OF IBADAN LIBRARY

CHAPTER FOUR

RESULTS

4.1. Socio-demographic characteristics and clinical features

The social and demographic characteristics of the study participants were as shown in Table 4.1. Study participants comprised 42 children with diagnosis of nephrotic syndrome (cases) and 42 healthy children (controls). There were 27 (64.3%) males and 15 (35.7%) females among cases and controls respectively. The ages of the case and control groups ranged from 40.0 to 140.0 months and 41 to 140.0 respectively. There was no significant difference in the mean age of nephrotic syndrome (103.5 ±32.7 months) and control (100.9 ±29.4 months); p = 0.740. The mean ages of mothers (38.5 ±6.4 years) and fathers (48.5 ± 10.9 years) of children with nephrotic syndrome were significantly higher than the mean ages of mothers (32.5 ±7.5 years) and fathers (38.9 ±6.4 years) of the controls. The distribution of study participants by parent marital status was not different in the nephrotic syndrome group compared with the control group (Table 4.1). The number of mothers' children ranged from 1 to 7 among nephrotic syndrome and 1 to 6 among control group with median value of 4 children for nephrotic syndrome and 3 children for control group. Also, the distribution of participants by parents' socioeconomic status was not different among cases and controls as shown in Table 4.1 (p=0.714). About two-third of the children in nephrotic syndrome (61.9%) and slightly over half of the control (54.8%) came from middle socioeconomic class while only 4 (9.5%) came from high socioeconomic

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

class. Figure 4.1 shows the distribution of study participants by their positions among mothers' children. The median number of mothers' children was 4 and 2 for nephrotic syndrome and control groups respectively.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Table 4.1: Social and demographic characteristics of the study participants

Characteristics	Nephrotic syndrome (n= 42)	Controls (n= 42)	P
Sex (Male/Female)	27/15	27/15	NA
Mean age in months (mean ±SD)	103.5±32.7	100.9±29.4	0.740
Mean mothers age in years (mean ±SD)	38.5 ±6.4	32.5 ±7.5	**0.002**
Mean fathers age in years (mean ±SD)	48.5 ±10.9	38.9 ±6.4	**0.001**
Parents' marital status, n (%)			
Never married	6 (14.2)	5 (11.9)	0.949
Married	34 (81.0)	35 (83.3)	
Divorced	2 (4.8)	2 (4.8)	
Median number of mother's children	4 (1 – 7)	3 (1 – 6)	**0.001**
Parents' social class, n (%)			
High	4 (9.5)	4 (9.5)	0.744
Middle	26 (61.9)	23 (54.8)	
Low	12 (28.6)	15 (35.7)	

NA – Not Applicable

n = number of subjects

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

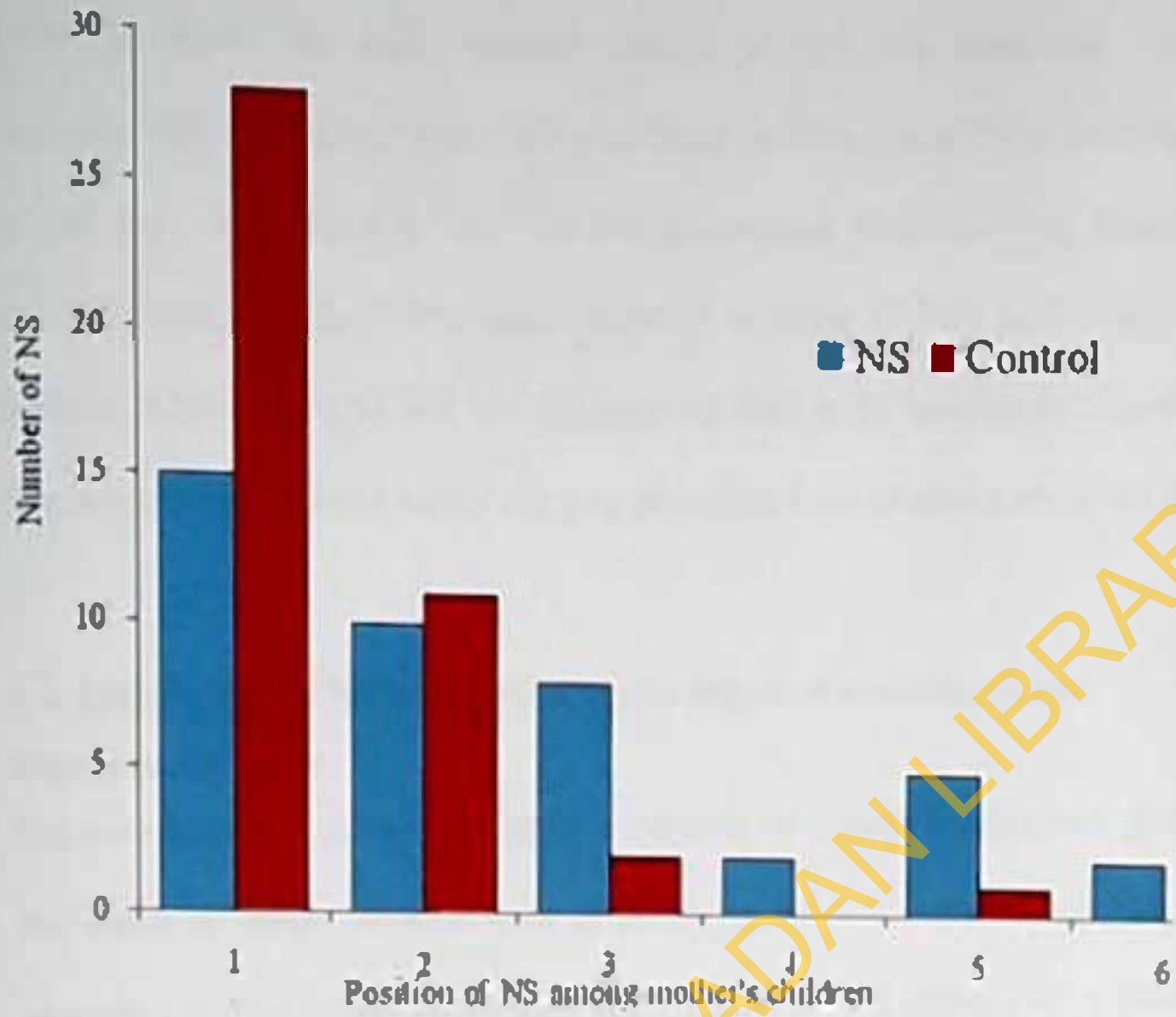


Figure 4.1: Distribution of study participants by their position among mothers' children

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

4.2. Major clinical features among the nephrotic syndrome group

Table 4.2 shows the major clinical features of nephrotic syndrome. The entire nephrotic syndrome group presented with facial swelling for a period ranging from 2 to 120 days. In addition 22 (52.4%) had generalized body swelling, diminution in urine (28.6%), cough (9.5%) and abdominal swelling (7.1%) were major clinical features. About two-third (66.7%) had been admitted to the hospital for illnesses other than nephrotic syndrome within one year preceding their participation in the study.

4.3. Distribution of NS and control by the degree of proteinuria and hypoalbuminaemia

The distribution of study subjects by the degree of proteinuria assessed qualitatively and levels of serum albumin were as shown in Table 4.3. Before supplementation, majority (76.2%) of the NS patients had proteinuria of 3 pluses (+++), 23.8% had 2 pluses (++). After supplementation (at three months), none of the NS had gone into remission. In the control group on the other hand, there was no one with significant proteinuria. Hypoalbuminaemia (<2.5g/dL) was found in all the NS patients. Only two (4.8%) of the subjects in the control group had serum albumin less than 2.5mg/dl.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Table 4.2: Major clinical features of 42 patients with Nephrotic Syndrome

Clinical features	n (%)	Median duration in days (Range)
Main presenting symptoms		
Facial swelling	42 (100.0)	36 (2 - 120)
Generalised body swelling	22 (52.4)	36 (2 - 120)
Abdominal swelling	3 (7.1)	17 (3 – 31)
Diminution in urine	12 (28.6)	14 (10 – 36)
Cough	4 (9.5)	5 (7 – 90)
Past hospital admissions		
Yes	28 (66.7)	6 (1 – 15)
No	14 (33.3)	-

n = number of subjects

UNIVERSITY OF IBADAN LIBRARY

Table 4.3: Distribution of NS and control by the degree of proteinuria and hypoalbuminaemia

Urine and serum protein	Nephrotic Syndrome		Control	
	n= 42	%	n =42	%
Degree of proteinuria				
Negative/Trace	0	0.0	42	100
1 plus (+)	0	0.0	0	0.0
2 pluses (++)	10	23.8	0	0.0
3 pluses (+++)	32	76.2	0	0.0
Serum albumin (g/dl)				
≥2.5	0	0.0	40	95.2
2.0 – 2.4	24	57.1	2	4.8
1.0 – 1.9	16	38.1	0	0.0
<1.0	2	4.8	0	0.0

n = number of subjects

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

4.4. Distribution of NS patients and controls into the upper-, inter- and lower-quartiles of the distribution of homocysteine

The distribution of patients and controls in the four quartiles of the distribution of homocysteine were as shown in Table 4.4. Majority (95.2%) of the participants in the nephrotic syndrome group fell into the upper quartile of the normal distribution for homocysteine compared with 26.2% in the control group. NS patients were 80 times more likely to have homocysteine level in the upper quartile than the control (OR=80.0, 95% CI = 9.24, 92.46; p<0.001). However for serum folate, vitamin B_{12} and B_6, there were no significant differences in the distribution of plasma level into quartiles between the two groups as shown in Table 4.4.

4.5. Prevalence of high plasma homocysteine and low vitamins in the study participants

Prevalence of high plasma homocysteine and low vitamins in the study participants are as shown in Table 4.5. Twenty-four subjects in the nephrotic syndrome group and only 3 participants among the control group had high plasma tHcy giving the prevalence of hyperhomocysteinaemia as 57.1% and 7.1% respectively. The nephrotic syndrome group was 17 times more likely to have high level of tHcy compared with the control group. Six (14.3%) of the children with nephrotic syndrome and none of the control children had low serum folate. Four (9.5%) participants in each of the nephrotic syndrome and control groups had low vitamin B_{12}.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Table 4.4: Distribution of subjects based on quartile values of homocysteine, folate, vitamins B_6 and B_{12} for control

Variables	No of patients (%) n = 42	No of control (%) n = 42	P	OR	95% CI
Homocysteine					
≤4.1	1 (2.4)	16 (38.1)	-	1	-
4.2 – 6.0	1 (2.4)	15 (35.7)	0.522	1.14	0.07, 20.02
≥6.1	40 (95.2)	11(26.2)	0.000	80.0	9.24, 92.46
Folate					
≤9.4	16 (38.0)	10 (23.8)	-	1	-
9.5 – 13.5	24 (57.2)	22 (52.4)	0.602	0.68	0.26, 1.82
≥13.6	2 (4.8)	10 (23.8)	0.014	0.13	0.02, 0.69
Vitamin B_{12}					
≤234.8	17 (40.5)	10 (23.8)	-	1	-
234.9 – 438.1	25 (59.6)	21 (50.0)	0.624	0.70	0.26, 1.85
≥438.2	0 (0.0)	11 (26.2)	0.000	0.0	0.0
Vitamin B_6					
≤66.9	15 (35.7)	10 (23.8)	-	1	-
67.0 – 90.4	26 (61.9)	22 (52.4)	0.804	0.79	0.30, 2.10
≥90.5	1 (2.4)	10 (23.8)	0.009	0.07	0.01, 0.61

n = number of subjects

UNIVERSITY OF IBADAN LIBRARY

Table 4.5: Prevalence of hyperhomocysteinaemia, low serum folate, low vitamin B_6 and low vitamin B_{12}

Variables	Nephrotic syndrome (n= 42)	Controls (n= 42)	P	OR (95% CI)
High Hcy (>10μmol/l)	24 (57.1%)	3 (7.1%)	<0.001	17.3 (4.6, 65.1)
Low folate (<3.4ng/mL)	6 (14.3)	0 (0.0)	0.026	-
Low vitamin B_6 (<20.0nmol/l)	0	0	-	-
Low vitamin B_{12} (<133pg/mL)	4 (9.5%)	4 (9.5%)	1.000	-

n = number of subjects

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

4.6. Baseline anthropometrics and skinfold thickness

Anthropometry and skinfold thickness measurements of study participants before supplementation were as shown in Table 4.6. The mean dry body weight of children in the nephrotic syndrome group (27.9 ±7.9 kg) was not significantly different from the corresponding value in the control group (24.7 ±7.3 kg); p = 0.082. However, the nephrotic syndrome group had a significantly lower mean height (110.3 ±26.0 cm) compared with control group (127 ±20.3 cm); p = 0.004. The mean Body Mass Index (BMI) of the nephrotic syndrome group (17.3 ±1.6kg/m^2) was significantly higher than the control group (15.9 ±1.2kg/m^2); p <0.001. The mean waist and hip circumferences of the nephrotic syndrome group (57.9±8.5cm; 62.6 ±10.5cm) were not significantly different from the comparative values in the control group (54.9 ±9.0cm; 60.6 ±11.7cm). There were no significant differences in the mean skinfold thicknesses measured at deltoid, sub-scapular, biceps, triceps, abdominal, thigh and calf areas in the nephrotic syndrome group compared with the mean values obtained at the corresponding sites in the control group (Table 4.6). Conversely, the skinfold thickness at the hip area was significantly lower in the nephrotic syndrome group (6.8 ±3.3mm) than control group (9.1 ±3.0mm); p = 0.004. The mean total body fat percentage was also significantly higher in NS (18.0 ±3.8%) than the control (16.2±2.7%); p = 0.015.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Table 4.6: Baseline anthroponietrics and skin-fold thicknesses of study participants

Biophysical parameters	Nephrotic syndrome (n= 42)	Controls (n= 42)	P
Mean dry weight in kg	27.9±7.9	24.7±7.3	0.082
Mean height in cm	110.3±26.0	127±20.3	0.004
Mean Body Mass Index in kg/m^2	17.3±1.6	15.9±1.2	<0.001
Mean waist circumference in cm	57.9±8.5	54.9±9.0	0.159
Mean hip circumference in cm	62.6±10.5	60.6±11.7	0.535
Mean Skin-fold thicknesses			
Deltoid area (mm)	7.9±3.5	7.4±2.2	0.451
Sub-scapular area (mm)	6.6±2.1	6.4±1.6	0.670
Biceps area (mm)	4.8±1.3	5.5±1.8	0.055
Triceps area (mm)	7.5±2.4	7.6±1.8	0.896
Abdominal area (mm)	5.7±2.0	6.5±1.9	0.109
Anterior right thigh area (mm)	9.3±3.8	8.4±2.4	0.258
Right hip area (mm)	6.8±3.3	9.1±3.0	0.004
Right calf area (mm)	9.1±4.1	8.9±2.7	0.865
Estimated body fat, BF (%)	18.0±3.8	16.2±2.7	0.015

n = number of subjects

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

4.7: Physical exercises and intake of fat-rich foods and salt

Table 4.7 shows responses of the study participants and caregivers to questions on exercise, intake of fat-rich foods and table salt before the study. The distribution of participants by frequency of their involvement in exercise sufficient to make them breathe faster or increase heart beat (>20 minutes) was not different among the nephrotic syndrome group and control. Majority (52.4%) in the nephrotic syndrome group compared with control (35.7%) occasionally or never took part in sufficient exercise. Similarly, there was no significant difference in the distribution of participants by the number of eggs participants ate when he/she had omelletes or scramble eggs. About two-third of the nephrotic syndrome group (57.1%) and slightly over half of the control (52.4%) would eat an egg when they had omelettes or scramble eggs. The NS patients were less likely to eat two eggs compared with control (OR=0.11, 95% CI = 0.02, 0.78; p=0.036). Slightly over half of the nephrotic syndrome group (52.4%) compared with 95.0% of the control reported the use of cow's full cream milk in at least three diets per week. The NS patients were less likely to use cow's full cream milk compared with control (OR = 0.06, 95% CI = 0.01, 0.46; p <0.001). However, over half (52.4%) of the nephrotic syndrome group compared with 23.8% of control group did not use any fat-rich spread over their foods (such as bread and sandwiches). The NS patients were less likely to use butter as spread compared with control (OR = 0.20, 95% CI = 0.23, 0.86; p = 0.006). In both groups, palm oil was usually used to cook food for the study participants: 59.5% of the nephrotic syndrome and 71.4% of control groups. All caregivers of participants in the control group reported that they added salt to food either during or after cooking with majority (95.2%) doing so during cooking while many (40.5%) of the nephrotic

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

syndrome group did not add salt. The proportions of participants whose mothers reported addition of salt during and after cooking were 54.7% and 4.8% respectively.

UNIVERSITY OF IBADAN LIBRARY

Table 4.7: Exercise and intake fat-rich foods among study participants three months before the study

Dietary pattern/lifestyle	Nephrotic syndrome (n= 42)	Controls (n= 42)	OR	95% CI	P
How often does your child take exercise sufficient to make him/her breathe faster or increase heart beat? (>20 minutes)					
Occasionally or never	22 (52.4)	15(35.7)	1	-	-
Once or twice a week	4 (9.5)	2 (4.8)	0.34	0.03,4.11	0.564
Three times a week or more	16 (38.1)	25(59.5)	0.44	0.18,1.08	0.114
How many eggs does your child eat when he/she has omelletes or scramble eggs?					
One egg	24 (57.1)	22(52.4)	0.55	0.14,2.07	0.116
Two eggs	2 (4.8)	9 (21.4)	**0.11**	**0.02,0.78**	**0.036**
More than 2 eggs	6 (14.3)	7 (16.7)	0.43	0.08,2.17	0.529
Do not eat at all	10 (23.8)	4 (9.5)	1	-	-
What kind of milk does your child often (at least 3 diets per week) use?					
Cow's full cream	22(52.4)	40(95.0)	**0.06**	**0.01,0.46**	**<0.001**
Cow's skimmed	10 (23.8)	1 (2.4)	1.0	0.05,8.30	0.458
Do not use milk	12 (28.6)	1 (2.6)	1	-	-
What type of spread does your child use to eat bread, sandwiches...?					
Butter	7 (16.7)	16(38.1)	0.20	0.23,0.86	**0.006**
Margarine	13 (31.0)	16(38.1)	0.37	0.13,1.05	0.073
He/she doesn't use spread	22 (52.4)	10(23.8)	1	-	-
What type oil do you most often use for your cooking?					
Palm oil	25 (59.5)	30(71.4)	0.61	0.25,1.51	0.390
Vegetable oil such as corn,	17 (40.5)	12(28.6)	1	-	-
When do you usually add salt to your child's food?					
During cooking	23 (54.7)	40(95.2)	0.57	0.08,4.36	**0.054**
After cooking	2 (4.8)	2 (4.8)	1	-	-
I do not add salt	17 (40.5)	0 (00)	-	-	**0.028**

n = number of subjects

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

4.8. Serum protein, creatinine, urea and estimated glomerular filtration rate (eGFR)

Table 4.8 shows the serum creatinine, serum urea, eGFR, serum albumin and serum total protein of the nephrotic syndrome and control groups. The mean serum creatinine and eGFR in the nephrotic syndrome group (0.7±0.4mg/dl and 78.0±33.9mL/min/1.73m^2 respectively) were not significantly different from the respective value in the control group (0.5±0.2mg/dl, ($p = 0.057$) and 86.5±38.4mL/min/1.73m^2, ($p = 0.284$). NS patients had a significantly higher mean urea (46.5±15.2mg/dl) compared with the values in the control group (31.0±10.1mg/dl), $p < 0.001$. On the other hand, the mean total serum protein and albumin concentrations in the nephrotic syndrome group (4.9±1.1g/dl and 2.2±1.1g/dl) were significantly lower than the corresponding values in the control group (6.5±0.6g/dl and 4.5±0.5g/dl); $p < 0.001$ for each.

4.9. Plasma lipids and apolipoprotein A1

The mean plasma lipids and apolipoprotein A1 concentrations of study participants at baseline were as shown in Table 4.9. Mean total plasma cholesterol, LDL cholesterol, triglycerides and Apo A1 concentrations as well as total cholesterol to HDL ratio in the nephrotic syndrome group (235.9±94.7mg/dl; 191.9±91.2mg/dl; 161.5±114.4mg/dl; 173.8±43.4mg/dl and 6.6±3.6 respectively) were significantly higher than the corresponding values in the control group (155.0±70.6mg/dl; 122.5±70.2mg/dl; 108.4±52.6mg/dl; 136.7±56.9mg/dl and 2.4±1.1 respectively). However, there was no significant difference in the mean HDL cholesterol levels of nephrotic syndrome and control groups.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Table 4.8: Serum protein, creatinine and creatinine clearance among study participants

Analytes	Nephrotic syndrome (n= 42)	Controls (n= 42)	p
Serum creatinine (mg/dl)	0.7±0.4	0.5±0.2	0.057
Urea (mg/dl)	46.5±15.2	31.0±10.1	<0.001
Estimated GFR (mL/min/1.73m^2)	78.0±33.9	86.5±38.4	0.284
Serum albumin (g/dl)	2.2±1.1	4.5±0.5	<0.001
Total serum protein (g/dl)	4.9±1.1	6.5±0.6	<0.001

n = number of subjects

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Table 4.9: Baseline plasma lipids of study participants

Plasma lipids	Nephrotic syndrome (n= 42)	Controls (n= 42)	P
Total cholesterol (mg/dl)	235.9±94.7	155.0±70.6	<0.001
LDL cholesterol (mg/dl)	191.9±91.2	122.5±70.2	<0.001
HDL Cholesterol (mg/dl)	12.7±10.2	9.8±8.3	0.163
Triglycerides (mg/dl)	161.5±114.4	108.4±52.6	0.014*
Total Cholesterol/HDL ratio	6.6±3.6	2.4±1.1	<0.001
Apolipoprotein A1 (mg/dl)	173.8±43.4	136.7±56.9	<0.001

*Mann-Whitney U test

n = number of subjects

UNIVERSITY OF IBADAN LIBRARY

4.10. Plasma homocysteine, serum folate, vitamins B_6 and vitamin B_{12} at baseline

The baseline levels of plasma homocysteine, serum folate, vitamins B_6 and B_{12} for nephrotic syndrome group and control are as shown in Table 4.10. The mean total homocysteine of 11.2±2.8μmol/L in the nephrotic syndrome group was significantly higher than respective mean value of 5.5±2.3 μmol/L in the control group ($p<0.001$). The mean serum folate and vitamin B_{12} concentrations in the nephrotic syndrome group (9.1±3.9ng/mL and 268.5±95.7pg/mL respectively) were significantly lower than corresponding values of 11.2±3.1ng/mL and 316.4±117.2pg/mL in the control group ($p = 0.009$; $p = 0.043$ respectively). Conversely, the slight reduction in the mean vitamin B_6 concentration in the nephrotic syndrome group (72.4±13.1 nmol/L) when compared with respective control value (75.8±15.2nmol/L) was not statistically significant.

