COLLEGE OF MEDICINE

Burden and predictors of metabolic syndrome in adolescents and young adults in Blantyre district, Malawi.

By

Queen Mwakhwawa

(MBBS)

(M201770074072)

Supervisor

Prof Kenneth Maleta

(MBBS, PhD)

A Research Project Dissertation Submitted in Partial Fulfilment of the Requirements of the Master of Public Health Degree

7th February 2020

DECLARATION

I Queen Mwakhwawa hereby declare that this dissertation is my original work and has not been presented for any other awards at the University of Malawi or any other University.

Name of Candidate	Queen Mwakhwawa
Signature	_____
Date	7th February 2020

CERTIFICATE OF APPROVAL

The Dissertation of Queen Mwakhwawa is approved by the Dissertation Examination Committee

_____

(Chairman, Post Graduate Committee)

_____

Prof Kenneth Maleta (Supervisor)

_____

Peggy Papathakis (Internal Examiner)

_____

Dr Charles Mangani (Head of Department)

ABSTRACT

Introduction: Metabolic syndrome (MetS) is a major public health concern as it is on the rise in developing countries. However, there is limited regional and national data specifically in adolescents to help drive interventions against MetS.

Broad Objective: To describe the burden and predictors of MetS in adolescents and young adults in Blantyre district.

Methodology: This was a cross sectional study that used secondary data. MetS related risk factors from socio-demographic characteristics, anthropometric indices and body composition by deuterium dilution technique (DDT) were assessed. MetS was diagnosed using 1) the international diabetes federation (IDF) criteria by the presence of three or more of the following components: raised blood pressure (BP), raised fasting blood glucose (FBG), reduced high-density lipoprotein (HDL), raised triglycerides and larger waist circumference (WC), 2) Metabolic syndrome severity score (MSSS) using these inputs: age, gender, race, weight, height, FBG, BP and HDL on an online calculator. Multivariate logistic regressions were applied to identify MetS -associated risk factors.

Results: The study enrolled 371 adolescents and young adults aged 10-28 years The most prevalent MetS component was raised FBG at 31.3 % and reduced HDL at 30 % while the least common component was raised blood pressure BP at 3%. The overall prevalence of MetS was 3.1% by MSSS and 2.5% by IDF criteria and all participants had mild MetS. There was no difference in MetS prevalence by all socio-demographic characteristics and anthropometric indices including history of treatment for acute malnutrition except for waist to height ratio (WHtR). High WHtR and excess fat % were associated with MetS in unadjusted regression analysis with Odds Ratio (OR) 95% Confidence interval (CI)) of 5.18 (0.33,0.91) and 8.87 (1.91,41.08) respectively. After adjusting for sex, age, maternal occupation, WHtR and WHR, participants with excess fat % had 6 times more risk of MetS compared to those with no excess fat%, OR (CI) of 5.88, (1.37,35.4).

Conclusions: MetS was relatively rare in this population at 3% prevalence. Abnormal body composition, especially presence of excess fat% increases the odds of presence of MetS.

Table of Contents

DECLARATION ii
CERTIFICATE OF APPROVAL iii
ABSTRACT iv
ACKNOWLEDGEMENTS 8
LIST OF TABLES 9
LIST OF FIGURES 11
ABBREVIATIONS AND ACRONYMS 12
CHAPTER 1: INTRODUCTION AND OBJECTIVES OF THE STUDY 15
1.1 Background 15
1.2 Statement of the problem 16
1.3 Literature review 24
1.3.1 Socio-demographic predictors of MetS 24
1.3.2 Nutritional predictors of MetS 26
1.3.3 Body composition predictors of MetS 29
1.4 Justification of the study 32
1.5 Objectives 33
1.5.1 Broad Objective 33
1.5.2 Specific objectives 33
CHAPTER 2: METHODS 34
2.1 Type of research study 34
2.2 Study place 34
2.3 Study population 35
2.4 Study period 36
2.5 Sample size 36
2.6 Inclusion and exclusion Criteria 37
2.7 Data collection 37
2.7.1 Data collection 37
2.7.2 Interviewer administered questionnaires 39
2.7.3 Assessment of body composition by DDT 39
2.7.4 Fasting blood glucose assessment 40
2.7.5 Blood pressure measurement 40
2.7.6 Blood sample collection and laboratory analysis for Trig and HDL 40

2.7.7 Anthropometry measurements 41
2.7.8 Assessment of MetS 44
2.8 Data Management 46
2.8.1 Data quality in the field and clinic 46
2.8.2 Data processing and cleaning 47
2.8.3 Statistical analysis 47
2.8.3 Results presentation 48
2.8.4 Dissemination of results 48
2.9 Study Limitations 48
2.10 Ethical Considerations 49
2.10.1 Research approval 49
2.10.2 Protection of human subjects 49
2.10.3 Informed consent 50
2.10.4 Respect for autonomy 50
2.10.5 Confidentiality 50
2.10.6 Benefits 50
CHAPTER 3: RESULTS 51
3.1 Socio-demographic, anthropometric and body composition characteristics 51
3.2 Prevalence of components of Mets 54
3.3 Prevalence and severity of MetS 58
3.4 Unadjusted association of MetS with socio-demographic, anthropometric and body composition predictors 61
3.5 Multivariate association of MetS with socio-demographic, anthropometric and body composition factors 65
CHAPTER 4. DISCUSSION 68
4.1 Prevalence of MetS 68
4.2 Socio-demographic predictors and MetS 69
4.3 Anthropometric predictors and MetS 71
4.4 Body composition predictors and MetS 72
CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS 73
5.1 Conclusions 73
5.2 Recommendations 73
References 74
Appendices 81