4.11: Socio-economic status and mean plasma homocysteine

The mean plasma homocysteine concentration in NS and control group classified by parents' socio-economic class are as shown in Table 4.11. The mean plasma Hcy concentrations in the middle and low socio-economic classes of NS were significantly higher than their corresponding control group, while the difference in the high socio-class was not statistically significant. Within each groups (that is NS and control), comparison of the three social classes did not reveal any statistically significant differences neither in the nephrotic syndrome nor control group respectively.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Table 4.10: Baseline plasma homocysteine, serum folate, vitamin B_{12} and Vitamin B_6 of study participants

Analytes	Nephrotic syndrome (n= 42)	Controls (n= 42)	p
tHcy (µmol/L)	11.2±2.8	5.5±2.3	<0.001
Folate (ng/mL)	9.1±3.9	11.2±3.1	0.009
Vitamin B_6 (nmol/L)	72.4±13.1	75.8±15.2	0.284
Vitamin B_{12} (pg/mL)	268.5±95.7	316.4±117.2	0.043

n = number of subjects

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Table 4.11: Socio-economic status and mean plasma homocysteine

Socio-economic class	Nephrotic Syndrome		Control		P value
	n=42	Mean	n=42	Mean	
High	4	8.36±3.54	4	5.04±2.04	0.262
Middle	26	11.01±2.71	23	6.03±2.81	0.000
Low	12	11.95±2.77	15	5.03±1.52	0.000
*P value		0.187		0.232	

*Compared values within each group

n = number of subjects

UNIVERSITY OF IBADAN LIBRARY

4.12. Serum creatinine, estimated GFR and protein levels before and after supplementation

Changes in serum creatinine, serum urea, estimated GFR and protein levels after supplementation are as shown in Table 4.12. After the 3months vitamins (folate, vitamin B_6 and B_{12}) supplementation there were significant reductions in the mean serum creatinine and urea concentrations with mean decrease of 0.2±0.4mg/dl and 15.5±5.1mg/dl respectively. On the other hand, the mean total protein and albumin increased following 3months of vitamins supplementation by mean of 0.4±0.8g/dl and 1.6±1.2g/dl respectively. The increase in estimated GFR 3months after vitamins supplementation by 4.7±1.8ml/min/1.73m^2 was not statistically significant

4.13. Plasma lipids and apolipoprotein A1 levels before and after supplementation

The mean plasma lipids, apolipoprotein A1, before and after vitamin and folate supplementation were as shown in Table 4.13. The mean total cholesterol, LDL cholesterol, triglycerides and Apo A1 concentrations as well as TC/HDL ratio reduced significantly by mean values of 6.2±3.0mg/dl, 40.2±16.3mg/dl, 42.2±105.6mg/dl, 21.7±5.4mg/dl, 2.9±1.6 respectively after supplementation. Although, there was slight reduction in the HDL cholesterol level by 1.9±7.2mg/dl, this was not statistically significant.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Table 4.12: Serum protein, creatinine and creatinine clearance pre and post vitamin and folate supplementation in nephrotic syndrome patients

Analytes	Nephrotic Syndrome					Control	p*
	Baseline n=42	After supplement n = 34	Mean difference	% change	p	n = 42	
Creatinine (mg/dl)	0.7±0.4	0.5±0.1	0.2±0.4	28.6	0.027	0.5±0.2	1.000
Urea (mg/dl)	46.5±15.2	31.0±10.1	15.5±5.1	33.4	<0.001	31.0±10.1	1.000
eGFR (ml/min/1.73m^2)	78.0±33.9	91.2±30.7	4.7±1.8	6.0	0.165	86.5±38.4	0.555
Albumin (g/dl)	2.5±1.1	4.1±0.7	1.6±1.2	64.1	0.002	4.5±0.5	0.007
Total protein (g/dl)	4.9±1.1	5.3±1.1	0.4±0.8	8.3	<0.001	6.5±0.6	<0.001

*Compared mean values of after supplementation with control.

n = number of subjects

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Table 4.13: Plasma lipids before and after vitamins supplementation

Plasma lipids	Nephrotic Syndrome					Control	p
	Baseline n =42	After supplement n = 34	Mean difference	% mean difference	p	n = 42	
TC (mg/dl)	235.9±94.7	188.2±51.3	6.2±3.0	21.1	<0.001	155.0±70.6	0.025
LDL (mg/dl)	191.9±91.2	154.3±50.6	40.2±16.3	21.2	0.018	122.5±70.2	0.025
HDL(mg/dl)	12.7±10.2	10.4±8.8	1.9±7.2	15.0	0.928*	9.8±8.3	0.761
Triglyceride (mg/dl)	161.5±114.4	117.6±37.6	42.2±105.6	26.1	0.023*	108.4±52.6	0.377
TC/HDL	6.6±3.6	3.7±1.4	2.9±1.6	12.5	0.025	2.4±1.1	<0.001
Apo A1 (mg/dl)	173.8±43.4	152.1±37.9	21.7±5.4	12.5	<0.001	136.7±56.9	0.163

*Wilcoxon Signed Rank Test used

*Compared mean values of after supplementation with control.

n = number of subjects

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

4.14. Plasma homocysteine, serum folate, vitamin B_{12} and vitamin B_6 levels before and after supplementation

In the nephrotic syndrome group, the mean plasma homocysteine significantly decreased following supplementations with folate and vitamin B by 6.1±3.0 µmol/l as shown in Table 4.14. On the other hand, there were significant increases in the levels of serum folate, vitamin B_6 and B_{12} by 2.4±2.1ng/mL, 11.1±15.6nmol/L and 41.3±23.1pg/mL respectively (Table 4.14).

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Table 4.14: Plasma Homocysteine, Serum folate, vitamins B_{12} and B_6 before and after vitamins supplementation

Analytes	Nephrotic Syndrome					Control n = 42	P
	Baseline n =42	After supplement n = 34	Mean difference	% mean difference	p		
tHcy (µmol/L)	11.2±2.8	5.1±1.5	6.1±3.0	52.6	<0.001	5.5±2.3	0.364
Folate (ng/mL)	9.1±3.9	11.5±4.2	2.4±2.1	26.4	<0.001	11.2±3.1	0.721
Vitamin B_6 (nmol/L)	72.4±13.1	83.2±9.2	11.1±15.6	15.3	<0.001	75.8±15.2	0.011
Vitamin B_{12} (pg/mL)	268.5±95.7	309.8±104.2	41.3±23.1	15.4	0.023*	108.4±52.6	0.377

n = number of subjects

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

4.15. Biophysical parameters in NS and control classified by homocysteine levels

The comparison of mean values of biophysical parameters between subjects who had tHcy above and below 10μmol/L in NS and control groups were as shown in Table 4.15. The Table shows that there were no differences in the mean age, weight, height, BMI and waist circumference of those whose tHcy levels were >10.0μmol/L compared with those whose tHcy were ≤10.0 μmol/L in both groups. Similarly, all the skinfold thicknesses were not significantly different between those whose tHcy levels were >10.0 μmol/L compared with those whose tHcy levels were ≤10.0 μmol/L except in the deltoid area. In the deltoid area, the NS with tHcy >10.0μmol/L had significantly higher skinfold thickness than those with tHcy ≤10.0 μmol/L. On the other hand, there were no significant differences in the mean age, weight, height, BMI, waist circumference, hip circumference and all skinfold thicknesses of those who had tHcy >10.0 μmol/L and those whose tHcy were ≤10.0 μmol/L among the control group. However, the mean estimated body fat proportion of NS whose tHcy levels were >10.0μmol/L was significantly higher than those whose tHcy were ≤10.0 μmol/L.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Table 4.15: Biophysical parameters in NS and control classified by homocysteine levels

Biophysical parameters	NS		Control	
	tHcy ≤10.0 n = 20	tHcy>10.0 n = 22	tHcy ≤10.0 n = 39	tHcy >10.0 n = 3
Mean age in months	101.5±30.4	109±32.6	105.8±10.5	101.11±30.0
Mean dry weight in kg	26.8±7.9	30.2±8.3	23.0±1.7	25.6±7.3
Mean height in cm	125±16.7	133±26.8	124.0±8.7	114.6±27.3
Mean Body Mass Index in kg/m^2	16.9±1.4	17.8±1.8	15.9±0.7	15.9±1.2
Mean waist circumference in cm	56.7±8.8	60.8±6.7	62.0±3.5	55.6±9.3
Mean hip circumference in cm	59.8±10.1*	67.0±9.4*	66.7±8.1	61.6±12.3
Mean Skin-fold thicknesses				
Deltoid area	7.2±3.3*	9.8±4.5*	7.9±2.7	7.0±2.0
Sub-scapular area	6.7±2.8	7.2±1.8	7.5±2.3	6.1±1.4
Biceps area	4.8±1.4	4.6±1.2	6.2±2.0	5.3±1.8
Triceps area	7.1±1.9	8.0±3.0	7.7±1.5	7.3±1.8
Abdominal area	5.2±1.9	6.3±2.3	6.8±2.3	6.4±2.0
Anterior right thigh area	9.2±4.4	10.7±3.8	8.7±0.2	7.9±2.5
Right hip area	7.3±4.2	6.7±2.4	9.9±0.5	8.7±3.1
Right calf area	9.3±3.4	9.7±6.5	11.1±0.6	8.5±2.7
Estimated body fat, BF (%)	15.3±2.6*	19.1±3.3*	16.1±2.7	17.2±2.4

*p<0.05

n = number of subjects

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

4.16. Biophysical parameters in NS and control classified by homocysteine levels at upper quartile of distribution

The comparison of mean biophysical parameters between subjects who had tHcy ≥6.1 μmol/L and tHcy <6.1μmol/L in NS and control groups were as shown in Table 4.16. The Table shows that there were no differences in the mean age, weight, height, BMI, waist and hip circumferences as well as all the skinfold thicknesses of those whose tHcy levels were ≥6.1 μmol/L compared with those whose tHcy were <6.1 μmol/L. Similarly in the control group, all these parameters were not significantly different between those who had tHcy ≥6.1 μmol/L compared with those whose tHcy were <6.1 μmol/L except the skinfold thickness in the biceps area where the control group with tHcy ≥6.1 μmol/L had significantly higher skinfold thickness than those with tHcy <6.1 μmol/L.

4.17: Biochemical parameters in NS and control classified by homocysteine levels

Tables 4.17 and 4.18 are showing the biochemical parameters in NS and control classified by homocysteine levels. Within the NS group, there were no significant differences in the values of albumin, creatinine, total cholesterol, triglycerides, HDL, LDL, folate, vitamin B_6 and vitamin B_{12} in those whose tHcy levels were >10.0 μmol/L compared with those whose tHcy were ≤10.0 μmol/L. Conversely, in the control group those whose tHcy were >10.0 μmol/L had significantly higher mean plasma creatinine (0.8±0.2 μmol/L) than those with tHcy ≤10.0μmol/L (0.5±0.1 μmol/L); p = 0.013. Also among control, the subgroup with tHcy >10.0 μmol/L had significantly lower mean vitamin B_6 (52.8±0.9 nmol/l, versus 77.5±14.3 nmol/L; p = 0.007) and vitamin B_{12} (124.6±10.1 pg/mL versus 331±108.0 pg/mL; p = 0.001) compared with the subgroup who had tHcy ≤10.0 μmol/L (Table 4.17).

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Table 4.16: Biophysical parameters in NS and control classified by homocysteine levels at upper quartile of normal distribution

Biophysical Parameters	NS		Control	
	tHcy ≥6.1 n = 40	tHcy <6.1 n = 2	tHcy ≥6.1 n = 11	tHcy <6.1 n = 31
Mean age in months	105.6±31.2	94.6±43.3	85.8±30.7	107.0±26.7
Mean dry weight in kg	28.5±8.1	22.8±9.5	26.3±9.9	25.1±6.0
Mean height in cm	129.2±21.0	96.0±15.0	118.8±35.0	114.0±23.3
Mean Body Mass Index in kg/m^2	17.2±1.6	18.8±2.1	16.4±1.9	15.8±0.8
Mean waist circumference in cm	58.526±8.2	52.0±7.2	59.0±12.3	54.9±7.7
Mean hip circumference in cm	62.9±10..4	55.0±9.4	64.2±16.2	61.1±10.3
Mean Skin-fold thicknesses				
Deltoid area	8.2±4.0	10.0±3.4	7.3±2.0	7.0±2.1
Sub-scapular area	6.9±2.4	6.4±1.8	6.4±1.4	6.2±1.6
Biceps area	4.7±1.3	4.2±1.2	6.5±1.8*	5.0±1.6*
Triceps area	7.5±2.4	6.4±3.0	7.7±1.5	7.3±1.8
Abdominal area	5.6±2.2	5.9±1.3	7.2±2.2	6.1±1.9
Anterior right thigh area	9.75±4.2	11.0±3.8	7.7±2.3	8.0±2.5
Right hip area	7.1±3.6	6.2±3.4	9.0±2.2	8.8±3.3
Right calf area	9.6±4.8	4.3±1.0	9.0±3.8	8.6±2.2

*p<0.05

n = number of subjects

UNIVERSITY OF IBADAN LIBRARY

Table 4.17: Biochemical parameters in NS and control classified by homocysteine levels at cut off

Biochemical parameters	NS		Control	
	tHcy ≥10.0 n = 22	tHcy ≤10.0 n = 20	tHcy ≥10.0 n = 3	tHcy ≤10.0 n = 39
Albumin (mg/dL)	2.8±1.2	2.2±1.0	4.2±0.1	4.4±0.6
Creatinine (mg/dL)	0.7±0.5	0.5±0.2	0.8±0.2*	0.5±0.1*
Total Cholesterol (mg/dL)	237.5±108.7	233.8±74.6	149.3±34.1	155.4±73.0
Triglycerides (mg/dL)	150.7±111.1	168.3±121.1	155.5±66.0	108.3±54.4
HDL (mg/dL)	10.8±8.0	14.3±11.7	11.3±9.2	10.1±9.0
LDL (mg/dL)	196.6±99.8	185±80.7	106.9±30.1	123.6±72.4
Folate (ng/mL)	152.8±36.0	150.8±41.6	176.6±1.2*	173.4±28.0*
Vitamin B_6 (nmol/L)	74.0±13.7	70.2±12.2	52.8±0.9*	77.5±14.3*
Vitamin B_{12} (Pg/mL)	277.4±98.4	256.5±93.4	124.6±10.1*	331.1±108.0*

*p<0.05

n = number of subjects

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

4.18. Biochemical parameters in NS and control classified by homocysteine levels at upper quartile of normal distribution

Within the NS group (in Table 4.18), there were no significant differences in the values of albumin, creatinine, total cholesterol, triglyceride, HDL, LDL, folate, vitamin B_6 and vitamin B_{12} of those whose tHcy levels were ≥6.1μmol/L compared with those whose tHcy were <6.1μmol/L. However, within the control group, the subgroup with tHcy ≥6.1μmol/L had significantly lower mean vitamin B_6 (63.4±13.7 nmol/L) and vitamin B_{12} (234.0±127.9 pg/mL) compared with 80.2±13.3 nmol/L (p = 0.001) and 345.6±99.7 pg/mL (p = 0.004), respectively in the subgroup with tHcy ≥6.1μmol/L.

4.19. Levels of homocysteine and GFR

The distribution of subjects by level of homocysteine and reduction in GFR are as shown in Table 4.19. Among NS group, 84.6% of those who had moderate-severe reduction in GFR also had hyperhomocysteinaemia with significant increased risk compared with those whose GFR was ≥90 mL/min/1.73 m^2 (OR = 12.38, 95% CI = 1.83, 83.77). A higher proportion of the NS who had mild reduction in GFR (56.3%) compared with those whose GFR was ≥90 mL/min/1.73 m^2 (30.7%) had hyperhomocysteinaemia, though the difference was not significant (p = 0.318). Among the control group, 33.3% of those who had moderate-severe reduction in GFR had hyperhomocysteinaemia while only 9.1% of those with mild reduction in GFR had hyperhomocysteinaemia.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Table 4.18: Biochemical parameters in NS and control classified by homocysteine levels at upper quartile of normal distribution

Biochemical parameters	NS		Control	
	tHcy ≥6.1 n = 40	tHcy <6.1 n = 2	tHcy ≥6.1 n = 11	tHcy <6.1 n = 31
Albumin (g/dL)	2.5±1.1	3.5±1.3	4.6±0.3	4.4±0.6
Creatinine (mg/dL)	0.6±0.4	0.6±0.3	0.6±0.2	0.5±0.1
Total Cholesterol (mg/dL)	241.1±94.0	133.5±19.1	155.8±19.2	154.6±81.8
Triglyceride (mg/dL)	160.4±116.8	115.4±31.2	130.4±76.6	105.0±46.0
HDL (mg/dL)	12.4±10.0	12.0±5.7	10.0±8.8	10.2±9.1
LDL (mg/dL)	196.6±90.9	98.4±18.4	119.7±25.2	123.4±80.7
Folate (ng/mL)	150.3±37.7	184.5±40.3	167.7±28.9	175.7±26.4
Vitamin B_6 (nmol/L)	72.1±13.2	77.9±13.3	63.4±13.7*	80.2±13.3*
Vitamin B_{12} (pg/mL)	266.2±95.3	313.9±129.1	234.0±127.9*	345.6±99.7*

*p<0.05

n = number of subjects

UNIVERSITY OF IBADAN LIBRARY

Table 4.19: Distribution of subjects by level of homocysteine and reduction in GFR

GFR class	tHcy >10.0µmol/L n (%)	tHcy ≤10.0µmol/L n (%)	P	OR	95% CI
Nephrotic syndrome (NS)					
15 - 59mL/min/1.73m^2	11 (84.6)	2 (15.4)	0.015*	12.38	1.83, 83.77
60 - 89mL/min/1.73m^2	9 (56.3)	7 (43.7)	0.318*	3.89	0.62, 13.46
≥90mL/min/1.73m^2	4 (30.7)	9 (69.3)	1	-	-
Control					
15 - 59mL/min/1.73m^2	2 (33.3)	4 (66.7)	0.514*	5.00	0.35, 38.91
60 - 89mL/min/1.73m^2	1 (9.1)	10 (90.9)	1	-	-
≥90 mL/min/1.73m^2	0 (0.0)	25 (100.0)	0.032*	-	-

*Fisher's exact test used. 15 - 59 mL/min/1.73 m^2: Moderate – severe reduction in GFR; 60-89 mL/min/1.73 m^2: Mild reduction in GFR; ≥90 mL/min/1.73 m^2: normal

n = number of subjects

UNIVERSITY OF IBADAN LIBRARY

4.20. Change in homocysteine after vitamin and folate supplementation in nephrotic syndronic patients classified by baseline levels

The mean plasma homocysteine pre and post vitamin and folate supplementation in nephrotic syndrome patients classified by baseline levels are as shown in Table 4.20. Among NS with tHcy above 10.0μmol/L, pre-supplementation mean tHcy significantly reduced from 13.3±1.3μmol/L to 5.4±1.5μmol/L post-supplementation ($p<0.001$). Similarly among NS who had tHcy of ≤10.0μmol/L, there was a reduction in the mean tHcy from 8.3±1.6μmol/L pre-supplementation to 4.8±1.5μmol/L post-supplementation ($p<0.001$).

Moreover, grouping the NS into two groups (using pre-supplementation tHcy cut-off of ≥6.1μmol/L and tHcy <6.1μmol/L), while the tHcy ≥6.1μmol/L group had significant reduction in tHcy following vitamins supplementation, there was no significant difference in the pre and post supplementation tHcy in the tHcy <6.1μmol/L group.