Appendix 1: COMREC approval for primary data collection 81
Appendix 2: Permission to use secondary data 82
Appendix 3: English Consent forms 83
Appendix 4: Chichewa consent forms 86
Appendix 5: Standard Operating Procedures for deuterium dilution technique 91
Procedure for assessing body composition in children and adults with analysis of deuterium enrichment using the Agilent 4500 Series Tumbl-IR spectrometer 91
Appendix 6: Data collection forms for primary data collection 113

ACKNOWLEDGEMENTS

Firstly, I would like to thank God for giving me wisdom and inspiration to write this paper. Secondly, I would like to thank my supervisor Professor Kenneth Maleta for the excellent supervision and guidance given to me throughout the process of writing this paper. I submit my gratitude to the International Atomic Energy Agency (IAEA), Department of Nutrition and HIV/AIDS (DNHA) and African Centre of Excellence in Public Health and Herbal Medicine (ACEPHEM) for the financial support. I am grateful to Public Health Nutrition Group, Masters in Public Health lecturers and classmates for their support during my MPH course. I am grateful to Oscar Divala and Jessie Khaki for their statistical input. Finally, I would like to thank the RAF6052 project team and the study participants for making it possible for me to have data to work with. Most importantly I would like to thank my family; my husband Brian for all the tremendous support, my kids Brian-Ryan and Don- Paul for their endless inspiration, my mother, sisters and brothers for their support and encouragement.

LIST OF TABLES

Table 1. Summary of approaches used to calculate the continuous metabolic syndrome score in adolescents and children 20

Table 2. Description of variables collected at the 12 year follow up indicating which variables were collected for the primary analysis and those that were included in secondary analysis 38

Table 3. Anthropometric indices cut-off points 43

Table 4: The IDF consensus definition of metabolic syndrome components' cut off points in children and adults 45

Table 5. Categorisation of severity of MetS derived from MSSS calculator 46

Table 6A: Socio-demographic characteristics of adolescents and young adults in Blantyre district 52

Table 6B: Anthropometric and body composition characteristics of adolescents and young adults in Blantyre district 53

Table 7A. Prevalence of MetS components by socio-demographic characteristics of adolescents and young adults in Blantyre district 55

Table 7B. Prevalence of MetS components by anthropometric and body composition in adolescents and young adults in Blantyre district 56

Table 8A. Prevalence of MetS by socio-demographic characteristics amongst adolescents and young adults in Blantyre district 58

Table 8B. Prevalence of MetS by anthropometric and body composition characteristics amongst adolescents and young adults in Blantyre district 59

Table 9A. Unadjusted association between socio-demographic predictors and prevalence of MetS amongst adolescents and young adults in Blantyre district 62

Table 9B. Unadjusted association between anthropometric and body composition predictors of MetS and MetS prevalence of MetS amongst adolescents and young adults in Blantyre district 64
Table 10. Adjusted association between predictors of MetS and MetS prevalence 66

LIST OF FIGURES

Figure 1: *Pathophysiology of the development of Metabolic syndrome* *17*

Figure 2: *Equations for the development of Sex and Race/Ethnic-Specific Childhood Metabolic syndrome severity score* *23*

Figure 3: *Glucose levels in oral glucose tolerance test over 120 minutes in adult survivors of severe acute malnutrition as well as their controls* *28*

Figure 4. *Schematic diagram of how deuterium is used to estimate total body water* *31*

Figure 5: *Schematic diagram of the study design indicating the relationship between the cross-sectional study and the parent cohort study.* *34*

Figure 6. *Prevalence of components of metabolic syndrome in children who were previously malnourished and age sex and community matched peers with no previous history of malnutrition.* *57*

ABBREVIATIONS AND ACRONYMS

1. BMI	Body Mass Index
2. CVD	Cardiovascular Disease
3.COMREC	College of Medicine Research and Ethics Committee
4. DoHA	Developmental Origins of Health and Disease
5. HDL	High-Density Lipoproteins
6. IDF	International Diabetes Federation
7. MetS	Metabolic Syndrome
8.MPH	Master of Public Health
9. MSSS	Metabolic Syndrome Severity Score
10. NCDs	Non-Communicable Diseases
11. SAM	Severe Acute Malnutrition
12. T2DM	Type 2 Diabetes Mellitus
13.FTIR	Fourier Transfer Infrared
15. WHtR	Waist to Height Ratio
16. WHR	Waist to Hip Ratio
17. Trig	Triglycerides
18. BP	Blood Pressure
19. FBG	Fasting Blood Glucose
20. WC	Waist Circumference

21. SES	Socio-Economic Status
22. cMetS	Continuous Metabolic Syndrome Score
23. DDT	Deuterium Dilution Technique
24. OR	Odds Ratio
25. CI	Confidence Interval
26. REDCap	Research Electronic Data Capture
27. TEM	Technical Error of Measurement
28. HIV	Human Immunodeficiency Virus
29. ART	Anti-Retroviral Therapy
30. LMIC	Low- and Middle-Income Countries
31. FFA	Free Fatty Acids
32. hsCRP	Highly Sensitive C Reactive Protein
33. PCA	Principal Component Analysis
34. CFA	Confirmatory Factor Analysis
35. FM	Fat Mass
36. FFM	Fat Free Mass
37.TBW	Total Body Water
38. 2C model	2 Compartment model
39. 3C model	3 Compartment model
40. 4C model	4 Compartment model

42. ROC	Receiver Operating Curve
43. SSA	Sub-Saharan Africa
44. QECH	Queen Elizabeth Central Hospital
45. WHO	World Health Organisation

CHAPTER 1: INTRODUCTION AND OBJECTIVES OF THE STUDY

1.1 Background

Metabolic syndrome (MetS) is a complex disorder which has tremendous impact and socio-economic burden on global health[1]. Different aetiological factors have been implicated in the development of MetS. These include genetic (age, sex, ethnicity), epigenetic factors (intra-uterine growth restriction (IUGR) and early childhood under-nutrition), geographical location, psycho-social factors, physical inactivity, intake of high caloric foods, high saturated fat foods and excess alcohol, smoking and drugs' side effects e.g. anti-retroviral therapy (ART) for the treatment of human immunodeficiency virus (HIV) [2] [3].

MetS leads to cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) which are part of non-communicable diseases (NCD) that contribute to 71% of all deaths globally [4] and 85% of all deaths in individuals aged between 30-69 years in low- and middle-income countries(LMIC) [4]. MetS increases the risk of developing CVD and T2DM by two- and five-fold respectively [5]. Adolescents with MetS grow up into adults with MetS that later develop CVD and T2DM [6] For every one-unit elevation in childhood MetS Z-score, the odds ratio of developing future T2DM was 2.7 for incident disease by the mean age of 38.5 years ($p<0.01$) [7].

Globally, the prevalence of MetS is estimated at 25% [8][9] . However, this prevalence ranges from as low as < 10.0% to as high as 89% depending on age, ethnicity, sex, setting and the definition of MetS used. MetS prevalence increases with age and is more common in urban populations and women. [10][11]. In addition, MetS has been shown to be more prevalent in individuals with T2DM (80%) and hypertension (21.2%) than the general population. For example, MetS prevalence was 2% amongst 25-74 year olds in Cameroon in the general population compared to 80 % in T2DM in Nigeria and Europe [8][3] In the general population, four decades ago, MetS was more prevalent in developed countries (17% to 25%) than developing countries (0% to 13%) [8]). However, in the past three decades, MetS has increased in developing countries making it comparable to developed countries. For example, the overall adult prevalence of MetS was 34.7% in the USA from 2003 to 2012[12] comparable to 37.1% in urban India in 2014[13] and 31% in South Africa in 2009[14].

Nutrition has been implicated in the development of MetS. Over-nutrition leads to dyslipidaemia which later leads to MetS [3]. Several studies have shown that under-nutrition in childhood most specifically stunting is associated with MetS because it leads to a state of chronic low grade inflammation which later leads to MetS [15]. However, there is paucity of data linking acute malnutrition in childhood (marasmus and kwashiorkor) to MetS.

MetS is a major public health big concern as it is on the rise in developing countries including Sub Saharan Africa (SSA). However, there is limited regional data specifically in adolescents that would help developing countries traditionally struggling with infectious diseases and undernutrition to now deal with the triple burden of infectious diseases, under- and over-nutrition including MetS. The objective of this study was therefore to describe the burden and understand some risk factors for the development of MetS in Blantyre district.

1.2 Statement of the problem

1.2.1. MetS

1.2.1.1 Definitions

MetS is defined as a clustering of risk factors that directly increase the risk of CVD and T2DM[1] independently as well as synergistically[16]. These risk factors include central obesity, hyperglycaemia, high blood pressure (BP), increased serum triglycerides (Trig) and reduced high-density lipoprotein cholesterol (HDL). These 5 risk factors are also called the components of MetS. MetS is diagnosed by the presence of three or more of these components with these cut offs; 1) high BP (systolic≥ 130 mmHg/diastolic ≥ 85mmHg, 2) raised fasting blood glucose (FBG) (≥ 100mg/Dl), 3) central obesity (waist circumference (WC) ≥ 90^{th} percentile), 4) low HDL (< 40mg/dL and 5) elevated Trig (≥ 150mg/Dl).

1.2.1.2 Pathophysiology of MetS

Mets is a state of chronic low-grade inflammation that is characterised by visceral adiposity, insulin resistance, atherogenic dyslipidaemia, endothelial dysfunction, genetic susceptibility, elevated BP, hyper-coagulable state and chronic stress. It develops from an interaction of genetic and environmental factors. Genetic predisposition such the thrifty phenotype hypothesis entails that babies with intrauterine malnutrition may adapt to under-nutrition and underutilise nutrients in times of plenty in adulthood leading to a state of excess nutrients in the body. At the same time,

environmental factors like high calorie and saturated fat food and physical inactivity lead to a state of excess nutrients or energy in the body. This state of excess nutrients stimulates adipose tissue cells in visceral fat to grow in size (hypertrophy) and numbers (hyperplasia). Progressive enlargement of adipocytes leads to hypoxia, necrosis and macrophage infiltration which lead to the production of free fatty acids (FFA) and adipokines. FFA lead to dyslipidaemia, atherosclerosis (which leads to hypertension), inhibit glucose uptake in skeletal cells leading to insulin resistance and impairs pancreatic β cells (which lead to hyperglycaemia) [17] The figure 1 summarises the pathophysiology of the development of MetS.