Figure 4.2 shows the graphical plot of the magnitude of reduction in plasma tHcy (that is pre minus post supplementation) and the baseline (pre-supplementation) homocysteine. The magnitude of the reduction following vitamin supplementation appears to increase with increasing level of tHcy before supplementation.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Table 4.20: Plasma homocysteine pre and post vitmins supplementation in nephrotic syndrome patients classified by baseline levels

Baseline homocysteine	Pre-supplementation	Post-supplementation	Mean difference	P
Classified by cut-off				
tHcy >10.0µmol/L	13.3±1.3	5.4±1.5	7.9±2.0	<0.001
tHcy ≤10.0µmol/L	8.3±1.6	4.8±1.5	3.5±2.3	<0.001
Classified as below or above upper quartile				
tHcy ≥6.1 µmol/L	11.5±2.4	5.1±1.5	6.4±2.6	<0.001
tHcy <6.1 µmol/L	5.1±2.3	4.1±0.6	0.9±0.7	0.573

UNIVERSITY OF IBADAN LIBRARY

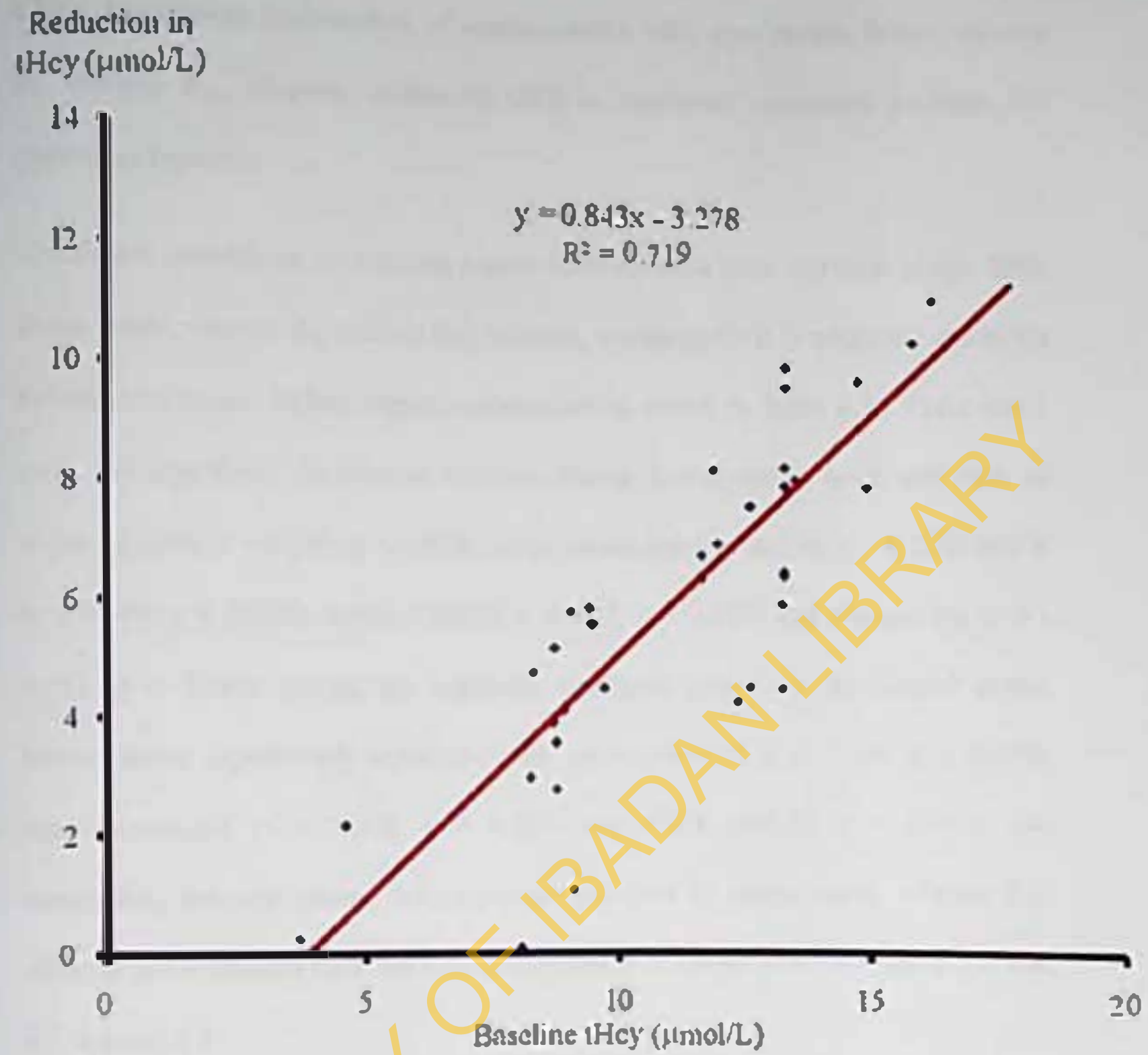


Figure 4.2: Graphical plot of the magnitude of reduction in plasma tHcy (that is pre- minus post- supplementation) and the baseline homocysteine

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

4.21. Coefficient Correlation of homocysteine with age, serum folate, vitamin B_6, vitamin B_{12}, albumin, estimated GFR in nephrotic syndrome patients and control at baseline

Coefficient correlations (r) between plasma homocysteine level and each of age, BMI, serum folate, vitamin B_6, vitamin B_{12}, albumin, estimated GFR in nephrotic syndrome patients and control before supplementation are as shown in Table 4.21. There was a weak but significant correlation between plasma homocysteine level and each of serum albumin ($r = 0.347$, $p = 0.024$), serum creatinine ($r = -0.336$, $p = 0.039$), eGFR ($r = 0.444$, $p = 0.020$), serum folate ($r = -0.607$, $p = 0.027$) and vitamin B_{12} ($r = -0.185$, $p = 0.045$) among the nephrotic syndrome group. In the control group, homocysteine significantly correlated with serum albumin ($r = 0.566$, $p = 0.029$), serum creatinine ($r = -0.330$, $p = 0.035$) and eGFR ($r=0.32$, $p = 0.037$). The correlations between plasma homocysteine and each of serum folate, vitamin B_{12}, albumin and estimated GFR are also demonstrated in scatter plots in Figures 4.3, 4.4, 4.5, 4.6 and 4.7.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Table 4.21: Correlation of homocysteine with age, serum folate, vitamin B_6, vitamin B_{12}, albumin, estimated GFR in nephrotic syndrome patients and control at baseline

Variables	Nephrotic syndrome patients		Control	
	r	p	R	p
Age in years	-0.189	0.237	-0.154	0.336
BMI	0.272	0.082	0.156	0.322
Serum albumin	0.347	**0.024**	**0.566**	**0.029**
Creatinine	**-0.336**	**0.039**	**-0.330**	**0.035**
Estimated GFR	0.444	**0.020**	0.323	**0.037**
Folate	-0.607	**0.027**	-0.111	0.416
Vitamin B_6	0.084	0.597	-0.292	0.061
Vitamin B_{12}	-0.185	**0.445**	-0.253	0.106
Apo A1	0.051	0.751	-0.087	0.586

Note: Spearman rho correlation was done because of the non-parametric nature of many of the variables

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

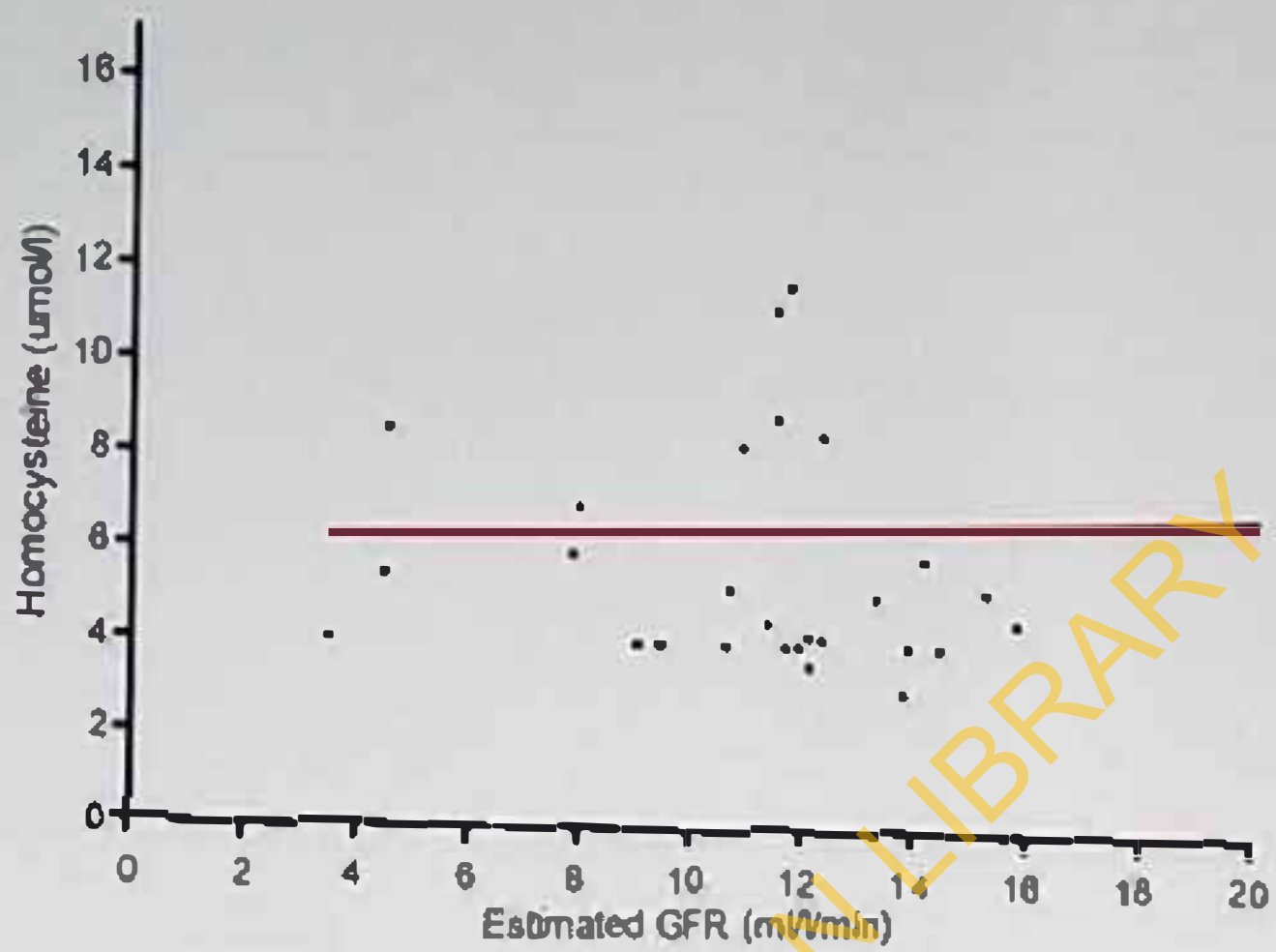


Figure 4.3: Scatter plot of plasma homocysteine and estimated GFR among NS patients

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

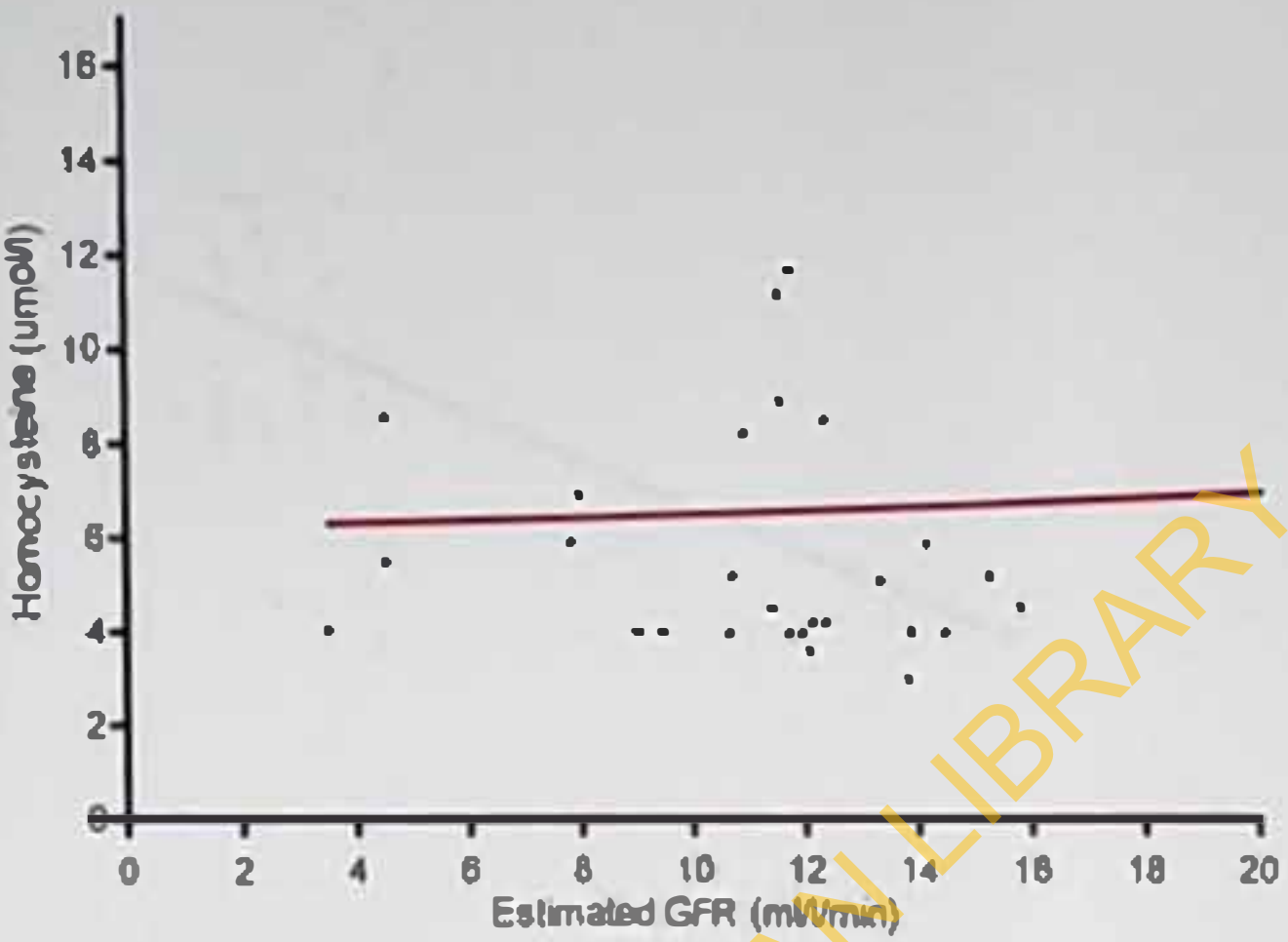


Figure 4.4: Scatter plot of plasma homocysteine and estimated GFR among control

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

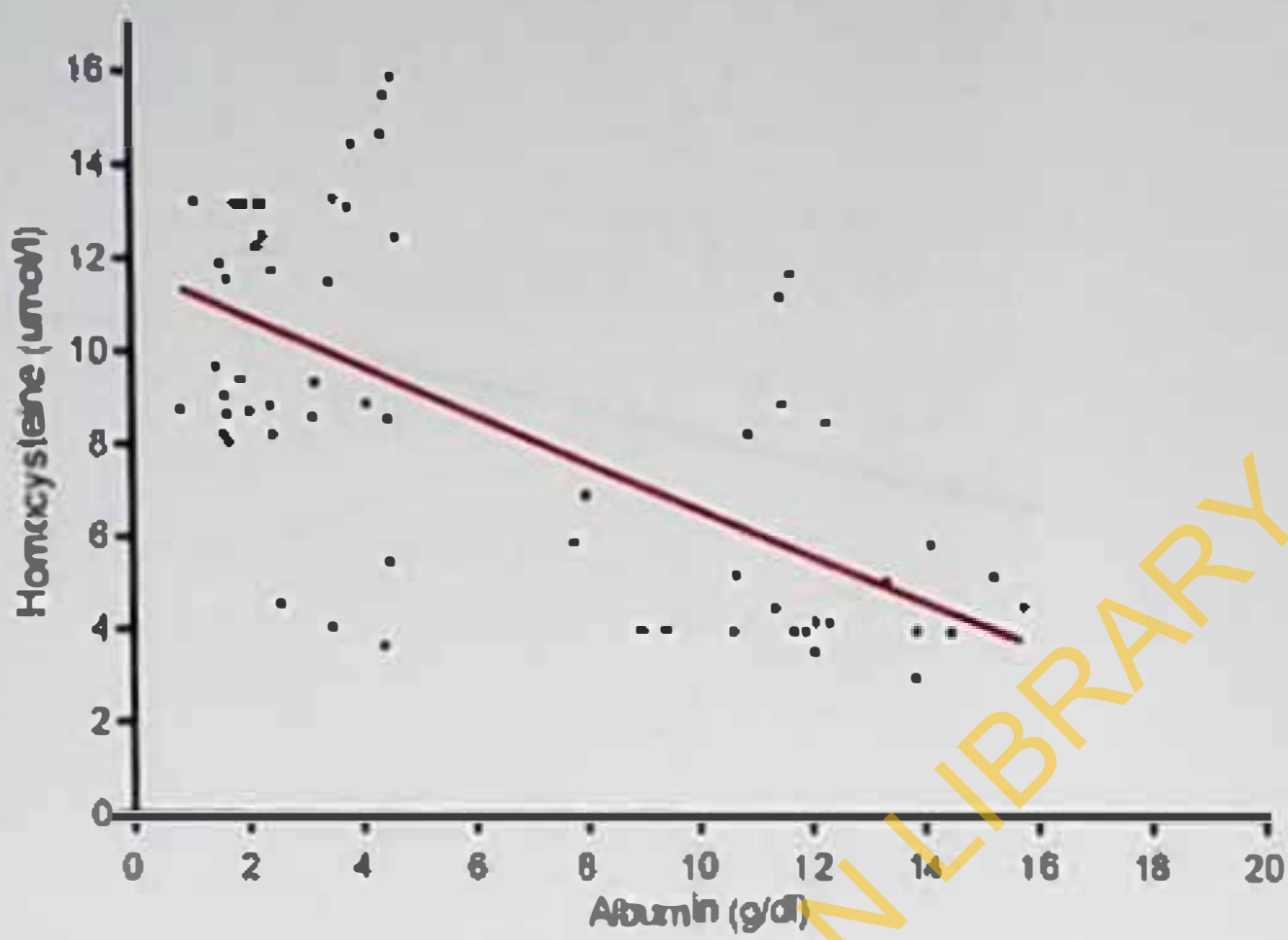


Figure 4.5: Scatter plot of plasma homocysteine and albumin among NS patients

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

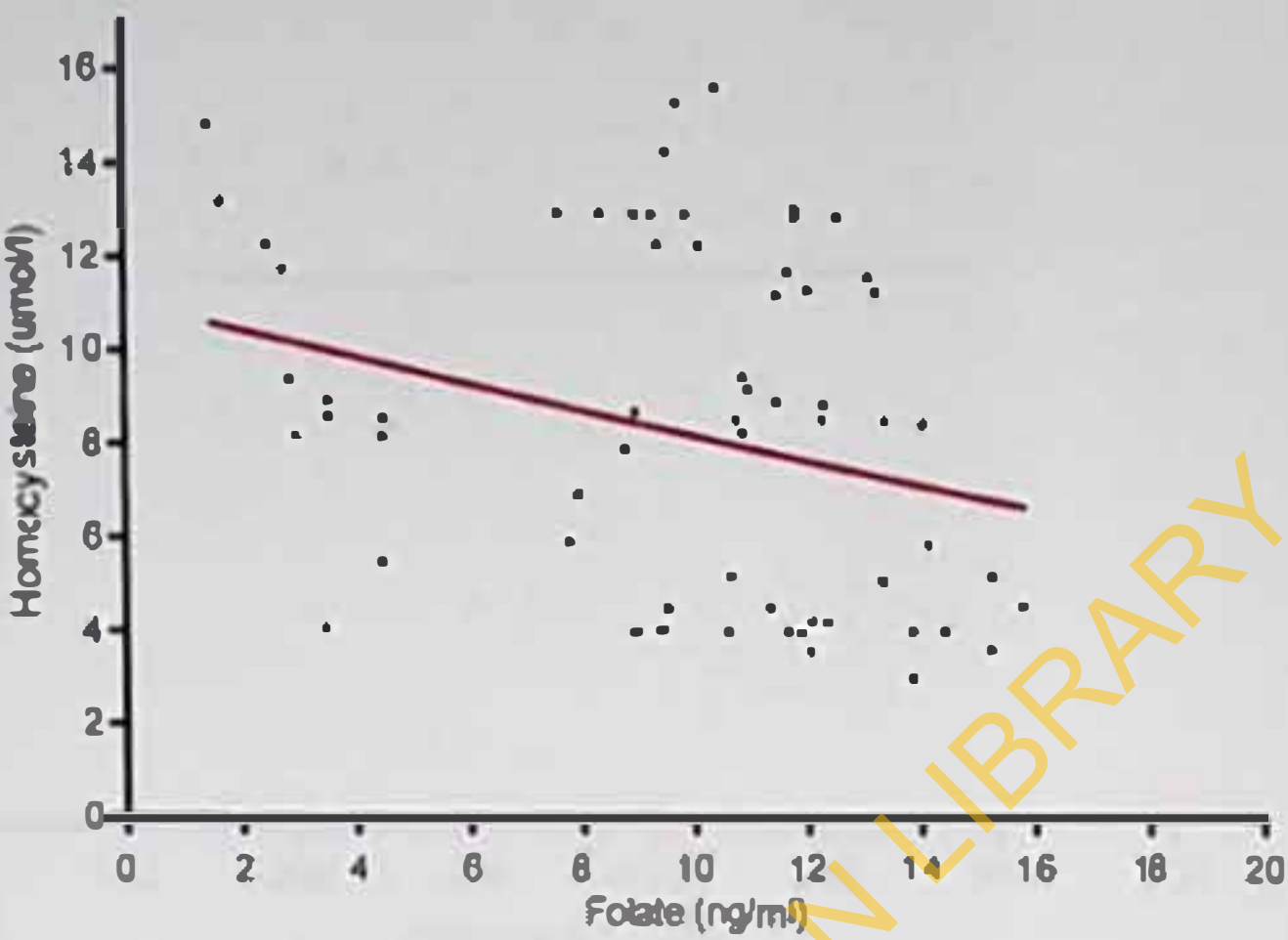


Figure 4.6: Scatter plot of plasma homocysteine and folate among NS patients

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

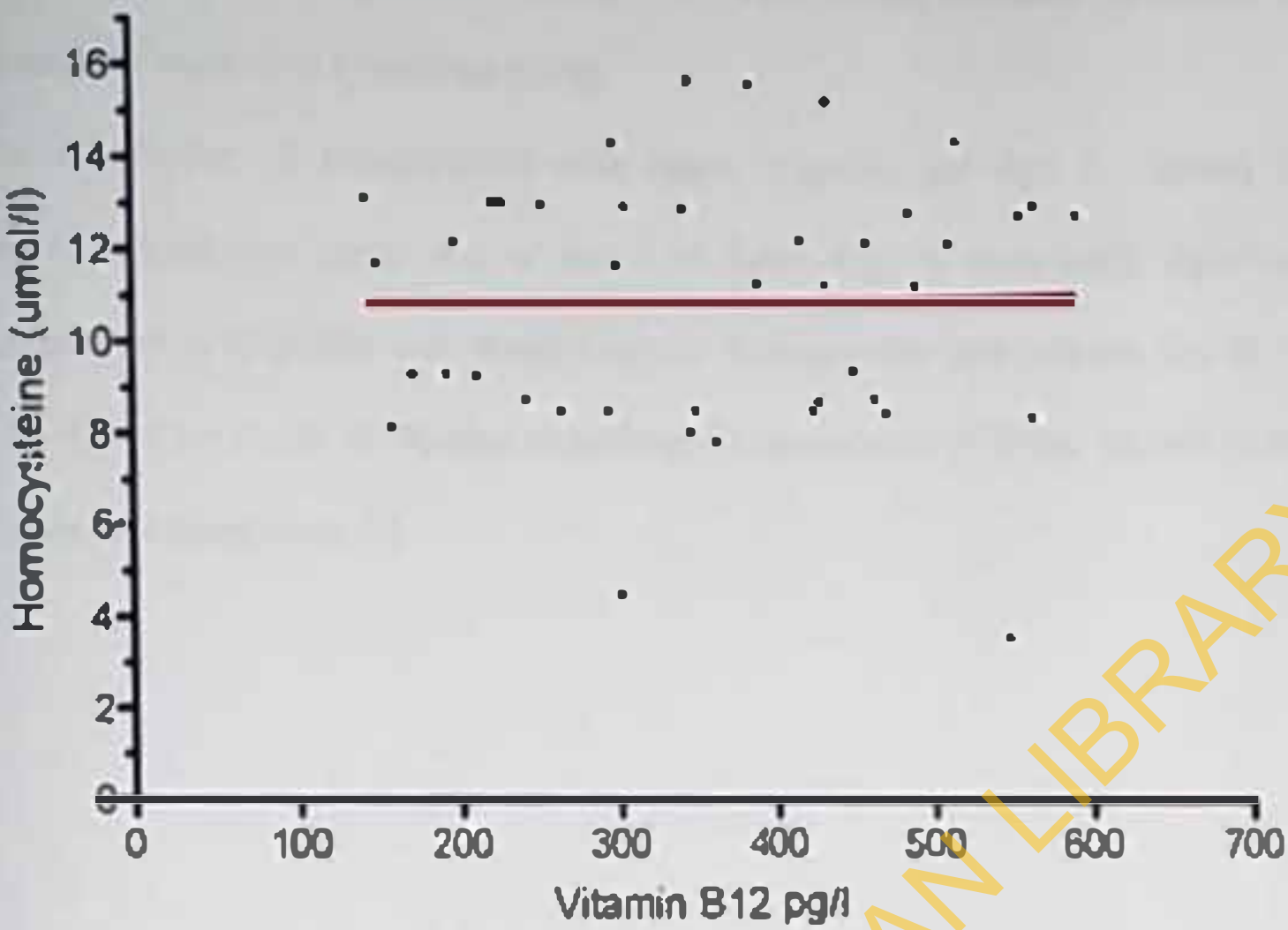


Figure 4.8: Scatter plot of plasma homocysteine and Vitamin B_{12} among NS patients

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

4.22: Relationship of plasma homocysteine with folate, vitamins and Apo A1 among the nephrotic syndrome group

The relationship of homocysteine with folate, vitamins and Apo A1 among the nephrotic syndrome group was as shown in Table 4.22. A statistically significant independent relationship was found between homocysteine and vitamin B_{12} (β = -0.64, 95% CI = -1.20, -0.08) after controlling for serum level of folate, vitamin B_6 and plasma Apolipoprotein A1.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Table 4.22: Multivariate analysis showing relationship of plasma homocysteine with folate, vitamins and Apo A1 among the nephrotic syndromic group

Variables	β	95% CI of β	p
Vitamin B_{12} (pg/mL)	-0.64	-1.20, -0.08	0.027
Vitamin B_6 (nmol/L)	0.84	-0.40, 2.09	0.180
Folate (ng/mL)	-0.17	-0.39, 0.05	0.127
Apo A1 (mg/dL)	0.00	0.00, 0.01	0.275
Constant	0.95	-0.25, 2.14	0.119

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

CHAPTER FIVE

DISCUSSION

5.1. Socio-demographic characteristics of study subjects

The results of this study showed that the children with nephrotic syndrome and the apparently healthy control were relatively similar in age, gender, socio-economic, family and cultural background. The similarities in age and gender characteristics of NS and control group could be attributed to the matching done during the selection of participants at the design stage of this study while the lack of differences in socio-economic, family and cultural background may imply the fact that the two groups were selected from the same population. Though the NS group was selected from the hospital, they are likely to have lived in the same or similar community as the control group. However, the mean and age range of the NS patients in this study is similar to those studied by *Asinobi et al* (1999) and *Anochie et al* (2006), at Ibadan and Port Harcourt in Nigeria respectively. The fact that the NS patients and control groups were comparable in age, gender and socio-economic background provide the basis for further comparisons with no significant concerns for the cofounding effects of these factors. The relatively higher number of male than female patients recruited in the study agrees with the reports from the same hospital and other tertiary health facilities in Nigeria. Ibadin and Abiodun (1998) in Benin and Asinobi *et. al.*, (1999) in Ibadan showed that nephrotic syndrome could be twice more common in boys than girls.

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

Homocysteine (tHcy) concentrations are known to increase with age among Nigerian children (Adebayo *et al.*, 2008), the plasma level approximately doubles the value of childhood by adulthood. However, the increasing trend in the level of homocysteine with age previously described was not apparent among the study participants because of the relatively narrow age range (mean age = 103.5±32.7months for NS and 100.9±29.4months for control). After puberty, males have higher mean tHcy concentrations than females (Must et al., 2003). There is also a known gender difference in tHcy with male having slightly higher level but it becomes less apparent with increasing age (Jacques et al., 1999, Must et al., 2003). Other factors considered as part of the baseline characteristics of participants in this study included the number of children in the family. The patients' mothers (nephrotic syndrome) had slightly higher number of children than control. This implied that those children with nephrotic syndrome are more likely to come from a relatively larger family size. Both patients and control majorly came from the middle socioeconomic class background. Data from this study showed no relationship between tHcy levels and the socio-economic status of the parents when NS patients and healthy children groups were considered separately. However, the level of tHcy was significantly higher in NS than control among those from low and middle socio-economic background but this difference was not observed among those from high socio-economic subgroup. In adults, few studies (Kim et al., 2003, Tovar et al., 2003) have examined the relationship between tHcy levels and the socioeconomic status with controversial results while limited data (Batty and Leon, 2002) exists for children.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

5.2. Diagnostic features and urinalysis findings at presentation

Expectedly, all the NS patients in this study had evidence of oedema at presentation, hypercholesterolemia, proteinuria, and hypoalbuminaemia which typically define nephrotic syndrome in children (Bergstain, 2000). While no one among the control had any of the clinical features of nephrotic syndrome, as many as 23.8% had traces of proteinuria and mild hypoalbuminaemia (2.0 to 2.4mg/dl) was found in 4.8%. These findings are not unusual as some physiological factors such as redistribution of fluid, posture, diet, exercise and drugs (especially steroid) could cause a slight loss of protein or reduction in serum albumin (Mayne, 1994). In this study, though there were no differences in the frequency of physical exercise and dietary pattern between NS patients and control in three-month period preceding the study, it could also be possible that information given by parents was not comprehensive enough to show any difference. Assessments of weight and height after patients were adjudged to be free from oedema showed that there was no difference in the mean dry weight but the control group were averagely taller (in height) than their NS patients counterparts. The relatively lower height of the NS patients compared with control may have been the consequence of the long standing nature of the disease. The average duration of illness among the NS patients was as long as four months. Children suffering from long standing illnesses such as renal diseases are known to have some degree of growth retardation (Mehis et al., 1989, Nissel et al., 2008).

5.3. Measured anthropometrics, estimated body fat and dietary pattern

This study revealed that the NS patients had averagely higher body mass index, body fat proportion and skinfold thickness at right hip areas than their control counterparts. These findings suggest that the NS patients have the tendency to have increased fat distribution in the hip areas more than healthy children from the same socio-economic

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

background. Studies have also shown that body fat distribution (measured by skinfold thickness) and/or body fat proportions are good indicators for atherosclerotic risk in adulthood (Bonora et al., 1992, Gutin et al., 1994). It is however, unclear from the present study how much of atherosclerotic risk could be attributed to the degree of adiposity and fat distribution among NS patients as no specific evidence of atherosclerosis such as measure of brachial artery flow dilatation or arterial wall thickness was measured as in previous study (Arnadottir *et al.*, 2001). Important factors that can explain the differences observed in the fat distribution and body fat proportion are the pattern of fat intake and physical exercise. However, the data showed no statistically significant differences in frequency of exercise and fat intakes between NS patients and control but the control group were more likely to eat two eggs and used butter as spread at a meal than their NS counterparts. The reason for this is likely to be the fact that many of the NS patients may have been counselled against excessive intake of cholesterol-rich foods and use of fat-rich spread in their meal by the attending clinicians.

5.4. Serum protein, creatinine and estimated glomerular filtration rate (eGFR)

This study showed that serum creatinine of NS patients and healthy children were not significantly different. This finding was corroborated by the similarity in the values of estimated GFR among NS patients and control. Serum creatinine and GFR being indices of renal function were expected to be affected in patients who have renal diseases such as nephrotic syndrome with increase in serum creatinine and decrease GFR. Creatinine is derived from muscle metabolism. Its production is relatively constant, and its excretion is primarily through glomerular filtration, although tubular secretion may become important in renal insufficiency. In contrast to

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

the concentration of blood urea nitrogen, this is affected by state of hydration and nitrogen balance, the serum creatinine level is primarily influenced by the level of glomerular function (Kon and Ichikawa, 2004). It is also known that the level of serum creatinine is of value only in estimating the GFR in the steady state. A patient may have a normal creatinine level without effective renal function very shortly after the onset of renal disease. In this clinical setting, serum creatinine may be an insensitive measure of decreased renal function because its level does not rise above normal until the GFR falls by 30-40%. This may explain why the serum creatinine levels of many of the NS patients in this study did not exceed 2.0mg/dl. Moreover, the precise measurement of the GFR could only be accomplished by determining the "clearance" of a substance that is freely filtered across the capillary wall and that is neither reabsorbed nor secreted by the tubules but this was not done in this study. As the GFR declines, an increasing proportion of the total creatinine in the urine is secreted by tubules, resulting in a creatinine clearance that progressively overestimates the actual GFR. Therefore, it is recommended that changes in renal function should be monitored by serum creatinine concentration when the serum creatinine level exceeds 2.0 mg/dL (180 μmol/L). None of the children who took part in this study had serum creatinine level above the recommended.

The fact that estimated glomerular filtration rate of the nephrotic syndrome patients and control were not significantly different, suggests that the patients' renal function were similar to normal (healthy) children. Though it was difficult to establish the exact onset of the illness, information obtained from the caregivers revealed that the longest duration of symptom was about four months. It is not unlikely that many of the patients may have been ill long before the parents or caregivers sought help in health facilities. Recent review of the burden of kidney diseases in Nigeria showed

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

that most patients present late to the hospital and they may attempt to deny it for reason of not wanting to be blamed (Okunola et al., 2012).

Low plasma albumin and/or low total protein are one of the cardinal findings in glomerular diseases because of the disruptions in the ultrafiltration system of the glomeruli in the kidneys. The low level of plasma albumin among the NS patients was therefore required as part of the diagnostic criteria for NS and exclusion criteria for the control group. This study also showed significant increase in GFR and reduction in the levels of creatinine and urea from the pre to post- vitamins supplementation values. Physiologically, these changes imply improvement in renal function among NS patients.

5.5. Plasma lipids and apolipoprotein A1

Previous studies (Abdurrahman et al., 1984, Erasmus et al., 1990, Ahaneku et al., 1999) have suggested that Nigerians with the nephrotic syndrome may represent a risk group for the development of ischaemic heart disease because of the various degrees of dyslipidaemia and dyslipoproteinaemia found among them. Similarly, in the present study, the level of total cholesterol, LDL-cholesterol, triglyceride and apolipoprotein A1 in NS patients were higher than their healthy counterparts. These findings were similar to previous reports from the same hospital by Agbedana *et al. (1990)*, which showed that plasma lipid concentrations within the childhood nephrotic syndrome group varies with elevation of both triglyceride and cholesterol. Unlike the report by Agbedana *et al. (1990)*, the mean levels of HDL-cholesterol and Total Cholesterol/HDL ratio were similar in both groups. The significant increase in apolipoprotein A1 concentration suggests the presence of an Apolipoprotein A-rich high density lipoprotein particle in children with nephrotic syndrome as speculated in earlier report in this community (Agbedana *et al.*, 1990). Abdurrahaman *et al.* (1984)

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

earlier reported similar elevation in both cholesterol and triglyceride concentration in childhood nephrotic syndrome but the changes in HDL-cholesterol and apolipoprotein were not investigated. Later, a similar but variable level of elevated total cholesterol and HDL-cholesterol was reported by Adigun *et al.* (1999) among adults with nephrotic syndrome in Ibadan. The causes of abnormal lipid levels in nephrotic syndrome patients are still largely unclear. Increase in synthesis of lipoprotein by the liver, in response to the massive protein loss, was suggested from liver perfusion experiments in rats with induced nephrosis (Marsh, 1960, Marsh and Drabkin, 1960). Previous studies have shown that the severity of risk of atherosclerosis is related to the HDL concentration and to the ratio of HDL-cholesterol to total cholesterol. Though it is difficult to make reasonable prediction of risk of developing coronary heart diseases in later life from the findings of the present study, a significant reduction in the levels of total cholesterol, triglycerides, LDL-cholesterol, Total Cholesterol/HDL ratio and apolipoprotein A1 were observed after three months of daily vitamin supplementation. It is likely that the reduction in the level of lipids and lipoprotein A1 observed in this study were direct effects of vitamins supplements given to NS patients and less likely to be a result of overall improvement in the disease condition. To the best of the researcher's knowledge, the only drug given to the patients who participated in the study apart from their regular prednisolone was the vitamin supplement. A counter argument may opt that the patients could have had reduction in the level of homocysteine as a result of the improvement in their renal function. It is worthy of note that none of these patients had significantly impared renal function at baseline. Moreover, Papandreou *et al* (2010), reported similar findings among healthy children in Greece. After the 2-month intervention with folate supplement, Hcy levels were significantly decreased; folate levels were significantly

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

increased, while total cholesterol levels were significantly improved from 183.8 to 160.8 mg/dL (Papandreou *et al.*, 2010). The authors concluded that folate supplementation reduces tHcy, serum folate, and total serum cholesterol levels in hyperhomocysteinaemic children as in the present study.

In other renal diseases, earlier studies on effects of vitamins supplementation on lipids and homocysteine, though among adult populations, gave controversial reports. McGregor et al (2000), treated 21 patients with end-stage renal disease (ESRD) with 5 mg/d folate for 3 months. The authors concluded that folate supplements given to patients with ESRD reduced serum plasma tHcy and may improve lipid profile (levels of total cholesterol, low-density lipoprotein, and triglycerides). Verhaar and colleagues (1999) examined the effects of folate supplements (5 mg/d) for 1 month on endothelium function and blood lipid profile. The researchers suggested that oral supplementation with folate may improve endothelium function and serum cholesterol levels. In contrast, Olthof et al (2005), reported that oral supplementation with folate (0.8 mg/d) for 2 weeks in healthy adults did not seem to affect blood lipids but reduced serum tHcy levels. The most important factors involved in the endothelial dysfunction in the nephrotic syndrome are LDL-cholesterol, total cholesterol and fibrinogen and their treatment is necessary to prevent atherosclerosis in patients with nephrotic syndrome (Caraba and Romosan, 2007).

In a previous study by Bayes et al (2003), the relation between homocysteine and lipid peroxidation was analyzed in haemodialysis patients and it was found that treatment with folic acid lowered plasma homocysteine levels and improved lipid peroxidation while decreasing ox-LDL antibody titre, although it did not reach the normal level. Despite the fact that previous studies were not able to show an improvement in endothelial function in uraemic patients treated with folic acid

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

(Thambyrajah et al., 2000). it has been demonstrated recently (Buccianti et al., 2002), that folic acid, in addition to reducing homocysteine concentration, improves endothelial function in haemodialysis patients.

All the NS patients in the present study were on steroid treatments (oral prednisolone) throughout the three-month period and none had remission. It is therefore difficult to attribute the improvement in dyslipidaemia wholly to the definitive treatments received during the study. It is worthy to note also that while there was significant reduction in other parameters there was no significant change in the level of HDL-cholesterol during supplementation.

5.6. Plasma homocysteine, folate, vitamins B_6 and B_{12}

This study showed that patients with the nephrotic syndrome had high plasma levels of tHcy, low serum levels of vitamin B_6 and low serum levels of vitamin B_{12} more frequently than healthy individuals. The two most obvious explanations for hyperhomocysteinemia in nephrotic syndrome patients would be a block in homocysteine remethylation and a disturbance in cysteine disposal. Support for the first one is based on trials showing that successful homocysteine-lowering regimens in chronic kidney disease always include folate. Randomized trials have shown that different forms of folate and different routes of administration are equally effective in lowering plasma homocysteine in ESRD patients (Abdelfatah et al., 2002, Ducloux et al., 2002). Suliman et al., (1999), proposed that a block in decarboxylation of cystinesulphinic acid the intermediate between cysteine and taurine, contributes to hyperhomocysteinemia in CRF.

Moreover, the relationship between tHcy and its metabolic cofactor vitamins has not been fully explored in patients with NS and controversial results have been reported

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

so far. Some studies showed that plasma tHcy levels were decreased (Arnadottir *et al.*, 2001), equal (Dogra *et al.*, 2001) and increased (Joven *et al.*, 2000) in NS patients compared to patients without NS and similar renal function (Arnadottir et al., 2001, Dogra et al., 2001) or to healthy controls (Joven *et al.*, 2000). There is sufficient evidence that plasma homocysteine is cleared from the body by urinary excretion after glomerular filtration, just like creatinine. However, the amount of homocysteine in the urine is minimal (about 6µmol/day) (Refsum et al., 1985). From a normal GFR of 180L/day and a free homocysteine concentration of 3µmol/L, it can be calculated that 99% of the filtered homocysteine is reabsorbed (van Guldener, 2006). The exact location of this uptake and the metabolic fate of homocysteine in the tubules are unknown. Homocysteine transulfuration and remethylation enzymes are present in human kidney tissue, indicating that metabolism of homocysteine within the kidney is possible. Studies in rats but not human have shown that homocysteine is taken up and metabolized by the kidney (House et al., 1998). Renal plasma flow does not largely affect GFR in humans (due to filtration disequilibrium), this is contrary to the general finding that plasma homocysteine is related to GFR. Also, a larger study failed to establish a relationship between homocysteine and effective plasma flow (Veldman et al., 2005). Thus an intrarenal homocysteine disposal has not been proven.

The results of this study also showed that children with nephrotic syndrome have had lower levels of serum folate, vitamin B_{12} and vitamin B_6 compared with their age and sex-matched healthy children. Low plasma levels of vitamin B_{12} might be due to urinary loss in nephrotic syndrome. The low level of vitamin B_6 in this study was also found in some other small sized studies of children with NS (van Buuren et al., 1987, Mydlik and Derzsiova, 2001). The loss of vitamin B_6 protein carriers in the urine

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

might influence vitamin B_6 status. Vitamin B_6 deficiency contributes to increase thrombotic risk in NS.

In the current study, a plasma tHcy level was inversely correlated with serum folate and vitamin B_{12} concentrations and this is consistent with the findings of other studies in children (Tonstad et al., 1996, Osganian et al., 1999, Bates et al., 2002). Reduced nutritional intakes in children may explain the decrease in serum level of this vitamin (Bjorke et al., 2003). There is a higher prevalence of folate deficiency and infectious diseases in Africans both of which impair folate assimilation (Rosenblatt and Whitehead, 1999). Nevertheless, the influence of vitamin B_{12} was less marked than the influence of folate. These findings indicate the need of folate to be adequate in children's diet to contain adequate folate. This could be achieved by increasing vegetables and fruit consumption, both good sources of folate, which will reduce tHcy levels and increase folate levels as reported in previous study (Stanger et al., 2003).

The findings from this study also showed no correlation between BMI and tHcy levels, but body fat proportion was on the average higher among NS patients with tHcy level >10.0μmol/L, than those who had tHcy ≤10.0 μmol/L. This difference was not found among the healthy children. These data are at variance with report by Gallisti et al (2000), who reported a significant correlation between BMI and tHcy concentrations. Also other workers confirmed a significant correlation between BMI, a measure of overweight and obesity and hyperhomocysteinaemia (Gallisti et al., 2000, Zuc et al., 2005). Moreover, in this study healthy children with tHcy above 10.0μmol/L had significantly lower values of serum folate, vitamins B_6 and B_{12} than the children with tHcy levels ≤10 mmol/L.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Folate deficiency occurs at all ages and is usually a result of poor diet in children. These findings suggest that these children should be in concern for possible medical problems regarding consequences of hyperhomocysteinaemia in later life. Adequate dietary folate maybe recommended for these individuals. In addition to poor dietary intake of folate itself, deficient intake of other B vitamins can contribute to folate deficiency. These vitamins include B1, B2, and B3 which are all involved in folate recycling.