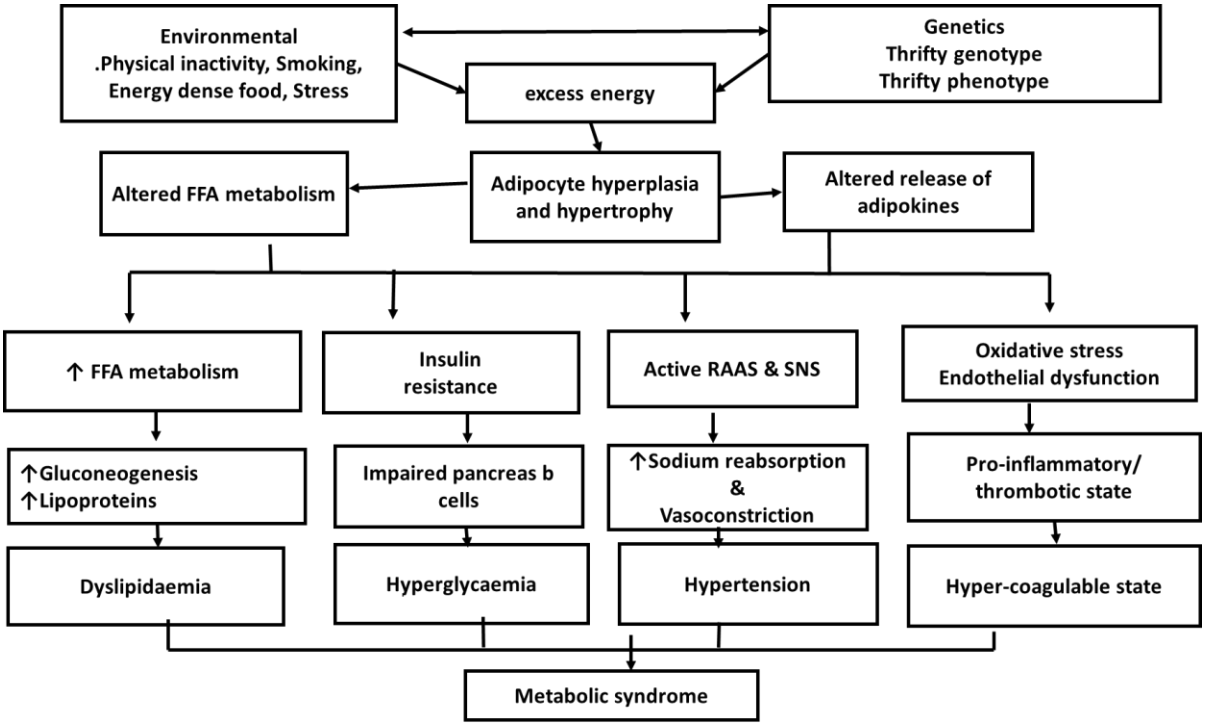


Figure 1: Pathophysiology of the development of Metabolic syndrome. Genetic and environmental factors lead to a state of excess energy that stimulate adipocytes to release FFA and adipokines. These stimulate a cascade of pathways that lead to dyslipidaemia, hyperglycaemia, hypertension and hyper-coagulable state that later lead to Metabolic syndrome. ATII = angiotensin II, PAI-1= plasminogen activator inhibitor-1, RAAS: renin-angiotensin aldosterone system, SNS = sympathetic nervous system.; FFA = free fatty acids Adapted from Kaur et, al 2019.[3].

1.2.1.3. Assessment of MetS

MetS has traditionally been diagnosed in a dichotomous criterion that quantifies the presence or absence of individual risk components. These components include insulin resistance, body weight, BP, glucose and lipids. Since 1998, five common criteria have been developed to diagnose MetS. Each new criterion builds on the shortfalls of the previous one and has different cut offs for the 5 components. Furthermore, some criteria include insulin resistance while others do not. These criteria include 1) World Health Organisation (WHO) (1998), 2) European Group for the Study of Insulin Resistance (EGIR1999), 3) National Cholesterol Education Program-Third Adult Treatment Panel (NCEP: ATPIII 2001), 4) American Association for Clinical Endocrinology (AACE, 2003) and 5) International Diabetes Federation (IDF 2005). Only the IDF and ATP III do not include insulin resistance. Since 2005, the IDF criteria has been widely used and no other criteria has been set. However, the initial IDF criteria used waist circumference as a prerequisite for MetS diagnosis. MetS then was diagnosed if an individual had higher WC and 2 of the other MetS components. However, since 2009, the IDF criteria diagnose MetS by the presence of three or more of the MetS components (raised BP, raised Trig, raised FBG, reduced HDL and higher WC) with cut offs as described above. Several reviews have suggested that using the dichotomised criteria may underestimate the presence of MetS [18]. For example, dichotomous criteria being a binary test may miss detection of individuals with abnormalities in multiple MetS components just shy of the cut-offs. In addition, MetS appears to manifest differently between males and females and among different races/ethnicities. Unfortunately the binary criteria are not sex and race specific which may lead to misdiagnosis of MetS[19] [17].

Continuous metabolic syndrome scores (cMetS) have thus been developed from the dichotomous criteria to overcome these limitations. Development of cMetS involves selection of variables to be incorporated in the score, (these are the MetS components/risk factors such as glucose and BP) and variables to be used to standardize the score (e.g. demographic factors like age, sex and race). Once selected, statistical procedures are initiated with the first being regressing the MetS components (dependent variables) on the demographic variables (independent variables). The standardized residuals of the MetS components (e.g. Z-FBG) are then saved as Z scores. These Z-scores are then later summed up to come up with the MetS score. Some approaches go further to use data reduction techniques such as principal component analysis (PCA) which measures

variance in a set of variables in a linear pathway or confirmatory factor analysis (CFA) which measures a latent variable to derive the score [9]. The scores being continuous do not only diagnose the presence of MetS but also its severity. For example, a score of 3 signifies severe MetS than a score of 2. In addition, the score is used to track MetS overtime if interventions like physical activity or balanced diets are used. In that scenario, an individual can move from a score of 3 to 1 overtime with interventions. In addition, continuous MetS scores have demonstrated significant associations with MetS risk factors, proposed pathway factors and protectors of MetS. For example cMetS was higher in individuals with adverse lifestyle factors such as smoking and drinking [7],[20],[21],[22] and those with raised high sensitive C-Reactive Protein (hsCRP) [23]. In addition cMetS showed to decrease with physical activity [24]The table 1 describes different methods or approaches for developing cMetS in adolescents and children.

Table 1. Summary of approaches used to calculate the continuous metabolic syndrome score in adolescents and children.

Study	**Obesity**	**Lipids**	**Glucose/ insulin**	**Blood pressure**	**Other**	**Statistical approach**
Bogolusa Heart Study	-	HDL	Insulin	SBP	-	Sum of the individual rankings by age-, sex-, and race-specific levels
Young Danes Study	Skinfolds	HDL, Trig	-	SBP and DBP	Smoking	Upper centiles
Cardiovascular Risk in Young Finns	-	TC and HDL	-	DBP	-	Upper tertile
European Youth Heart Study	Skinfolds	HDL, Trig	Glucose and insulin	Average f SBP and DBP	-	Sum of six Z scores divided by 6
Corpus Christi Child Heart Study	BMI	HDL, Trig	Insulin	SBP	-	2 approaches: 1) sum of Z scores, and 2) PCA
Quebec Family Study	Skinfolds	HDL, and Trig TC:HDL	Glucose	MAP	-	PCA
Aerobic Center longitudinal Study	WC	HDL and Trig	Glucose	MAP	-	Sum of age-standardized residuals

Australian Health and Fitness Study	WC	HDL and Trig	-	MAP	-	Sum of age-standardized residuals
Physical Activity across the Curriculum	WC	HDL and Trig	HOMA	MAP	-	Sum of age-, sex-, and race-standardized residuals
National Health and Nutrition Examination Study (MSSS)	BMI	HDL	Glucose	SBP	-	2 approaches: 1. Sum of age-, sex-, and race-standardized residuals 2. CFA

BMI = body mass index; WC = waist circumference; TC = total cholesterol; HDL = high density lipoprotein-cholesterol; Trig = triglycerides; HOMA = homeostasis model assessment; SBP = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean arterial pressure; CFA = confirmatory factor analysis, PCA = principal component analysis. Adapted from Eisenmann et, al 2008 [9]

MSSS

MSSS is one example of a cMetS. MSSS was developed using cross-sectional data from the National Health and Nutrition Examination Survey (NHANES) in USA on 4,174 adolescents age 12–19 years and 6,870 adults age 20–64 years [13,14]. It uses a CFA that evaluates how the 5 components of MetS interact together to give a MetS score considering that each component contributes differently to the score according to its strength of association with MetS [19]. As such, for each of these 5 components, it uses different weightings and incorporates sex and race/ethnicity in its equation.[25] [19][26]. MSSS has been validated for use in adolescents [21]. A national school-based surveillance in Iran in 2015 in 3843 students aged 7-18 years found that MSSS was highly accurate in predicting children and adolescents with MetS in all gender and age groups with its sensitivity at 93% and specificity at 82% and area under the receiver operating curve (ROC) curve at 94%. The score can be calculated using the developed equations or using an online calculator accessed on http://metscalc.org. using the following inputs: weight, height, birth date, FBG, systolic blood pressure, HDL, patient's race/ ethnicity, patient's gender as described in table 1. The figure 5 describes the equations for the development of MSSS from the 5 components of MetS in male and female children. As shown in the figure, different races have different weightings.