In this study, in patients with NS and healthy children there were negative correlations between tHcy and creatinine clearance. The data also showed that plasma tHcy correlates with serum albumin as previously reported by Bostom *et al.* (2001). This is also suggested by the finding that the plasma levels of tHcy in the NS patients are independent of both creatinine clearance and serum albumin. The low serum levels of vitamin B_{12} and B_6 that was observed in the NS patients certainly contributed to the development of hyperhomocysteinaemia. It is possible that other features of the NS, for example the loss of vitamin B_6 protein carriers in the urine, might influence vitamin B_6 status.

5.7. Effects of vitamin supplementation

Some of the major findings of this study were the reduction of plasma cholesterol and homocysteine levels, both of which are known to be risk factors of cardiovascular disease (CVD) in adults and children. In addition, vitamins supplementation was significantly associated with rise in serum albumin, folate, vitamin B_{12} and vitamin-B_6. These findings agree with previous studies which showed that treatment with folic acid reduce plasma tHcy levels (Refsum et al., 2004, Araki et al., 2006, Anderson et al., 2010). Araki et al. (2006), earlier showed among 32 healthy male Japanese volunteers aged 20-29 years that folate supplementation as short as 2 weeks decreased

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

total homocysteine concentration significantly and increased the concentration of serum and red blood cell folate. The participants in this study had the benefit of receiving both folate and vitamins B complex supplementation for 3 months. Therefore, marked reduction in the levels of plasma homocysteine found in this study was not surprising. Apart from the ameliorative effects of folate on homocysteine, folate supplements have been found to improve vascular function in children with vascular diseases (Pena et al., 2004, Schroder et al., 2004). It has also been shown to improve endothelial dysfunction in children with vascular diseases (Pena et al., 2004, McKenzie et al., 2006). The use of 5mg folic acid in this study was purely based on the clinicians' experience. However, Anderson et al., (2010), had shown in a previous study that the decrease they was not dependent on the dose of folic acid given to patients.

It appears that folate is the most important supplement needed by children with nephrotic syndrome because of the consistent association between high levels of homocysteine and low serum folate in this study. The use of combination of folate and vitamin B_{12} supplements was beneficial. Dickes *et al.*, (1999), in another study reported that the close interrelation between vitamin B_{12} and folate metabolism justified why vitamin B_{12} supplementation effectively decreases both plasma they in patients with renal disease who had low B_{12} levels. Moreover, vitamin B_{12} supplements are useful in folate treated patients to prevent cobalamin deficiency and its neurological consequences (Billion et al., 2002). In addition vitamin B_{12} supplementation has been shown to abolish the increased risk of thrombotic event both arterial and venous in patients with NS (Podda et al., 2007).

Folate deficiency can lead to impaired cell division and alterations of protein synthesis. Folate is needed in the conversion of homocysteine to methionine (Figure

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

2.2). The elevated plasma homocysteine levels observed in this study is as a result of low serum concentration of folate (Selhub and Miller, 1992). Under normal metabolic circumstances 50% of homocysteine derived from methionine is remethylated to methionine. The remethylation of homocysteine requires two key enzymes: methionine synthase (MS) (E.C.2.1.1.13) and methylenetetrahydrofolate reductase (MTHFR). It uses vitamin B_{12} as a cofactor and 5-methyltetrahydrofolate as a methyl donor. The MTHFR is needed in the formation of 5-methyltetrahydrofolate. When there is an excess of protein or methionine, homocysteine is metabolised by irreversible transulfuration pathway, which degrade homocysteine to cysteine. In the transulfuration, homocysteine is first sulf-conjugated to cystathionine by cystathionine beta-synthase (CβS) (E.C.4.2.1.22). Cystathionine is further cleaved into cysteine and alpha-ketobutyrate by cystathionine γ-lyase. Both enzymes need vitamin B_6 as a cofactor. Cysteine may be utilised in the protein synthesis or as a precursor of the antioxidant glutathione. When homocysteine accumulates and cannot be metabolised due to low concentrations of folate and vitamin B_{12} or vitamin B_6, it is excreted from the cells leading to elevated concentrations of homocysteine in the plasma or in the urine (Ueland et al., 1993).

Overall, findings from the present study are of public health importance, considering the fact that cardiovascular diseases are gradually becoming a common cause of death in adults Nigerians. With increasing survivors among children suffering from nephrotic syndrome, it is not unlikely that the risk of cardiovascular diseases may be heightened by the prevailing hyperhomocysteinaemia if the situation is not addressed early.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

CHAPTER SIX

SUMMARY AND CONCLUSIONS

6.1. Summary of findings

In this study the prevalence of hyperhomocysteinaemia (plasma homocysteine concentration >10μmol/L) among children with nephrotic syndrome was determined, concentration of plasma homocysteine in children suffering from nephrotic syndrome was compared with those who were apparently healthy and effects of giving oral 5mg folic acid and a tablet of vitamin B complex daily to children with nephrotic syndrome on plasma homocysteine. lipids as well as Apo A1. The results this research showed that:

1. The prevalence of hyperhomocysteinaemia was 57.1% and 7.1% in NS patients and healthy children respectively.
2. The prevalence of low serum folate (<3.4ng/ml.) among NS patients was 14.3% while low serum vitamin B_{12} (<133pg/mL) was found in 9.5% of both NS patients and healthy children. None of the study participants had low serum vitamin B_6 (<20.0nmol/L).
3. The average level of plasma homocysteine in NS patient was higher than healthy children.
4. The average reduction in total plasma homocysteine was 6.1 μmol/L after daily vitamins supplementation for 3 months.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

5. NS Patients with high level of homocysteine (tHcy > 10.0) had relatively high values of hip circumference, skinfold thickness in the deltoid area and body fat proportion.

6. Plasma homocysteine had positive but weak correlations with albumin and GFR. However, the correlations of between homocysteine and vitamin B_{12} and folate inverse and relatively weak as well.

6.2. Conclusions

From the above findings, the following conclusions may be drawn:

1. Many Nigerian children with nephrotic syndrome have hyperhomocysteinaemia, a known independent risk factor for cardiovascular diseases and the severity increases with decreasing glomerular filtration rate.

2. The elevated plasma homocysteine among children with nephrotic syndrome is associated with low serum folate, cyanocobalamin and pyridoxine.

3. tHcy if established during early life, may be modulated by diet and lifestyle, thereby providing opportunity for public health intervention.

4. Nephrotic syndrome patient may benefit from folate and vitamin B_{12} fortification of foods to reduce tHcy, possibly in the prevention of future atherogenic disease.

6.3. Further studies

1. Long term prospective studies in NS patients are desirable to define the effect of vitamin supplementation on lipids and lipoprotein abnormalities.
2. Information from this study provides useful data towards various stages of planning clinical trials to test the efficacy and effectiveness of vitamin B

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

supplementation in children with nephrotic syndrome. In such instances, it has provided the basis for ensuring that the research question is clearly formulated and clinically relevant to the target population.

6.4. Limitntions of study findings

1. Sixteen percent of eligible patients were unable to complete the three month follow up period and data on effect of vitamin supplementation were not collected.
2. Plasma tHcy levels are also influenced by genetic variables. This study is limited by the absence of measuring the MTHFR polymorphism.
3. There was no evidence from the data to show that changes in level of tHcy over the three-month period among NS patients would not have happened irrespective of vitamin supplementation. For example, NS patients may, on the average, improve or deteriorate between the first day they were recruited and the day a repeat blood sample for reassessment of tHcy. However, within the limit of information collected and reported by study participants, the only measurable change during this interval was the vitamins supplementation. The pre- and post-nature of the study design, in which each patient served as control for his/herself strengthen the claim that change in tHcy could not have been as a result of unmeasured baseline characteristics such as concomitant medications, lifestyle, diet and genetic factor (MTHFR 677C3T homozygosity).

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT
UNIVERSITY OF IBADAN LIBRARY

REFERENCES

Abdelfatah A., Ducloux D., Toubin G., Motte G., Alber D. and Chalopin J. M. 2002. Treatment of hyperhomocysteinemia with folic acid reduces oxidative stress in renal transplant recipients. *Transplantation* 73.4:663-665.

Abdurrahman M. B., Suvamabai P. C., Aikhionbare H. A. and Timoszyk J. 1984. Serum cholesterol and triglycerides in childhood nephrotic syndrome in northern Nigeria. A preliminary report. *Tropical Geographical Medicine* 36.1:7-11.

Adebayo K. J., Madu E. F. and Adebayo-Kay V. C. 2008. Serum total homocysteine concentrations in children and adolescents in Jos, Nigeria. *Journal of Tropical Pediatrics* 54.4:282-283.

Adigun M. O., Agbedana E. O., Kadiri S. and Taylor G. O. 1999. Increased high density lipoprotein cholesterol in adult nephrotic syndrome in Nigeria. *African Journal of Medicine and Medical Sciences* 28.1-2:97-100.

Agbedana E. O., Taylor G. O. and Folami A. O. 1990. Apolipoprotein A and high density lipoprotein cholesterol in childhood nephrotic syndrome. *East African Medical Journal* 67.4:279-285.

Ahaneku J. E., Nwosu M. C., Ahaneku G. I. and Okugba P. C. 1999. Utilisation of clinical chemistry tests, with special reference to lipid profile, in disease management in a Nigeria setting. *East African Medical Journal* 76.3:172-175.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Allain C. C., Poon L. S., Chan C. S., Richmond W. and Fu P. C. 1974. Enzymatic determination of total serum cholesterol. *Clinical Chemistry* 20.4:470–475.

Allen R. H., Stabler S. P., Savage D. G. and Lindenbaum J. 1990. Diagnosis of cobalamin deficiency I: usefulness of serum methylmalonic acid and total homocysteine concentrations. *American Journal of Hematology* 34.2:90-98.

Aminzadeh M. A., Gollapudi P. and Vaziri N. D. 2010. Effect of nephrotic syndrome on homocysteine metabolism. *Nephrology Dialysis Transplantation 17.3:121-124.*

Anderson C. A., Jee S. H., Charleston J., Norrett M. and Appel L. J. 2010. Effects of folic acid supplementation on serum folate and plasma homocysteine concentrations in older adults: a dose-response trial. *American Journal of Epidemiology* 172.8:932-941.

Anochie I., Eke F. and Okpere A. 2006. Childhood nephrotic syndrome: change in pattern and response to steroids. *Journal of the National Medical Association* 98.12:1977-1981.

Araki R., Maruyama C., Igarashi S., Yoshida M., Maruyama T., Satoh T. and Umegaki K. 2006. Effects of short-term folic acid and/or riboflavin supplementation on serum folate and plasma total homocysteine concentrations in young Japanese male subjects. *European Journal of Clinical Nutrition* 60.5:573-579.

Arnadottir M., Brattstrom L., Simonsen O., Thysell H., Hultberg D., Andersson A. and Nilsson-Ehle P. 1993. The effect of high-dose pyridoxine and folic acid

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

supplementation on serum lipid and plasma homocysteine concentrations in dialysis patients. *Clinical Nephrology* 40.4:236-240.

_____, Hultberg B. and Berg A. L. 2001. Plasma total homocysteine concentration in nephrotic patients with idiopathic membranous nephropathy. *Nephrology Dialysis Transplantation* 16.1:45-47.

_____, _____, Nilsson-Ehle P. and Thysell H. 1996. The effect of reduced glomerular filtration rate on plasma total homocysteine concentration. *Scandinavian Journal of Clinical & Laboratory Investigation* 56.1:41-46.

Arruda V. R., von Zuben P. M., Chiaparini L. C., Annichino-Bizzacchi J. M. and Costa F. F. F. 1997. The mutation Ala677→Val in the methylene tetrahydrofolate reductase gene: a risk factor for arterial disease and venous thrombosis. *Journal of Thrombosis and Haemostasis* 77.5:818-821.

Asinobi A. O., Gbadegesin R. A., Adeyemo A. A., Akang E. E., Arowolo F. A., Abiola O. A. and Osinusi K. 1999. The predominance of membranoproliferative glomerulonephritis in childhood nephrotic syndrome in Ibadan, Nigeria. *West African Journal of Medicine* 18.3:203-206.

Austin R. C., Lentz S. R. and Werstuck G. H. 2004. Role of hyperhomocysteinemia in endothelial dysfunction and atherothrombotic disease. *Cell Death & Differentiation* 11 Suppl 1:S56-64.

Bailey L. B. and Gregory J. F., 3rd 1999. Polymorphisms of methylenetetrahydrofolate reductase and other enzymes: metabolic significance, risks and impact on folate requirement. *Journal of Nutrition* 129.5:919-922.

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

Bao X. M., Wu C. F. and Lu G. P. 2010. Atorvastatin inhibits homocysteine-induced oxidntive stress and apoptosis in endothelial progenitor cells involving Nox4 and p38MAPK. *Atherosclerosis* 210.1:114-121.

Bates C. J., Mnnsoor M. A. and J G. 2002. Correlates of plasma homocysteine, cysteine and cysteinyl-glycine in respondents in the British National Diet and Nutrition Survey of young people aged 4–18 years, and a comparison with the survey of people aged 65 years and over. *British Journal of Nutrition* 87:71-79

Batty G. D. and Leon D. A. 2002. Socio-economic position and coronary heart disease risk factors in children and young people. Evidence from UK epidemiological studies. *European Journal of Public Health* 12:263-272.

Bayes B., Pastor M. C., Bonal J., Junca J., Hernandez J. M., Riutort N., Forasier A. and Romero R. 2003. Homocysteine, C-reactive protein, lipid peroxidation and mortality in haemodialysis patients. *Nephrology Dialysis Transplantation* 18.1:106-112.

Bellowski J., Jamroz-Wisniewska A. and Tokarzewska D. 2010. Hydrogen sulfide and its modulation in arterial hypertension and atherosclerosis. *Cardiovascular and Hematological Agents in Medicinal Chemistry* 8.4:173-186.

Bergstain J. M. 2000. Nephrotic syndrome. In Behrman, R. E., Kliegmnn, R. M. and Jenson, H. B. Eds. *Nelson textbook of pediatrics* 16th ed. Philadephia, W. B. Saunders Company pp 1592-1596.

Billion S., Tribout B., Cadet E., Queinnec C., Rochette J., Wheatley P. and Bataille P. 2002. Hyperhomocysteinaemia, folate and vitamin B12 in unsupplemented

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

haemodialysis patients: effect of oral therapy with folic acid and vitamin B12. *Nephrology Dialysis Transplantation* 17.3:455-461.

Bjorke M. A. L., Refsum H., Markestad T. and Ueland P. M. 2003. Cobalamin status and its biochemical markers methylmalonic acid and homocysteine in different age groups from 4 days to 19years. *Clinical Chemistry* 49:2067 2075.

Bonora E., Zenere M., Branzi P., Bagnani M., Maggiulli L., Tosi F., Travia D., Cacciatori V., Qucrena M., Moghetti P., Micciolo R., Ferrari A., and Muggeo M. 1992. Influence of body fat and its regional localization on risk factors for atherosclerosis in young men. *American Journal of Epidemiology* 135.11:1271-1278.

Bostom A. G., Kronenberg F., Jacques P. F., Kuen E., Ritz E. and Konig P. 2001. Proteinuria and plasma total homocysteine levels in chronic renal disease patients with a normal range serum creatinine: critical impact of true glomerular filtration rate. *Atherosclerosis* 159:219-223.

_____ and Lathrop L. 1997. Hyperhomocysteinemia in end-stage renal disease: prevalence, etiology, and potential relationship to arteriosclerotic outcomes. *Kidney International* 52.1:10-20.

_____, Rosenberg I. H., Silbershatz H., Jacques P. F., Selhub J., D'Agostino R. B., Wilson P. W. and Wolf P. A.1999. Nonfasting plasma total homocysteine levels and stroke incidence in elderly persons: the Framingham Study. *Annals of Internal Medicine* 131.5:352-355.

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

Boushey C. J., Beresford S. A., Omenn G. S. and Motulsky A. G. 1995. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. *Journal of the American Medical Association* 274.13:1049-1057.

Brattstrom L., Wilcken D. E., Ohrvik J. and Brudin L. 1998. Common methylenetetrahydrofolate reductase gene mutation leads to hyperhomocysteinemia but not to vascular disease: the result of a meta-analysis. *Circulation* 98.23:2520-2526.

Bremner W. F., Holmes E. W., Kanabrocki E. L., Hermida R. C., Ayala D., Garbincius J., Third J. L., Ryan M. D., Johnson M., Foley S., Shirazi P., Nemchausky B. A. and Scheving L. E. 2000. Circadian rhythm of serum total homocysteine in men. *American Journal of Cardiology* 86.10:1153-1156, A1159-1110.

Buccianti G., Rosetti S. and Baragetti I. 2002. 5Methyltetrahydrofolate restores endothelial function in uraemic patients on convective haemodialysis. *Nephrology Dialysis and Transplantation* 17:857-864.

Cafolla A., Dragoni F., Girelli G., Tosti M. E., Costante A., De Luca A. M., Funaro D. and Scott C. S. 2002. Effect of folic acid and vitamin C supplementation on folate status and homocysteine level: a randomised controlled trial in Italian smoker-blood donors. *Atherosclerosis* 163.1:105-111.

Caraba A. and Romosan I. 2007. Endothelial dysfunction in the nephrotic syndrome. *Medicinski Pregled* 60 Suppl 2:66-69.

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

Carey M., Fennelly J. J. and FitzGerald O. 1966. Folate metabolism in homocystinuria. *Ireland Journal of Medical Science* 6.491:488-492.

_____, _____ and _____. 1968. Homocystinuria. II. Subnormal serum folate levels, increased folate clearance and effects of folic acid therapy. *American Journal of Medicine* 45.1:26-31.

Caridi G., Bertelli R., Carrea A., Di Duca M., Catarsi P., Artero M., Carraro M., Zennaro C., Candiano G., Musante L., Seri M., Ginevri F., Perfumo F. and Ghiggeri G. M. 2001. Prevalence, genetics, and clinical features of patients carrying podocin mutations in steroid-resistant nonfamilial focal segmental glomerulosclerosis. *Journal of the American Society of Nephrology* 12.12:2742-2746.

Carmel R., Green R., Jacobsen D. W., Rasmussen K., Florea M. and Azen C. 1999. Serum cobalamin, homocysteine, and methylmalonic acid concentrations in a multiethnic elderly population: ethnic and sex differences in cobalamin and metabolite abnormalities. *American Journal of Clinical Nutrition* 70.5:904-910.

Carson N. A. and Neill D. W. 1962. Metabolic abnormalities detected in a survey of mentally backward individuals in Northern Ireland. *Archive of Diseases in Childhood* 37:505-513.

Casanueva V., Cid X., Cancino M., Borzone L. and Cid L. 2003. [Serum homocysteine in children and adolescents. Relation with family history of cardiovascular disease]. *Revista médica de Chile* 131.9:997-1002.

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

Cattaneo M. 1999. Hyperhomocysteinemia, atherosclerosis and thrombosis. *Journal of Thrombosis and Haemostasis* 81.2:165-176.

_____. 2000. Hyperhomocysteinaemia and atherothrombosis. *Annals of Medicine* 32 Suppl 1:46-52.

Chamberlain K. L. 2005. Homocysteine and cardiovascular disease: a review of current recommendations for screening and treatment. *Journal of the American Academy of Nurse Practitioners* 17.3:90-95.

Chauveau P., Chadefaux B., Coude M., Aupetit J., Hannedouche T., Kamoun P. and Jungers P. 1992. Increased plasma homocysteine concentration in patients with chronic renal failure. *Mineral and Electrolyte Metabolism* 18.2-5:196-198.

_____, _____, _____, Kamoun P. and Jungers P. 1996. Long-term folic acid but not pyridoxine supplementation lowers elevated plasma homocysteine level in chronic renal failure. *Mineral and Electrolyte Metabolism* 22.1-3:106-109.

Christensen B., Refsum H., Vinterstyr O. and Ueland P. M. 1991. Homocysteine export from cells cultured in the presence of physiological or superfluous levels of methionine: Methionine loading of nontransformed, transformed, proliferating, and quiescent cells in culture. *Journal of Cell Physiology* 146:52-56.

_____ and Ueland P. M. 1993. Methionine synthase inactivation by nitrous oxide during methionine loading of normal human fibroblasts. Homocysteine remethylation as determinant of enzyme inactivation and homocysteine

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

export. *Journal of Pharmacology and Experimental Therapeutics* 267.3:1298-1303.

Ciaccio M. and Bellia C. 2010. Hyperhomocysteinemia and cardiovascular risk: effect of vitamin supplementation in risk reduction. . *Current Clinical Pharmacology* 5:30-36.

Clarke R., Daly L., Robinson K., Naughten E., Cahalane S., Fowler B. and Graham I. 1991. Hyperhomocysteinemia: an independent risk factor for vascular disease. *New England Journal of Medicine* 324:1149 - 1155.

Cockcroft D. W. and Gault M. H. 1976. Prediction of creatinine clearance from serum creatinine. *Nephron* 16.1:31-41.

Dastani Z., Dangoisse C., Boucher B., Desbiens K., Krimbou L., Dufour R., Hegele R. A., Pajukanta P., Engert J. C., Genest J. and Marcil M. 2006. A novel nonsense apolipoprotein A-I mutation apoA-IE136X causes low HDL cholesterol in French Canadians. *Atherosclerosis* 185.1:127-136.

den Heijer M., Koster T., Blom H. J., Bos G. M., Bricl E., Reitsma P. H., Vandenbroucke J. P. and Rosendaal F. R. 1996. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. *New England Journal of Medicine* 334.12:759-762.