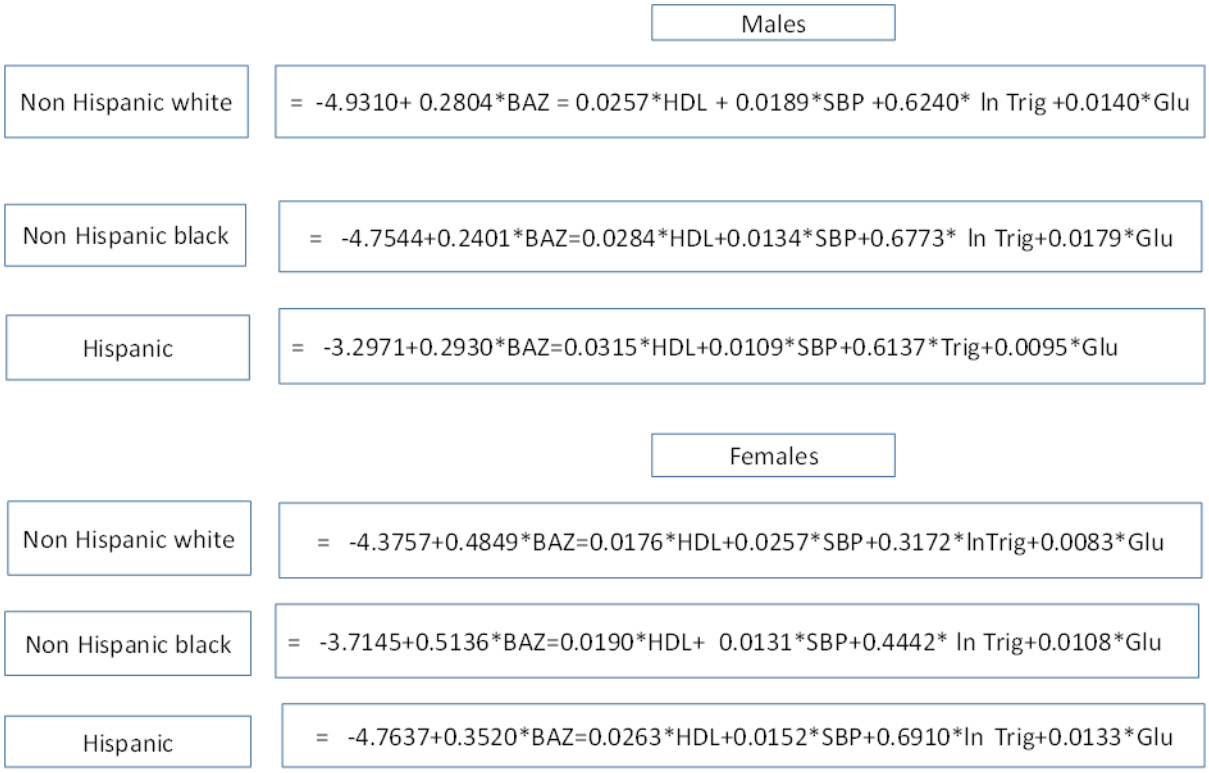


Figure 2: *Equations for the development of sex and race/ethnic-specific childhood Metabolic syndrome severity score. Key: BAZ = body mass index Z-score; HDL= high-density lipoprotein, SBP: systolic blood pressure, Trig= triglycerides; Glu= glucose. Adapted from Gurka et, al 2012 [25].*

1.2.1.4 Global and regional prevalence of MetS

The global prevalence of MetS is estimated at 25% [9]. The prevalence of MetS varies by age, sex, ethnicity and setting. MetS increases with age, tends to be more common in women especially postmenopausal, obese people, diabetic and hypertensive individuals and urban populations. As regards to age differences in MetS prevalence, in the USA, in 2003-2012, the prevalence of MetS was 9.8% in adolescents aged 12–19 years [27], 19% in adults 20–39 years, 34% in adults 40–59 years, and 47% in adults ≥60 years[12]. In keeping with this, in Nigeria, MetS prevalence increased from 11% in subjects aged 20-29 years to 89% in those aged 70- 79 years [8].A study done in Cape Town, South Africa found that among 25–74-year-old urban Africans, 31% of the population had MetS, with women having a higher prevalence (43.5%) compared to men (16.5%). They found central obesity the most common component followed by reduced HDL then high BP[14]. The prevalence of MetS in adolescents in Africa was 7.4% in Egypt and 4.5% in South Africa [8].

1.2.1.5 Epidemiology of MetS in Malawi

In Malawi in 2013-2016, a health survey on NCDs in urban and rural adult populations ≥ 18 years of age showed that overweight and obesity, hypertension, and diabetes were highly prevalent. The prevalence was even higher in urban residents, the less poor, and better educated than in rural, the poorest, and least educated participants. The prevalence of overweight and obesity was at 18% in urban men, 44% in urban women, 9% in rural men and 27% in rural women [28]. The findings also showed that the overall prevalence of diabetes was 2% and highest in urban residents while hypertension was 13% and highest in men. [10]. There have been no studies known to the author that have looked at MetS as a whole syndrome in Malawi in either adults or adolescents.

1.3 Literature review

1.3.1 Socio-demographic predictors of MetS

1.3.1.1 Genetic predictors

Genetics have been implicated in the development of MetS. Mets is more common in Hispanics, followed by non-Hispanic white then Blacks. Mets increases with age. For example, in the USA in 2003-2012 the prevalence of MetS was 9.8% in adolescents aged 12–19 year, 19% in adults 20–39 years, 34% in adults 40–59 years, and 47% in adults ≥60 years[12]. Mets prevalence also varies with sex. Mets is more common in females than males in adulthood but more common in males than females in adolescence. For the specific MetS components, obesity has shown to be higher in females whereas hypertension tends to be more common in males [12][14]. In the USA from 2003-2012, there was a male vs female predominance in adolescence (10.9% vs 6.29%) that reversed by mid-adulthood (18% vs 20% at age 20–39 years) and widens thereafter in later adulthood (42% in males and vs 51% in females by age ≤60 years) [12]. A study done in Cape Town, South Africa found that among 25–74-year-old urban Africans,31% of the population had MetS, with women having a higher prevalence (43.5%) compared to men (16.5%) [14].