Deschenes G. 2004. [Why doesn't glomerular filtration stop?]. *Archives of Pediatrics* 11.7:858-859.

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

Deurenberg P., Weststrate J. A. and Seidell J. C. 1991. Body mass index as a measure of body fatness: age- and sex-specific prediction formulas. *British Journal of Nutrition* 65:105-114.

Dierkes J., Domrose U., Ambrosch A., Schnede J., Guttormsen A. B., Neumann K. H. and Luley C. 1999. Supplementation with vitamin B12 decreases homocysteine and methylmalonic acid but also serum folate in patients with end-stage renal disease. *Metabolism* 48.5:631-635.

Dogra G., Irish A. B. and Watts G. F. 2001. Homocysteine and nephrotic syndrome. *Nephrology Dialysis Transplantation* 16.8:1720-1721.

Doucet C., Mooser V., Gonbert S., Raymond F., Chapman J., Jacobs C. and Thillet J. 2000. Lipoproteins in the nephrotic syndrome: molecular analysis of lipoproteins and apolipoproteins fragments in plasma and urine. *Journal of the American Society of Nephrology* 11.3:507-513.

Ducloux D., Aboubakr A., Motte G., Toubin G., Fournier V., Chalopin J. M., Drucke T. and Massy Z. A. 2002. Hyperhomocysteinaemia therapy in haemodialysis patients: folinic versus folic acid in combination with vitamin B6 and B12. *Nephrology Dialysis Transplantation* 17.5:865-870.

Dwivedi J. and Sarkar P. D. 2010. Lipoprotein a, homocysteine, lipid profile with oxidative stress in nephrotic syndrome and cardiovascular nephropathy. *International Journal of Pharmacy and Biological Sciences* 1.4:B340-350.

Eke F. U. and Eke N. N. 1994. Renal disorders in children: a Nigerian study. *Pediatric Nephrology* 8.3:383-386.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

El-Melegy N. T., Mohamed N. A. and Sayed M. M. 2008. Oxidative modification of low-density lipoprotein in relation to dyslipidemia and oxidant status in children with steroid sensitive nephrotic syndrome. *Pediatric Research* 63.4:404-409.

Elia M. 1998. Oral or parenteral therapy for B12 deficiency. *Lancet* 352.9142:1721-1722.

Erasmus R. T., Arije A. and Uyot C. 1990. Dyslipoproteinaemia in Nigerians with the nephrotic syndrome an increased risk for ischaemic heart disease?. *Central Africa Journal of Medicine* 36.8:195-198.

Farrell R. J., Collins R., Goggins M., Forkin C., Gaffney E., Keogh J. A. and Weir D. G. 1995. Nephrotic syndrome, renal vein thrombosis, and folate deficiency in a young man: is there a relationship to homocysteine metabolism? *Nephrology Dialysis Transplantation* 10.11:2130-2132.

Faure-Delanef L., Quere I., Chasse J. F., Guerassimenko O., Lesaulnier M., Bellet H., Zittoun J., Kamoun P. and Cohen D. 1997. Methylenetetrahydrofolate reductase thermolabile variant and human longevity. *American Journal of Human Genetics* 60.4:999-1001.

Ferechide D. and Radulescu D. 2009. Hyperhomocysteinemia in renal diseases. *Journal of Medical Life* 2:53-59.

Finkelstein J. D. 1990. Methionine metabolism in mammals. *Journal of Nutritional Biochemistry* 1:228–237.

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

_____ and Martin J. J. 1984a. Inactivation of betaine-homocysteine methyltransferase by adenosylmethionine and adenosylethionine. *Biochemical and Biophysical Research Communications* 118.1:14-19.

_____ and _____. 1984b. Methionine metabolism in mammals. Distribution of homocysteine between competing pathways. *Journal of Biological Chemistry* 259.15:9508-9513.

Folsom A. R., Nieto F. J., McGovern P. G., Tsai M. Y., Malinow M. R., Eckfeldt J. H., Hess D. L. and Davis C. E. 1998. Prospective study of coronary heart disease incidence in relation to fasting total homocysteine, related genetic polymorphisms, and B vitamins: the Atherosclerosis Risk in Communities ARIC study. *Circulation* 98.3:204-210.

Franceschini G., Sirtori M., Gianfranceschi G. and Sirtori C. R. 1981. Relation between the HDL apoproteins and AI isoproteins in subjects with the A1Milano abnormality. *Metabolism: clinical and experimental* 30.5:502-509.

Frantzen F., Faaren A. L., Alfheim I. and Nordhei A. K. 1998. Enzyme conversion immunoassay for determining total homocysteine in plasma or serum. *Clinical Chemistry* 44.2:311-316.

Friedewald W. T., Levy R. I. and Fredrickson D. S. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry* 18.6:499502.

Friedman A. N., Hunsicker L. G., Selhub J. and Bostom A. G. 2002. Proteinuria as a predictor of total plasma homocysteine levels in type 2 diabetic nephropathy. *Diabetes Care* 25.11:2037-2041.

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

Frosst P., Blom H.J., Milos R., Goyette P., Sheppard C.A., Matthews R.G., Boers G.J., den Heijer M., Kluijtmans L.A., van den Heuvel L.P. 1995. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nature Genetics* 10.1:111-113.

Fuiano G., Mazza G., Comi N., Coglioti A., De Nicola L., Iodice C., Andreucci M. and Andreucci V. E. 2000. Current indications for renal biopsy: a questionnaire-based survey. *American Journal of Kidney Diseases* 35.3:448-457.

Gallisti S., Sudi K., Mangge H., Erwa W. and Borkenstein M. 2000. Insulin as an independent correlate of plasma homocysteine levels in obese children and adolescents. *Diabetes Care* 23:1348-1351.

Garibotto G., Sofia A., Saffioti S., Russo R., Deferrari G., Rossi D., Verzola D., Gandolfo M. T. and Sala M. R. 2003. Interorgan exchange of aminothiols in humans. *American Journal of Physiology - Endocrinology and Metabolism* 284.4:E757-763.

Gerritsen T., Vaughn J. G. and Waisman H. A. 1962. The identification of homocystine in the urine. *Biochemical and Biophysical Research Communications* 9:493-496.

Gokkusu C., Tulubas F., Unlucerci Y., Ozkok E., Umman B. and Aydin M. 2010. Homocysteine and pro-inflammatory cytokine concentrations in acute heart disease. *Cytokine* 50.1:15-18.

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Gubler M. C. 2003. Podocyte differentiation and hereditary proteinuria/nephrotic syndromes. *Journal of the American Society of Nephrology* 14 Suppl 1:S22-26.

Gutin B., Islam S., Manos T., Cucuzzo N., Smith C. and Stachura M. E. 1994. Relation of percentage of body fat and maximal aerobic capacity to risk factors for atherosclerosis and diabetes in black and white seven- to eleven-year-old children. *Journal of Pediatrics* 125.6 Pt 1:847-852.

Guttormsen A. B., Schneede J., Fiskerstrand T., Ueland P. M. and Refsum H. 1994. Plasma concentrations of homocysteine and other aminothiol compounds are related to food intake in healthy human subjects. *Journal of Nutrition* 124:1934-1941.

_____, Ueland P. M., Lonning P. E., Mella O. and Refsum H. 1998. Kinetics of plasma total homocysteine in patients receiving high-dose methotrexate therapy. *Clinical Chemistry* 44.9:1987-1989.

_____, _____, Svarstad E. and Refsum H. 1997. Kinetic basis of hyperhomocysteinemia in patients with chronic renal failure. *Kidney International* 52.2:495-502.

Hankey G. J. and Eikelboom J. W. 1999. Homocysteine and vascular disease. *Lancet* 354.9176:407-413.

Herrmann W., Schorr H., Geisel J. and Riegel W. 2001. Homocysteine, cystathionine, methylmalonic acid and B-vitamins in patients with renal disease. *Chemistry and Laboratory Medicine* 39.8:739-746.

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

Holven K. B., Aukrust P., Holm T., Ose L. and Nenseter M. S. 2002. Folic acid treatment reduces chemokine release from peripheral blood mononuclear cells in hyperhomocysteinemic subjects. *Arteriosclerosis, Thrombosis, and Vascular Biology* 22.4:699-703.

Homocysteine Lowering Trialists' Collaboration 1998. Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. *British Medical Journal* 3:894-898.

Hong S. Y., Yang D. H. and Chang S. K. 1998. Plasma homocysteine, vitamin B6, vitamin B12 and folic acid in end-stage renal disease during low-dose supplementation with folic acid. *American Journal of Nephrology* 18.5:367-372.

Hoogeveen E. K., Kostense P. J., Jager A., Heine R. J., Jakobs C., Bouter L. M., Donker A. J. and Stehouwer C. D. 1998. Serum homocysteine level and protein intake are related to risk of microalbuminuria: the Hoorn Study. *Kidney International* 54.1:203-209.

Hortin G. L., Sean N. and Hoehn G. T. 2006. Bound homocysteine, cysteine, and cysteinylglycine distribution between albumin and globulins. *Clinical Chemistry* 52.12:2258-2264.

House J. D., Brosnan M. E. and Brosnan J. T. 1997. Characterization of homocysteine metabolism in the rat kidney. *Biochemical Journal* 328. Pt 1:287-292.

_____, _____ and _____ 1998. Renal uptake and excretion of homocysteine in rats with acute hyperhomocysteinemia. *Kidney International* 54.5:1601-1607.

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

Huang J. T., Wang L., Prabakaran S., Wengenroth M., Lockstone H. E., Koethe D., Gerth C. W., Gross S., Schreiber D., Lilley K., Wayland M., Oxley D., Leweke F., M and Bahn S. 2007. Independent protein-profiling studies show a decrease in apolipoprotein A1 levels in schizophrenia CSF, brain and peripheral tissues. *Molecular Psychiatry* 13.12:1118-1128.

Hultberg B., Andersson A. and Arnadottir M. 1995. Reduced, free and total fractions of homocysteine and other thiol compounds in plasma from patients with renal failure. *Nephron* 70.1:62-67.

Ibadin M. O. and Abiodun P. O. 1998. Epidemiology and clinicopathologic characteristics of childhood nephrotic syndrome in Benin-City, Nigeria. *Journal of the Pakistan Medical Association* 48.8:235-238.

Jacobsen D. W. 1998. Homocysteine and vitamins in cardiovascular disease. *Clinical Chemistry* 44.8 Pt 2:1833-1843.

Jacques P. F., Rosenberg I. H., Rogers G., Selhub J., Bowman B. A., Gunter E. W., Wright J. D. and Johnson C. L. 1999. Serum total homocysteine concentrations in adolescent and adult Americans: results from the third National Health and Nutrition Examination Survey. *American Journal of Clinical Nutrition* 69.3:482-489.

Jakubowski H. 1999. Protein homocysteinylation: possible mechanism underlying pathological consequences of elevated homocysteine levels. *FASEB Journal* 13.15:2277-2283.

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

Joven J., Arcelus R., Camps J., Ordonez-Llanos J., Vilella E., Gonzalez-Sastre F. and Blanco-Vaca F. 2000. Determinants of plasma homocysteine in patients with nephrotic syndrome. *Journal of Molecular Medicine* 78.3:147-154.

Jungers P., Joly D., Massy Z., Chauveau P., Nguyen A. T., Aupetit J. and Chadefaux B. 1999. Sustained reduction of hyperhomocysteinaemia with folic acid supplementation in predialysis patients. *Nephrology Dialysis Transplantation* 14.12:2903-2906.

Kanani P. M., Sinkey C. A., Browning R. L., Allaman M., Knapp H. R. and Haynes W. G. 1999. Role of oxidant stress in endothelial dysfunction produced by experimental hyperhomocysteinemia in humans. *Circulation* 100.11:1161-1168.

Kannel W. B., Wolf P. A., Castelli W. P. and D'Agostino R. B. 1987. Fibrinogen and risk of cardiovascular disease. The Framingham Study. *Journal of the American Medical Association* 258.9:1183-1186.

Karle S. M., Uetz B., Ronner V., Glaeser L., Hildebrandt F. and Fuchshuber A. 2002. Novel mutations in NPHS2 detected in both familial and sporadic steroid-resistant nephrotic syndrome. *Journal of the American Society of Nephrology* 13.2:388-393.

Kaysen G. A. and de Sain-van der Velden M. G. 1999. New insights into lipid metabolism in the nephrotic syndrome. *Kidney International Supplement* 71:S18-21.

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

Kim M. K., Ordovas J. M., Selhub J. and Campos H. B. 2003. Vitamins and plasma homocysteine concentrations in an urban and rural area of Costa Rica. *Journal of the American College of Nutrition* 22:224-231.

Klee G. G. 2000. Cobalamin and folate evaluation: measurements of methulmalonic acid and homocysteine vs vitamin B12 and folate. *Clinical Chemistry* 46:1277–1283.

Kniazewska M. H., Obuchowicz A. K., Wielkoszynski T., Zmudzinska-Kilczak J., Urban K., Marek M., Witanowska J. and Sieron-Stoltny K. 2009. Atherosclerosis risk factors in young patients formerly treated for idiopathic nephrotic syndrome. *Pediatric Nephrology* 24.3:549-554.

Kon V. and Ichikawa I. 2004. Glomerular filtration and diseases. IN Avner ED, H. W., Ninudet P Ed. *Pediatric Nephrology* 5th ed. Baltimore, Lippincon Williams & Wilkins 25-44.

Krohn R. I. 2002. *The colorimetric detection and quantitation of total protein* New York, John Wiley & Sons, Inc. 265-275.

Kuzma E. and Roszkowska B. M. 2006. Lipid abnormalities in children with refractory nephrotic proteinuria. *Medicinski Pregled* 63.Suppl 3:201-204.

Lamb E., Newman D. J. and Price C. P. 2006. Kidney Function Tests. IN Burtis, C. A., Ashwood, E. R. and Bruns, D. E. Eds. *Tietz Textbook of clinical chemistry and molecular diagnostics* 4th ed., Elsievier Inc.

Lentz S. R. 2005. Mechanisms of homocysteine-induced atherothrombosis. *Journal of Thrombosis and Haemostasis* 3.8: 1646-1654.

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

_____ and Sadler J. E. 1991. Inhibition of thrombomodulin surface expression and protein C activation by the thrombogenic agent homocysteine. *Journal of Clinical Investigations* 88.6:1906-1914.

_____ and _____ 1993. Homocysteine inhibits von Willebrand factor processing and secretion by preventing transport from the endoplasmic reticulum. *Blood* 81.3:683-689.

Loehrer F. M., Angst C. P., Brunner F. P., Haefeli W. E. and Fowler B. 1998. Evidence for disturbed S-adenosylmethionine : S-adenosylhomocysteine ratio in patients with end-stage renal failure: a cause for disturbed methylation reactions? *Nephrology Dialysis Transplantation* 13.3:656-661.

Malinow M. R. 1994. Homocysteine and arterial occlusive diseases: a mini review.. *Clinical Chemistry* 173-176.

_____, Duell P. B., Hess D. L., Anderson P. H., Kruger W. D., Phillipson B. E., Gluckman R. A., Block P. C. and Upson B. M. 1998. Reduction of plasma homocysteine levels by breakfast cereal fortified with folic acid in patients with coronary heart disease. *New England Journal of Medicine* 338.15:1009-1015.

Marsh J. B. 1960. Increased net synthesis of plasma low density LP by perfused liver of nephrotic rats. *Federation proceedings* 19:149.

_____ and Drabkin D. L. 1960. Experimental reconstruction of metabolic pattern of lipid nephrosis. Key role of hepatic protein synthesis in hyperlipidemia. *Metabolism* 9:946-950.

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

Maruthamuthu M. and Kishore S. 1988. Bromocresol green as a new spectrophotometric probe for serum albumins. *Proceedings of Indian Academy of Science (Chem Sci)* 100.6:525-533.

Mayne D. P. 1994. Proteins in plasma and urine. *Clinical Chemistry in Diagnosis and Treatment* 6th ed. London. Edward Arnold. pp 311-335

McGregor D., Shand B. and Lynn K. 2000. A controlled trial of the effect of folate supplements on homocysteine, lipids and hemorheology in end-stage renal disease. *Nephron* 85.3:215-220.

McKenzie K. E., Wiltshire E. J., Gent R., Hirt C., Piotto L. and Couper J. 2006. Folate and vitamin B6 rapidly normalize endothelial dysfunction in children with type 1 diabetes mellitus. *Pediatrics* 118:242-253.

McKinley M. C., Strain J. J., McPartlin J., Scott J. M. and McNulty H. 2001. Plasma homocysteine is not subject to seasonal variation. *Clinical Chemistry* 47.8:1430-1436.

Medina M., Urdiales J. L. and Amores-Sanchez M. I. 2001. Roles of homocysteine in cell metabolism: old and new functions. *European Journal of Biochemistry* 268.14:3871-3882.

Medina M. A. and Amores-Sanchez M. I. 2000. Homocysteine: an emergent cardiovascular risk factor? *Journal of Clinical Investigations* 30.9:754-762.

Mehls O., Wingen A. M., Bonzel K. E., Ruder H. and Tonshoff B. 1989. Protein restriction in children with chronic renal failure? *Blood Purification* 7.1:46-51.

UNIVERSITY OF IBADAN LIBRARY
AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Miller J. W., Ribaya-Mercado J. D., Russell R. M., Shepard D. C., Morrow F. D., Cochary E. F., Sadowski J. A., Gershoff S. N. and Selhub J. 1992. Effect of vitamin B-6 deficiency on fasting plasma homocysteine concentrations. *American Journal of Clinical Nutrition* 55.6:1154-1160.

Molloy, A. M., Daly, S., Mills, J. L., Kirke, P. N., Whitehead, A. S., Ramsbottom, D., Conley, M. R., Weir, D. G. and Scott, J. M. 1997. Thermolabile variant of 5,10-methylenetetrahydrofolate reductase associated with low red-cell folates: implications for folate intake recommendations. *Lancet* 349.9065:1591-1593.

Monnerat C. and Hayoz D. 1997. [Homocysteine and venous thromboembolism]. *Schweizerische medizinische Wochenschrift* 127.36:1489-1496.

Mouline B., Ollier S. and Olmer M. 1992. Disturbances of lipid metabolism during nephrotic syndrome. *Nephrologie* 13.5:193-199.

Mudd S. H., Finkelstein J. D., Irreverre F. and Laster L. 1964. Homocystinuria: An Enzymatic Defect. *Science* 143:1443-1445.

_____, _____, Refsum H., Ueland P. M., Malinow M. R., Lentz S. R., Jacobsen D. W., Brattstrom L., Wilcken B., Wilcken D. E., Blom H. J., Stabler S. P., Allen R. H., Selhub J. and Rosenberg I. H. 2000. Homocysteine and its disulfide derivatives: a suggested consensus terminology. *Arteriosclerosis, Thrombosis, and Vascular Biology* 20.7:1704-1706.

_____ and Poole, J. R. 1975. Labile methyl balances for normal humans on various dietary regimens. *Metabolism* 24:721-735.

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

Must A., Jacques P. F., Rogers G., Rosenberg I. H. and Selhub J. 2003. Serum total homocysteine concentrations in children and adolescents: results from the third National Health and Nutrition Examination Survey NHANES III. *Journal of Nutrition* 133.8:2643-2649.

Mydlik M. and Derzsiova K. 2001. Renal replacement therapy and secondary hyperoxalemia in chronic renal failure. *Kidney International Supplements* 78:S304-307.

_____, _____ and Zemberova E. 1997. Metabolism of vitamin B6 and its requirement in chronic renal failure. *Kidney International Supplements* 62:S56-59.

Nakano E., Higgins J. A. and Powers H. J. 2001. Folate protects against oxidative modification of human LDL. *British Journal of Nutrition* 86.6:637-639.

Nath K. A. 1998. Homocysteine and atherothrombosis. *New England Journal of Medicine* 339.7:478-479.

Nissel R., Lindberg A., Mehls O. and Haffner D. 2008. Factors predicting the near-final height in growth hormone-treated children and adolescents with chronic kidney disease. *Journal of Clinical Endocrinology and Metabolism* 93.4:1359-1365.

Nissen S. E., Tsunoda T., Tuzcu E. M., Schoenhagen P., Cooper C. J., Yasin M., Eaton G. M., Lauer M. A., Sheldon W. S., Grines C. L., Halpern S., Crowe T., Blankenship J. C. and Kerensky R. 2003. Effect of recombinant ApoA-I Milano on coronary atherosclerosis in patients with acute coronary syndromes.

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

a randomized controlled trial. *Journal of the American Medical Association* 290.17:2292-2300.

Nurk E., Tell G. S., Nygard O., Refsum H., Ueland P. M. and Vollset S. E. 2001. Plasma total homocysteine is influenced by prandial status in humans: the Hordaland Homocysteine Study. *Journal of Nutrition* 131.4:1214-1216.

Nygard O., Refsum H., Ueland P. M. and Vollset S. E. 1998. Major lifestyle determinants of plasma total homocysteine distribution: the Hordaland Homocysteine Study. *American Journal of Clinical Nutrition* 67.2:263-270.

Okunola O., Akinsola A. and Ayodele O. 2012. Kidney diseases in Africa: aetiological considerations, peculiarities and burden. *African Journal of Medicine and Medical Sciences* 41.2:119-133.

Olthof M. R., van Vliet T., Verhoef P., Zock P. L. and Katan M. B. 2005. Effect of homocysteine-lowering nutrients on blood lipids: results from four randomised, placebo-controlled studies in healthy humans. *PLoS Medicine* 2.5:e135.

Ordonez J. D., Hiatt R. A., Killebrew E. J. and Fireman B. H. 1993. The increased risk of coronary heart disease associated with nephrotic syndrome. *Kidney International* 44:638-642.