1.3.1.2 Epigenetic predictors

These occur in utero and early childhood and affect the development of MetS in adulthood. The thrifty genotype theory suggests that genes derived from times of deprivation may result in adaptive processes that have adverse effects in times of plenty For example, some schools of thought suggest that poor nutrition in utero may lead to β cell dysfunction of pancreas cells that

may predispose to the development of T2DM [29] [30] The Developmental Origins of Health and Disease (DOHaD) concept postulates that developing foetuses and potentially young children undergo adaptive epigenetic changes that have long term effects on metabolism and other processes [31][32]. In early childhood, excess or deficits in nutrients, hormones, or metabolites may trigger changes in DNA or histone methylation, which in turn suppresses or enhances gene expression for the development of MetS. In addition, a mismatch between the early environment in utero or early childhood (undernutrition) and later environment in late childhood and adulthood (excess calories) renders detrimental for the development of MetS. Children who IUGR have shown to have increased risk of developing MetS than those who did not have IUGR[33][34].

1.3.1.3 Social predictors

For any expression of a gene, there are environmental factors that suppress or enhance its phenotype. In MetS development, environmental factors have been implicated in its development. There are direct social factors that have shown to increase MetS such as 1) high calorie and high fat diet 2) reduced physical activity,3) smoking, 4) alcohol intake and 5) stress. These factors are driven by other (indirect) social factors[11][5][35] [36] .These include, 1) economic factors where individuals with higher income tend consume unhealthy food which has a lot of sugar and fat and have sedentary lifestyles 2) psychological studies have shown that depressed individuals tend to consume more unhealthy food which has a lot of sugar and fat, chronic stress releases cortisol that stimulates FFA synthesis that leads to the development of MetS[3], 3) legislative factors; reduced costs of unhealthy food on the market invite more individuals to consume it; few health promotion policies and adverts on importance of physical activity and the risks of unhealthy food 4) social factors, like peer pressure for smoking and drinking alcohol,5) cultural perceptions that elite individuals in society need to be fat in appearance and that a good looking woman needs to be fat in appearance 4) education- usually individuals with higher education tend to have higher economic status which is associated with sedentary lifestyle.5) geographical location, developed countries have higher rates of physical inactivity and high calorie intake compared to developing countries and7) urbanization; life styles in urban areas are sedentary in nature because of use of vehicles as transport modes unlike in rural areas where individuals walk long distances

A few studies have looked at the association between the described social factors and MetS (CVD and T2DM). In Malawi, in 2013-2016, a cross sectional study done in adults investigating the

association of general and central adiposity with glycaemia and BP by gender and area of residence found that smoking was more prevalent in the urban than rural and urban settlers were in the highest fifth of wealth and had the lowest physical activity as compared to the rural population. In addition, hypertension, diabetes and higher body mass index (BMI) were more prevalent in urban than rural populations.[10]. In the same population, higher education attainment was associated with overweight and obesity; p value <0.0001 [28]. Similar findings were found in Iran in 2014 amongst 30-70 years' adults where MetS prevalence was higher in urban than rural populations; p value <0.002. Furthermore, a cross-sectional study amongst Brazilian adolescents aged 14-19 years in 2014, found that adolescents that watched television daily for two or more hours (OR=2.11, 95%CI 1.08-4.13) had a higher chance of having abdominal obesity. They further found out that adolescents whose mothers had fewer than eight years of schooling (OR=0.56; 95%CI from 0.35 to 0.91) had a lower chance of having abdominal obesity [35]. Finally, a cross sectional study in a Korean adult population aged 20-79 years in 2013, found that suicidal thoughts in men (OR 1.64, CI 1.03–2.61) and perceived stress in women (OR 1.26, CI 1.01–1.59) were associated with MetS [36].

1.3.2 Nutritional predictors of MetS

1.3.2.1 Definitions

Nutrition is the intake of food for growth, metabolism and repair. It encompasses the interaction between food and the body. Malnutrition, classified as over-nutrition and under-nutrition, refers to deficiencies, excesses or imbalances in a person's intake of energy and/or nutrients. Under-nutrition includes stunting (low height for age) wasting (low weight for height), underweight (low weight for age) and micronutrient deficiencies. Under-nutrition is further classified as acute or chronic malnutrition, where acute malnutrition ordinarily results from sudden reduction in food intake or diet quality and/or pathological causes while chronic malnutrition occurs from sustained episode or episodes of under-nutrition[37]. Acute malnutrition is further classified as severe or moderate. On the other hand, over-nutrition includes overweight and obesity.

1.3.2.2 Assessment of nutritional status by anthropometry

Anthropometry is the measurement of size, weight and proportions. It is a widely used simple, inexpensive and non-invasive tool for the assessment of the general nutritional status of a child,

adult or population [38]. Anthropometry mainly uses length (or height) and weight in combination with age and sex. These measurements are used to develop indices and indicators for individuals and populations respectively. Indices are recorded as Z-scores. Z-scores are measured as standard deviations and describes how far and in which direction an individual's anthropometric measurement deviates from the measurement of a healthy person of the same sex and age (median)[38] Weight for height of <-3 SD is severe wasting(marasmus) and ≤-2 & >-3 is moderate wasting Kwashiorkor is characterised by oedema and loss of appetite. Height for age of <-2 is severe stunting [39].

BMI is measured by dividing weight in kilograms by height in m^2. Obesity is the accumulation of excess fat over time which manifests as increased weight or larger WC also known as central obesity. Central obesity as defined by WHO as WC of ≥ 102 cm and ≥ 88 cm for males and females, respectively, while generalized obesity is BMI of ≥ 30 kg/m^2, overweight is ≥ 25 & <30 kg/m^2 [8].

1.3.2.3 Global and regional prevalence of wasting, stunting and obesity

Severe acute malnutrition (SAM) affects millions of children and is the leading cause of death in early life in (LMIC)[33] accounting for about 45% of mortality among children under the age of 5 years[40]. Stunting is the most common type of undernutrition and affects 155 million of under 5 children worldwide. Approximately 25% of under-5 children are stunted and 90% of these live in SSA and Asia[41]. In 2016, worldwide obesity had tripled since 1975 and 39% of adults were overweight and 13% were obese. In Southern Africa in 2015, the prevalence of obesity was 21% representing a 330% increase in 25 years and was the highest compared to other African regions [42]

1.3.2.4 Malawi prevalence of stunting, wasting and obesity

The prevalence of stunting in Malawi has remarkably decreased from 55% in 1992 to 37% in 2015-2016. At the same time, wasting has decreased from 6% to 3% in the same period. In 2015-2016, 21% of women aged 15-49 years were obese or overweight and 5 % of under-5 children were overweight [43].

1.3.2.4 The role of nutrition status as assessed by anthropometry in the development of MetS

A few studies have been undertaken exploring the long-term effects of under-nutrition in childhood and the development of MetS, CVD and T2DM in adulthood The SPECT- China study in 2014 found that women who were exposed to the great famine in their foetal period or early childhood in 1959-1962 had significantly higher prevalence of MetS as adults (50-55 years post-famine) than women who were born after the famine from 1963-1974 (non-exposed) [44].In 2010 in Jamaica, a case control study between adults who had marasmus or kwashiorkor at 6-18 months from 1963-1992 were assessed against age-, sex-, and body mass index-matched community controls; and age- and birth weight-controls who never suffered from acute malnutrition. The findings showed that adult survivors of marasmus tended to be less insulin sensitive with significantly lower insulin secretion and more glucose intolerance compared with kwashiorkor survivors and the two controls p value < 0.05 [29]. The Figure 3 demonstrates glucose concentrations in marasmus and kwashiorkor survivors compared with controls during an oral glucose tolerance test (OGTT) in the study [29].

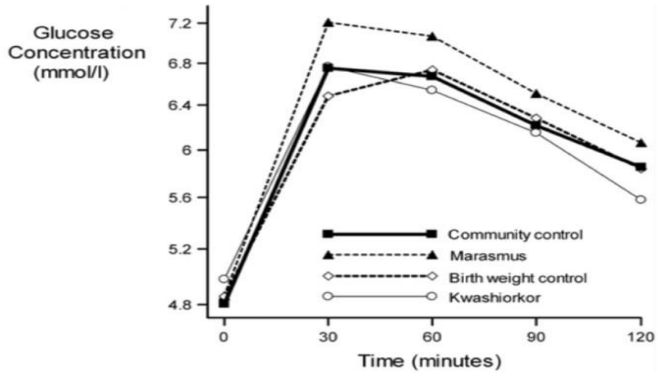


Figure 3: Glucose levels in oral glucose tolerance test over 120 minutes in adult survivors of severe acute malnutrition as well as their controls (matched for age, sex, birthweight and body mass index), N=191 adults. Glucose levels in marasmus survivors were significantly higher than kwashiorkor survivors and controls at 30, 60, and 120 minutes (P-value<0.05). Adapted from Patrice et, al 2014 [29].

In Malawi in 2013, severe and moderate acute malnutrition survivors who had acute malnutrition before the age of 2 years were assessed 7 years' post-treatment against their age and sex matched community controls who never suffered from acute malnutrition. The results showed that acute

malnutrition survivors had less lean mass and higher diastolic BP compared to their community controls.[45].

Individuals with central obesity have fat around their abdominal viscera such as the liver and pancreas. which is the dangerous type of fat as it predisposes them to MetS, CVD and T2DM. This is because visceral fat produces FFA that lead to insulin resistance and later T2DM. In addition, fat deposits in endothelium of blood vessels lead to atherosclerosis and narrowing of blood vessels which later lead to hypertension [3]. As such obese adults are at high risk of developing MetS, CVD and T2DM. In addition, higher WC is a component of MetS itself meaning those who are obese already have one component of MetS and therefore only require two more components to have MetS. It is known that obese children and adolescents grow into obese adults who are at high risk of developing MetS, CVD and T2DM [46]. In Beijing in 2015, a prospective BP cohort study which followed children for 22 years showed that the incidence of obesity in adults was 60% in those who had childhood obesity compared to 13.4% in those who did not have childhood obesity. In addition, the odds of having raised FBG and abdominal obesity in adulthood were 2.7 and 2.8 respectively if