Osganian S. K., Stampfer M. J., Spiegelmann D., Rimm E., Cutler J. A., Feldman H. A., Montgomery D. H., Webber L. S., Lytle L. A., Bausserman L., Nader PR 1999. Distribution and factors associated with serum homocysteine levels in U.S. school children: The Child and Adolescent Trial for Cardiovascular Health. Journal of the American Medical Association 281.13: 1189-1196.

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

Oyedeji G. A. 1985. Socio-economic and Cultural Background of Hospitalised Children in Ilesha. *Nigerian Journal of Paediatrics* 12.4:111-117.

Papandreou D., Rousso I., Malindretos P., Makedou A. and Arvanitidou M. 2010. Effects of oral folate supplementation on serum total homocysteine and cholesterol levels in hyperhomocysteinemic children. *Nutrition in Clinical Practice* 25.4:390-393.

Pena A. S., Wiltshire E., Gent R., Hirte C. and Couper J. 2004. Folate improves endothelial function in children and adolescents with type 1 diabetes. *Journal of Pediatrics* 144:500-504.

Perna A. F., Ingrosso D., De Santo N. G., Galletti P., Brunone M. and Zappia V. 1997. Metabolic consequences of folate-induced reduction of hyperhomocysteinemia in uremia. *Journal of American Society of Nephrologist* 8.12:1899-1905.

_____, _____, Violetti E., Luciano M. G., Sepe I., Lanza D., Capasso R., Ascione E., Raiola I., Lombardi C., Stenvinkel P., Massy Z. and De Santo N. G. 2009. Hyperhomocysteinemia in uremia—a red flag in a disrupted circuit. *Seminar on Dialysis* 22.4:351-356.

Phillips A., Shaper A. G. and Whincup P. H. 1989. Association between serum albumin and mortality from cardiovascular disease, cancer, and other causes. *Lancet* 2.8677:1434-1436.

Piolot A., Nadler F., Perez N. and Jacotot B. 1996. [Homocysteine: relations to ischemic cardiovascular diseases]. *Revue de Médecine Interne* 17.1:34-45.

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

Podda G. M., Lussana F., Moroni G., Faioni E. M., Lombardi R., Fontana G., Ponticelli C., Maioli C. and Cattaneo M. 2007. Abnormalities of homocysteine and B vitamins in the nephrotic syndrome. *Thrombosis Research* 120.5:647-652.

Radhakrishnan J., Appel A. S., Valeri A. and Appel G. B. 1993. The nephrotic syndrome, lipids, and risk factors for cardiovascular disease. *American Journal of Kidney Diseases* 22.1:135-142.

Refsum H., Guttormsen A. B., Fiskerstrand T. and Ueland P. M. 1998a. Hyperhomocysteinemia in terms of steady-state kinetics. *European Journal of Pediatrics* 157.Suppl 2:S45-S49.

_____, Helland S. and Ueland P. M. 1985. Radioenzymic determination of homocysteine in plasma and urine. *Clinical Chemistry* 31:624-628.

_____, Smith A. D., Ueland P. M., Nexo E., Clarke R., McPartlin J., Johnston C., Engbaek F., Schneede J., McPartlin C. and Scott J. M. 2004. Facts and recommendations about total homocysteine determinations: an expert opinion. *Clinical Chemistry* 50.1:3-32.

_____, Ueland P. M., Nygard O. and Vollset S. E. 1998b. Homocysteine and cardiovascular disease. *Annual Review of Medicine* 49:31-62.

Rifai N. 2000. Lipoproteins and apolipoproteins, composition, metabolism and associated with coronary heart disease. *Archive of Pathology and Laboratory Medicine* 110:694-704.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Robinson K., Arheart K., Refsum H., Brattstrom L., Boers G., Ueland P., Rubba P., Palma-Reis R., Meleady R., Daly L., Witteman J. and Graham I. 1998. Low circulating folate and vitamin B6 concentrations: risk factors for stroke, peripheral vascular disease, and coronary artery disease. European COMAC Group. *Circulation* 97.5:437-443.

Rosenblatt D. S. and Whitehead V. M. 1999. Cobalamin and folate deficiency: acquired and hereditary disorders in children. *Seminars in Hematology* 36.1:19-34.

Santucci L., Candiano G., Petretto A., Pavone B., Bruschi M., Gusmano R., Federici G., Urbani A. and Ghiggeri G. M. 2001. Protein-protein interaction heterogeneity of plasma apolipoprotein A1 in nephrotic syndrome. *Molecular BioSystems* 7.3:659-666.

Schroder H., Marrugat J., Covas M., Elosua R., Pena A., Weinbrenner T., Fito M., Vidal M. A. and Masia R. 2004. Population dietary habits and physical activity modification with age. *European Journal of Clinical Nutrition* 58.2:302-311.

Selhub J. 1999. Homocysteine metabolism. *Annual Review of Nutrition* 19:217-246.

_____ and Miller J. W. 1992. The pathogenesis of homocysteinemia: interruption of the coordinate regulation by S-adenosylmethionine of the remethylation and transsulfuration of homocysteine. *American Journal of Clinical Nutrition* 55.1:131-138.

Sengupta S., Wehbe C., Majors A. K., Ketterer M. E., DiBello P. M. and Jacobsen D. W. 2001. Relative roles of albumin and ceruloplasmin in the formation of

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

homocystine, homocysteine-cysteine-mixed disulfide, and cysline in circulation. *Journal of Biological Chemistry* 276.50:46896-46904.

Shemin D., Bostom A. G. and Selhub J. 2001. Treatment of hyperhomocysteinemia in end-stage renal disease. *American Journal of Kidney Diseases* 38.4 Suppl 1:S91-94.

Stabler S. P., Estacio R., Jeffers B. W., Cohen J. A., Allen R. H. and Schrier R. W. 1999. Total homocysteine is associated with nephropathy in non-insulin-dependent diabetes mellitus. *Metabolism* 48.9:1096-1101.

Stanger O., Herrmann W., Pietrzik K., Fowler B., Geisel J., Dierkes J. and Weger M. 2003. DACH-LIGA homocystein german, austrian and swiss homocysteine society: consensus paper on the rational clinical use of homocysteine, folic acid and B-vitamins in cardiovascular and thrombotic diseases: guidelines and recommendations. *Clinical Chemistry and Laboratory Medicine* 41.11:1392-1403.

Stevenson R. E., Schwartz C. E., Du Y. Z. and Adams M. J., Jr. 1997. Differences in methylenetetrahydrofolate reductase genotype frequencies between Whites and Blacks. *American Journal of Human Genetics* 60.1:229-230.

Stoycheff N., Stevens L. A., Schmid C. H., Tighiouart H., Lewis J., Atkins R. C. and Levey A. S. 2009. Nephrotic syndrome in diabetic kidney disease: an evaluation and update of the definition. *American Journal of Kidney Diseases* 54.5:840-849.

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

Suliman M. E., Barany P., Kalantar-Zadeh K., Lindholm B. and Stenvinkel P. 2005. Homocysteine in uraemia--a puzzling and conflicting story. *Nephrology Dialysis Transplantation* 20.1:16-21.

_____, Divino Filho J. C., Barany P., Anderstam B., Lindholm B. and Bergstrom J. 1999. Effects of high-dose folic acid and pyridoxine on plasma and erythrocyte sulfur amino acids in hemodialysis patients. *Journal of the American Society of Nephrology* 10.6:1287-1296.

_____, Qureshi A. R., Barany P., Stenvinkel P., Filho J. C., Anderstam B., Heimburger O., Lindholm B. and Bergstrom J. 2000. Hyperhomocysteinemia, nutritional status, and cardiovascular disease in hemodialysis patients. *Kidney International* 57.4:1727-1735.

_____, Stenvinkel P., Heimburger O., Barany P., Lindholm B. and Bergstrom J. 2002. Plasma sulfur amino acids in relation to cardiovascular disease, nutritional status, and diabetes mellitus in patients with chronic renal failure at start of dialysis therapy. *American Journal of Kidney Diseases* 40.3:480-488.

Tehlivets O., Malanovic N., Visram M., Pavkov-Keller T. and Keller W. 2013. S-adenosyl-L-homocysteine hydrolase and methylation disorders: yeast as a model system. *Biochimica et Biophysica Acta* 1832.1:204-215.

Thambyrajah J., Landray M. J., McGlynn F. J., Jones H. J., Wheeler D. C. and Townend J. N. 2000. Does folic acid decrease plasma homocysteine and improve endothelial function in patients with predialysis renal failure? . *Circulation* 102:871-875.

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

Tkaczyk M., Czupryniak A., Nowicki M., Chwatko G. and Bald E. 2009. 2009 [Homocysteine and glutathione metabolism in steroid-treated relapse of idiopathic nephrotic syndrome]. *Polski merkuriusz lekarski* 26.154:294-297.

Togawa A., Yamamoto T. and Hishida A. 2004. [Nephrotic syndrome: pathophysiology, classification and diagnostic criteria]. *Nihon Rinsho* 62.10:1777-1783.

Tonstad S., Refsum H., Sivertsen M., Christophersen B., Ose L. and Ueland P. M. 1996. Relation of total homocysteine and lipid levels in children to premature cardiovascular death in male relatives. *Pediatric Research* 40:47-52.

Tousoulis M., Zingraff J., Jungers P., Chadefaux-Vekemans B., Drueke T. and Messy Z. A. 1999. Effective correction of hyperhomocysteinemia in hemodialysis patients by intravenous folinic acid and pyridoxine therapy. *Kidney International* 56.6:2292-2296.

Tovar A. R., Torres N., Barrales-Benitez O., Lopez A. M., Diaz M. and Rosado J. L. 2003. Plasma total homocysteine in Mexican rural and urban women fed typical model diets. *Nutrition* 19:826-831.

Ubbink J. B., Delport R. and Vermaak W. J. 1996. Plasma homocysteine concentrations in a population with a low coronary heart disease prevalence. *Journal of Nutrition* 126.4 Suppl:1254S-1257S.

_____, Vermaak W. J., van der Merwe A. and Becker P. J. 1992. The effect of blood sample aging and food consumption on plasma total homocysteine levels. *Clinica Chimica Acta* 207.1-2:119-128.

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

Ueland P. M. 1995. Homocysteine species as components of plasma redox thiol status. *Clinical Chemistry* 41:340-342.

_____ and Refsum H. 1989 Plasma homocysteine, a risk factor for vascular disease: plasma levels in health, disease, and drug therapy. *Journal of Laboratory and Clinical Medicine* 114.5:473-501.

_____, _____, Stabler S. P., Malinow M. R., Andersson A. and Allen R. H. 1993. Total homocysteine in plasma or serum: methods and clinical applications. *Clinical Chemistry* 39.9:1764-1779.

van Buuren A. J., Louw M. E., Shephard G. S. and Labadarios D. 1987. The effect of pyridoxine supplementation on plasma pyridoxal-5'-phosphate levels in children with the nephrotic syndrome. *Clinical Nephrology* 28.2:81-86.

_____, Shephard G. S. and Labadarios D. 1985. Low plasma pyridoxal-5'-phosphate levels in children with the nephrotic syndrome. *South African Medical Journal* 67:329-332.

van Guldener, C. 2006 Why is homocysteine elevated in renal failure and what can be expected from homocysteine-lowering? *Nephrology Dialysis Transplantation* 21.5:1161-1166.

_____, Janssen, M. J., Lambert, J., ter Wee, P. M., Jakobs, C., Donker, A. J. and Stehouwer, C. D. 1998 No change in impaired endothelial function after long-term folic acid therapy of hyperhomocysteinaemia in haemodialysis patients. *Nephrology Dialysis Transplantation* 13.1:106-112.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

_____, Stam F. and Stehouwer C. D. 2001. Homocysteine metabolism in renal failure. *Kidney International* 78:S234-237.

Veldman B. A., Vervoort G., Blom H. and Smits P. 2005. Reduced plasma total homocysteine concentrations in Type 1 diabetes mellitus is determined by increased renal clearance. *Diabetic Medicine* 22.3:301-305.

Verhaar M. C., Wever R. M., Kastelein J. J., van Loon D., Milstien S., Koomans H. A. and Rabelink T. J. 1999. Effects of oral folic acid supplementation on endothelial function in familial hypercholesterolemia. A randomized placebo-controlled trial. *Circulation* 100.4:335-338.

Verhoef P., Stampfer M. J. and Buring J. E. 1996. Homocysteine metabolism and risk of myocardial infarction: Relation with vitamins B6, B12 and folate. *American Journal of Epidemiology* 143:845-859.

Vermeulen E. G., Stehouwer C. D., Twisk J. W., van den Berg M., de Jong S. C., Mackaay A. J., van Campen C. M., Visser F. C., Jakobs C. A., Bulterjis E. J. and Rauwerda J. A. 2000. Effect of homocysteine-lowering treatment with folic acid plus vitamin B6 on progression of subclinical atherosclerosis: a randomised, placebo-controlled trial. *Lancet* 355.9203:517-522.

Vilaseca M. A., Moyano D., Ferrer I. and Artuch R. 1997. Total homocysteine in pediatric patients. *Clinical Chemistry* 43.4:690-692.

Vollbach H., Heun R., Morris C. M., Edwardson J. A., McKeith I. G., Jessen F., Schulz A., Maier W. and Kolsch H. 2005. APOA1 polymorphism influences risk for early-onset nonfamilial AD. *Annals of neurology* 58.3:436-441.

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

Vollset S. E., Refsum H., Tverdal A., Nygard O., Nordrehaug J. E., Tell G. S. and Ueland P. M. 2001. Plasma total homocysteine and cardiovascular and noncardiovascular mortality: the Hordaland Homocysteine Study. *American Journal of Clinical Nutrition* 74.1:130-136.

Weisberg I., Tran P., Christensen B., Sibani S. and Rozen R. 1998. A second genetic polymorphism in methylenetetrahydrofolate reductase MTHFR associated with decreased enzyme activity. *Molecular Genetics and Metabolism* 64.3:169-172.

Wilcken D. E. and Wilcken B. 1976. The pathogenesis of coronary artery disease. A possible role for methionine metabolism. *Journal of Clinical Investigations* 57.4:1079-1082.

Winkelmayer W. C., Kramar R., Curhan G. C., Chandraker A., Endler G., Fodinger M., Horl W. H. and Sunder-Plassmann G. 2005. Fasting plasma total homocysteine levels and mortality and allograft loss in kidney transplant recipients: a prospective study. *Journal of the American Society of Nephrology* 16.1:255-260.

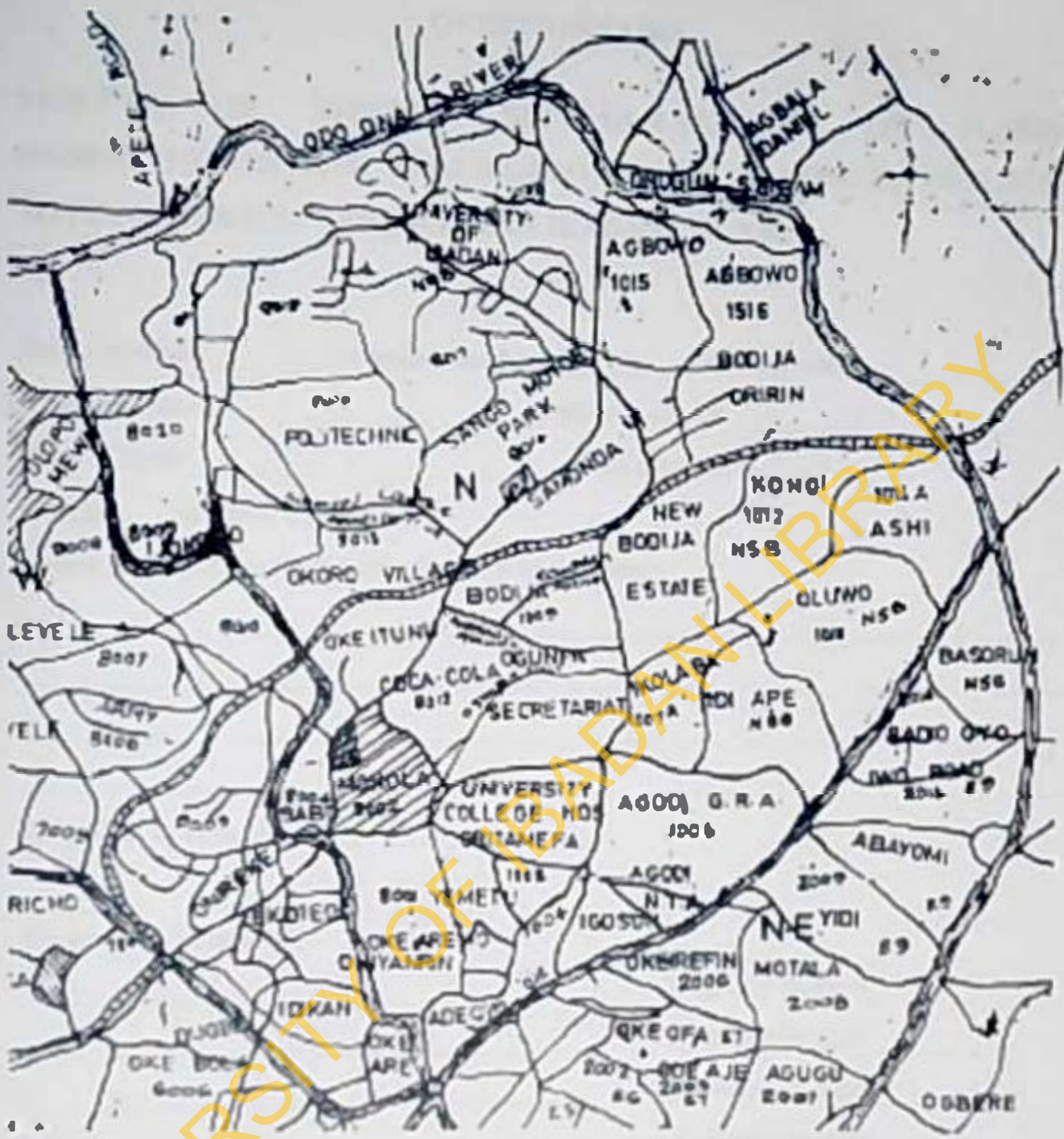
Zhu X., Wu C., Zeng W., Xue H. and Chen B. 2005. Cysteine mutants of human apolipoprotein A-I: a study of secondary structural and functional properties. *Journal of lipid research* 46.6:1303-1311.

Zue W., Huang X., Li M. and Neubauer H. 2005. Elevated plasma homocysteine in obese schoolchildren with early atherosclerosis. *European Journal of Pediatrics* 5:33-38.

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

APPENDIX I



Map show the location of UCH within Ibadan North Local Government Area

Source: Ibadan North Local Government Area Secretariat

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

APPENDIX II

QUESTIONNAIRE

EFFECTS OF VITAMIN SUPPLEMENTATION ON PLASMA HOMOCYSTEINE AND APOLIPOPROTEIN A1 LEVELS IN CHILDREN WITH NEPHROTIC SYNDROME IN IBADAN, NIGERIA

Study number: _____ Hospital number: _____ Date enrolled: __/__/____

Mother's phone: _____ Father's phone: _____

Date of birth: __/__/_____ Age (months): _____

Gender Male=1, Female=2 ☐

Home address (please describe the way to get there):

_____

_____

_____

_____

Date first seen in UCH: __/__/_____

Presenting complaints:

1. _____ Duration in days_____
2. _____ Duration in days _____
3. _____ Duration in days _____
4. _____ Duration in days _____

Other symptoms (specify): _____

_____

Past admissions? [ ] Yes=1, No=2: If yes, how many? [ ].

Haemoglobin [ ] A=1, AS=2, AC=3, SS=4, SC=5, CC=6, Sβthal=7

Family history: Number of siblings: [_____]

Position of patient among mother's children: [_____]

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Marital Status of parents

Not yet married = 1

Married = 2 [ ]

Divorced/Separated = 3

Widowed = 4

If Married

Monogamous = 1 [ ]

Polygamous = 2

Mother's age: |__|__| years

Father's age: |__|__| years

Education:

No formal education = 5

Primary/incomplete secondary = 4

Secondary/Technical/Grade II = 3

Post secondary but not University = 2

University/Postgraduate = 1

Father's education: |__|

Mother's education: |__|

Occupation:

Unemployed, fulltime house wife, student, subsistence farmer = 5

Petty trader, labourer, messenger, similar grades = 4

Junior school teacher, driver, artisan = 3

Intermediate grade, public servant, senior school teachers = 2

Senior public servant, professional, manager, contractor, large scale trader = 1

Mother's Occupation: |__| Father's Occupation: |__|

Dietary assessment

1. Over the last three months would you say your child's weight has?
   a. Remained about the same
   b. Been going up
   c. Been going down
2. How often do you (your child) take part in exercise (sufficient to make you slightly breathless and your heart beat faster) which lasts for 20 minutes or more?
   a. Occasionally or never
   b. Once or twice a week
   c. Three times a week or more

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

3. When you have omelettes or scrambled eggs how many eggs do you give your child?

   a. 1 egg
   b. Eggs
   c. More than 2
   d. Do not eat omelettes or scrambled eggs

4. What type of cheese do you eat most often?

   a. Full fat (stilton, gorgonzola. etc.)
   b. Cheddar type (cheddar, cheshire etc)
   c. Low fat (cottage cheese, edam. brie, etc)
   d. Soft, cream or processed cheese
   e. Half fat cheeses
   f. Do not eat cheese

5. What kind of milk do you usually use, for example, in tea, coffee, on cereals etc?

   a. Cow's full cream
   b. Cow's semi-skimmed
   c. Cow's skimmed
   d. Soya or non dairy type
   e. Do not use milk
   f. Other type _____

6. What type of spread do you normally use for your child, for example, on bread, toast, sandwiches etc?

   a. Butter
   b. Margarine
   c. Olive oil based spread
   d. Low Fat Spread
   e. Very low or 'little'
   f. Cholesterol lowering spread e.g. Benecol
   g. Do not use spread
   h. Other type _____

7. Which of the following is your food normally cooked in?

   a. Palm oil
   b. Butter

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

c. Margarine

d. Vegetable oils e.g. corn oil, sunflower oil

e. Olive Oil

f. No fat used

8. Is the bread you normally eat

a. White

b. Brown

c. Wholemeal/granary

d. Other type _____

9. What type of salad dressing do you usually use?

a. Oil & vinegar/vinaigrette

b. Mayonnaise

c. Salad Cream

d. Low caloric dressing

e. Do not use salad dressing

10. Which of the following do you usually drink?

a. Fruit squashes

b. Sugar free/low sugar squashes

c. Fruit juice

d. Carbonated drinks (e.g. colas, lemonade, etc.)

e. Low caloric carbonated drink (e.g. diet colas, etc)

f. Other type _____

11. Do you usually add salt to your food?

a. In cooking

b. At the table

c. Do not add salt

d. Use salt substitute

12. Do you regularly take any dietary supplements?

a. Combined multi-vitamins & mineral supplement

b. Individual vitamin supplement(s) e.g. vitamin C, vitamin E

c. Individual mineral supplement(s) e.g. iron, calcium

d. Fish oils e.g. cod liver oil

e. Garlic

f. Do not take supplements

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

Relevant Clinical findings on the day of enrolment

Parameter	Date	Present	Absent	Comment
Oedema				
Cyanosis				
Dehydration				
Dyspnoea				
X-ray evidence of pneumonia				
Fever				

Other relevant signs:

_____

_____

Anthropometry:

Parameter	Date	Reading 1	Reading 2	Reading 3
Weight at enrolment (kg)				
Height (cm)				
Waist circumference (cm):				
Hip circumference (cm)				
Skinfold thicknesses				
• Sub-scapular area				
• Deltoid area				
• Triceps area				
• Biceps area				
• Abdominal area				
• Hip area				
• Thigh area				
• Calf area				

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Laboratory findings:

	Set 1		Set 2		Not done?	Comment
Parameter	Date	values	Date	values		
Serum sodium						
Serum potassium						
Serum bicarbonate						
Serum urea						
Serum chloride						
Serum calcium						
Serum phosphate						
Serum creatinine						
Serum albumin						
Total Serum protein (mg/dl)						
Serum cholesterol						
Serum LDL						
Serum HDL						
Serum TG						
Serum Homocysteine						

Clinical diagnosis:

Treatments being given:

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

APPENDIX III

Apparatus and Reagents for Determination of Vitamin B_{12} (cyanocobalamin) and serum Folate

Apparatus and reagents for vitamin B_{12}

- Beakers
- Pipette
- Shaker
- Centrifuge
- $KMnO_4$ solution
- HCL solution
- Vitamin B_{12} standards
- Acetic acid solution
- H_2O_2 solution
- H_2SO_4 solution

Apparatus and reagents for folate:

- Glass vials
- Micropipettes
- Hand gloves
- Syringe, shaker
- Centrifuge
- Centrifuge tubes
- Ultrapure water
- Ascorbic acid
- 5,10-methenyltetrahydrofolic acid, hydrochloric acid
- Diglutamate tri-hydrochloride.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

APPENDIX IV

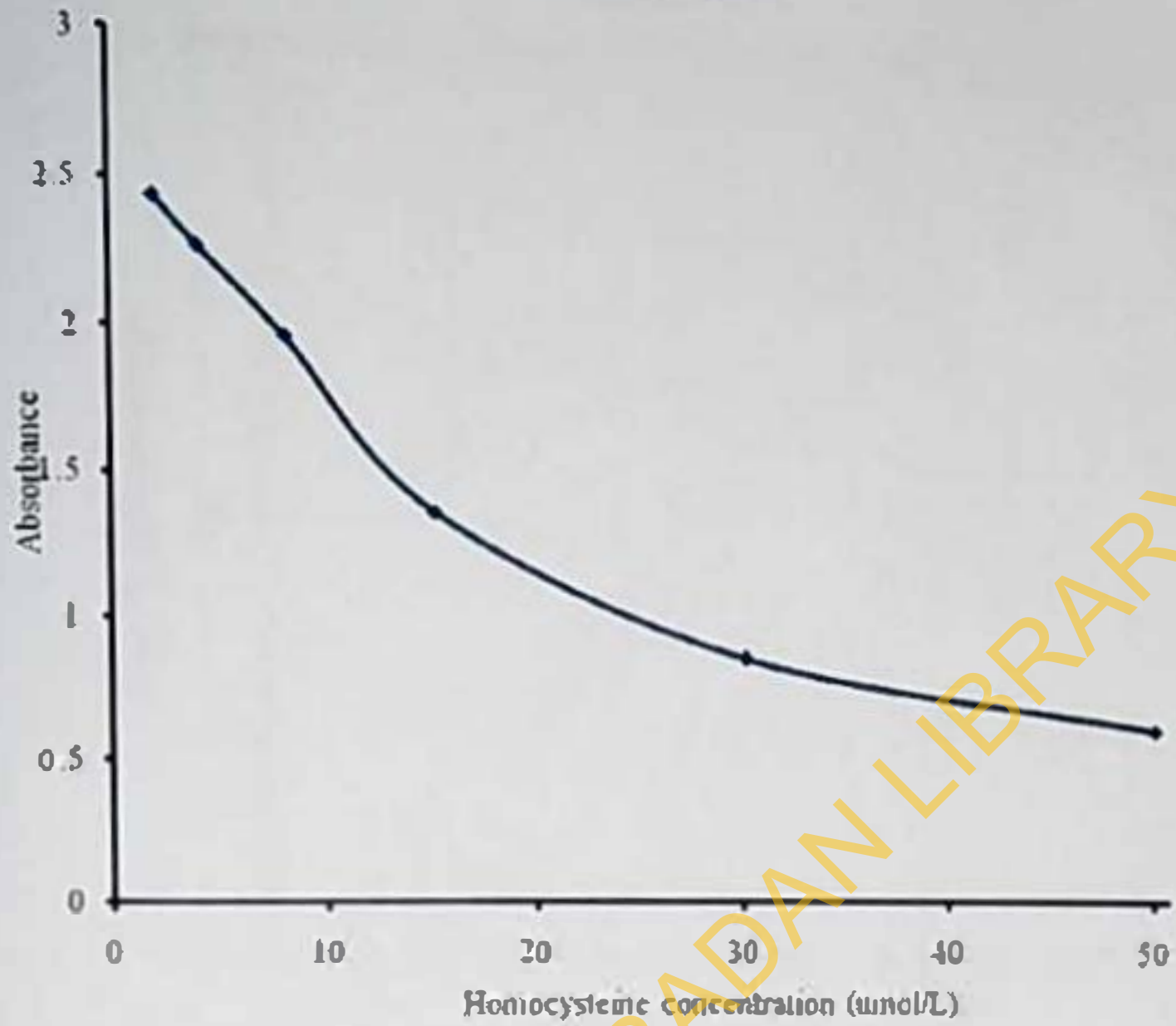


Figure 6.1: Standard homocysteine concentration and absorbance

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

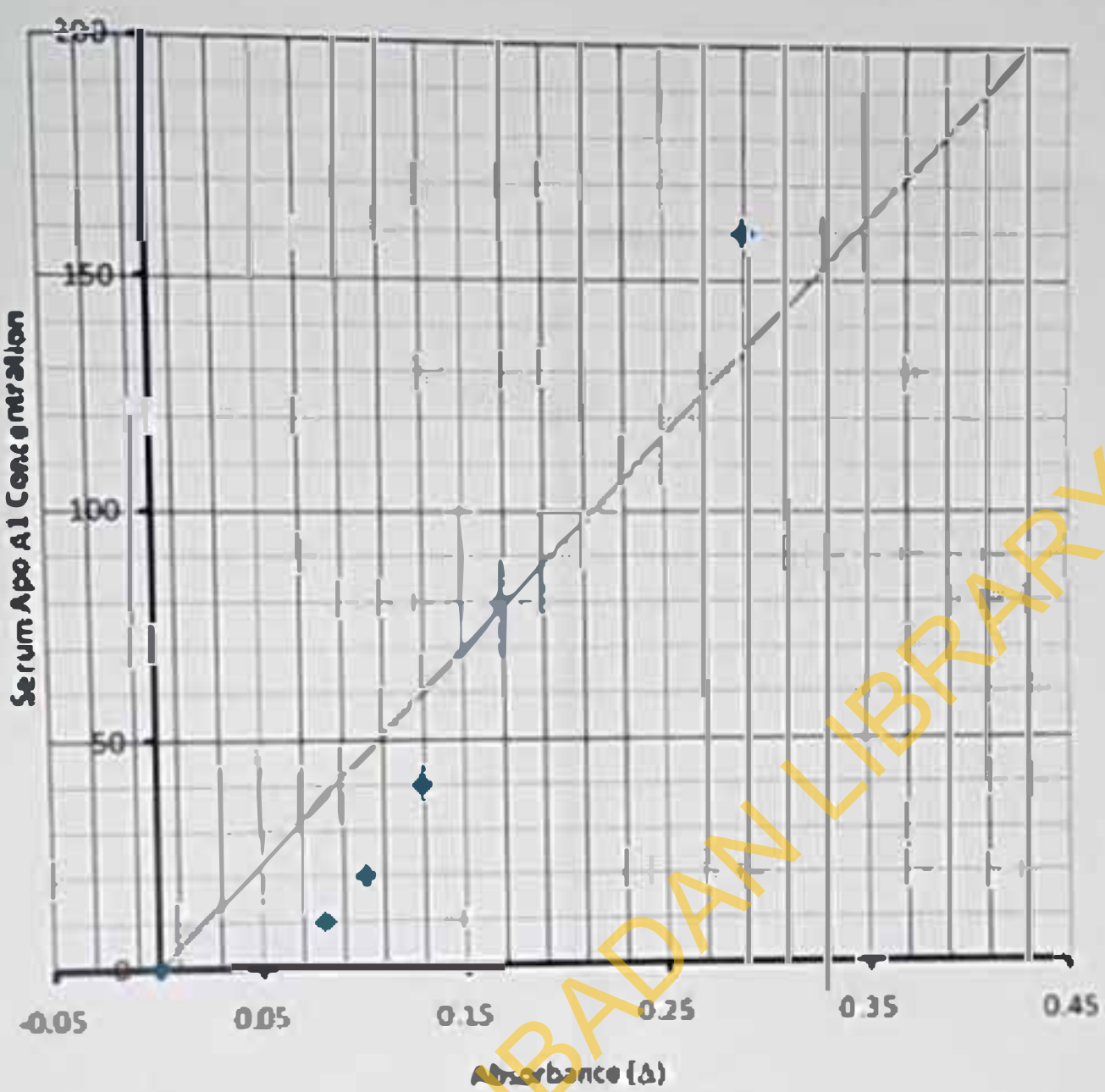


Figure 6.2: Standard apo A1 concentration and absorbance

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Appendix VI

Picture of the stadiometer

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Appendix VII – SKINFOLD THICKNESSES AND CIRCUMFERENCES

TRICEPS AREA

SUBSCAPULAR

DELTOID AREA

ABDOMINAL

BICEPS AREA

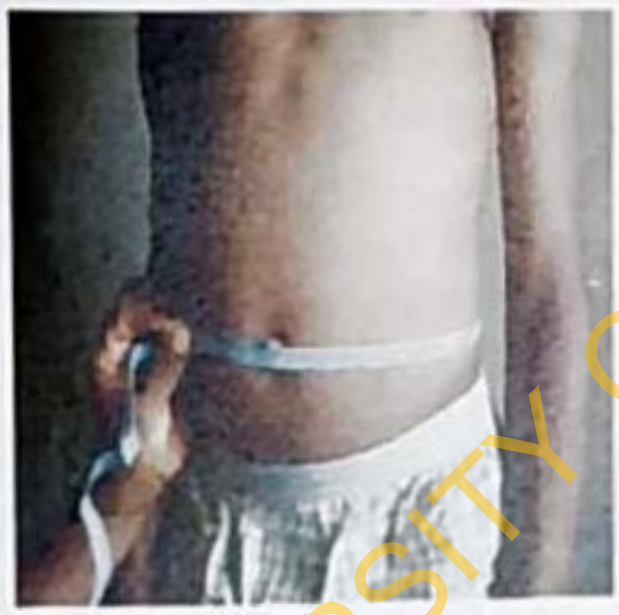
HIP AREA

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

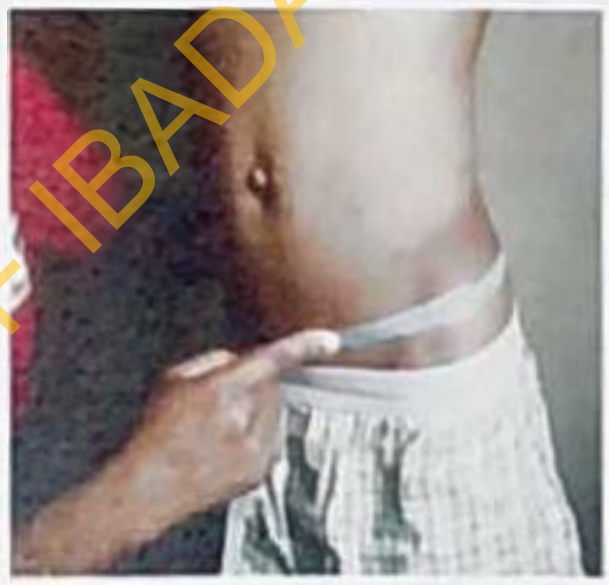
UNIVERSITY OF IBADAN LIBRARY

THIGH AREA

CALF AREA



WAIST CIRCUMFERENCES



HIP CIRCUMFERENCE

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

INSTITUTE FOR ADVANCED MEDICAL RESEARCH AND TRAINING (IMRAT)
COLLEGE OF MEDICINE, UNIVERSITY OF IBADAN, IBADAN, NIGERIA.
Telefax: 234-2-2410088/3310, 3120, 3114, 3594, Fax: 234-2-2413645,
07028383980, 07028383039. E-mail: imratcomui@yahoo.com
DIRECTOR: Prof. C. A. Adebamowo BMChB Hons (Jos), FWACS, FACS, Dsc (Harvard)

UI/UCH EC Registration Number: NIREC/05/01/2008a

NOTICE OF FULL APPROVAL AFTER FULL COMMITTEE REVIEW

Re: Plasma Homocysteine and Effects of Folate and Vitamin B Supplementation in Nigerian Children with Nephrotic Syndrome

UI/UCH Ethics Committee assigned number: UI/EC/09/0179

Name of Principal Investigators: Bose E. Orimadegun

Address of Principal Investigater: Department of Chemical Pathology,
College of Medicine, University of Ibadan

Date of receipt of valid application: 24/07/2008

Date of meeting when final determination of research was made: N/A

This is to inform you that the research described in the submitted protocol, the consent forms and other participant information materials have been reviewed and *given full approval by the UI/UCH Ethics Committee*

This approval dates from 08/06/2009 to 07/06/2010. If there is delay in starting the research, please inform the UI/UCH Ethics Committee so that the dates of approval can be adjusted accordingly. Note that no participant access or activity related to the research may be conducted outside of these dates. *All important current terms used in this study must carry the UI/UCH EC assigned number and duration of UI/UCH EC approval of the study.* In multicenter research, endeavour to submit your annual report to the UI/UCH EC early in order to obtain renewal of your approval and avoid disruption of your research.

The Institutional Code for Health Research Ethics requires you to comply with the institutional guidelines, rules and regulations and with the country wide Code including ensuring that all adverse events are reported promptly to the UI/UCH EC, that changes are instituted in the research without prior approval by the UI/UCH EC except in circumstances outlined in the Code. The UI/UCH EC reserves the right to conduct compliance visits to the research with unannounced visits.

UI/UCH EC
10 JUN 2009
APPROVED

Dr. A. A. Akinkugbe,
Chairman, Medical Advisory Committee,
University College Hospital, Ibadan.
For Chairman, UI/UCH Ethics Committee

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

APPENDIX IX

INFORMED CONSENT FORM

Title of research:

EFFECTS OF VITAMIN SUPPLEMENTATION ON PLASMA HOMOCYSTEINE AND APOLIPOPROTEIN A1 LEVELS IN CHILDREN WITH NEPHROTIC SYNDROME IN IBADAN, NIGERIA

Names and affiliation of researchers;

Investigator: Bose E. Orimadegun

Department of Chemical Pathology

University of Ibadan

Supervisor Prof. E. O. Agbedana

Department of Chemical Pathology

University of Ibadan

Purpose of research:

The purpose of this research is to find out if Nigerian children who develop nephrotic syndrome also have abnormally high level of homocysteine, a derivative of an amino acid in the body which could be harmful and also worsen illness in those with nephrotic syndrome. The research also hopes to find out if the use of folic acid, vitamin B_{12} and vitamin B_6 could lower high homocysteine in patients with nephrotic syndrome. If this is true, it means such cheap and safe drugs can be used to help (reduce morbidity and mortality) Nigerian children with nephrotic syndrome.

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

Procedure of the research:

If you agree that your child participates in this research, your child will be examined. you and your child will be asked some questions pertaining to the illness and general well-being of your child. Your child will be required to come fasting (no meal after dinner) until blood sample is collected the following morning. 5 millilitre of blood will be taken using sterile needle and syringe in a manner that will cause very little or minimal pain. Your child will be required to take a tablet of folic acid (5mg) and one tablet of vitamin B complex daily for the next three months. You will be required to bring him/her to the hospital for review at interval of 2 weeks during the period of the 3 months vitamin supplementations. These visits are different from the required regular Nephrology clinic attendance but it will be schedule such that it coincides with the appointments given by the Consultant Nephrologist. You may also bring the child for review (s) at any time in case you need urgent attention for any reason or you observe any unusual symptoms during the study period. Another 5mL of blood will be collected 3months after vitamin supplementation. Please, note that the vitamin supplements to be given as part of the study are NOT meant to replace the actual treatment for nephrotic syndrome prescribed by the Nephrologist. These drugs are harmless and hope to reduce high level of homocysteine.

Expected duration of research:

Your child is expected to be part of this study for just a period of 3 months. After this period further treatment of the illness will be based on the decisions of the Consultant Nephrologist.

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Foreseeable Risks and Discomfort:

The use of Folic acid and Vitamin B complex as supplements in health and disease has been shown to be harmless but beneficial. You child is not expected to experience any significant side effect or unusual symptoms during the study. The only thing you or your child stands to lose by participating in this study is time taken for the interview and examination. The researcher will ensure that this time is minimized and your time will not be wasted.

Cost to the participants:

You will not need to pay for tests and drugs meant for the purpose of this study.

Benefits of the study:

The goal of this study is to provide data that may be used to formulate policies and strategies to reduce morbidity and mortality as well as improve case management of nephrotic syndrome through exploration of the relationship of plasma homocysteine and relevant biochemical parameters in nephrotic syndrome patients and the assessment of the implications of giving folic acid and vitamin B supplements to children with nephrotic syndrome who have hypercholesterolaemia. It is hoped that the measured outcomes from this study will provide data that will be made available to physicians, policy makers, local and national government, non-governmental organisation involved in the care of patients with nephrotic syndrome.

Confidentiality

All information collected in this study will be given code numbers and no name or other identity traceable to your child will be recorded. This information cannot be

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT
UNIVERSITY OF IBADAN LIBRARY

linked to you or your ward in anyway and your child's name or identifiers will not be used in any publication or reports from this study. As part of our efforts to conduct proper research, officials of the ethical review board may have access to these records.

Voluntariness and alternative to participation

Your child's participation in this study is entirely voluntary. You also have the right to withdraw you child's participation at any given time if you choose to do so. We will like to assure you and your child that if you choose not to take participate or withdraw from the study your child/ward will still receive all the necessary treatment. However, we will appreciate your help if you/your ward voluntarily respond to take part in the study.

Due inducement:

You will be refunded only your transportation costs at each visit made to the clinics for the purpose of this study. No payment or gift will be given to you or your child for taking part in the study.

What happen after study is completed?

Your child will continue the normal clinic attendance and treatments (which will not stop even during the study period) after the research is over. Any of the results or outcome of this study that may benefit your child will be promptly made available to the physicians for immediate decision making.

UNIVERSITY OF IBADAN LIBRARY

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

Sharing of benefits:

The immediate benefits of this study were as stated above. On the long term, the principal investigator hopes to present the data for the award of a degree.

Conflict of interest: None to be declared.

Person to Contact for answer to any question:

Bose Etaniamhe Orimadegun

Address:

Department of Chemical Pathology

College of Medicine,

University of Ibadan,

Ibadan, Nigeria

Mobile phone number: 08060660894

e-mail: boorimadegun@yahoo.com

If you have any question about your right to participate or ethical issues relating to this study you can contact:

The Chairman

University of Ibadan/University College Hospital Joint Ethical Review Committee

IMRAT

College of Medicine,

University College Hospital

Ibadan.

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

Statement of investigator obtaining the consent:

I have fully explained this research to Mrs/Mr _____

On or both parent (s) of _____ and

have given sufficient information, including about risk and benefits, to make an informed decision.

_____ _____

Name/Signature of investigator Date

Statement of person giving the consent:

I (names) _____ the father/ mother/

guardian of _____ have

read or had the description of the research read to me or have had the detail translated into the language I understand. I have also talked it over with the doctors and investigator. I understand that my ward's / child's participation is voluntary. I know enough about the purpose, methods, risks and benefits of the research study to judge that I want my child to take part in it. I have received a copy of this consent form.

Date: _____ Signature/Thumb printing _____

_____ _____

Witness's signature/thumbprint Date

AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY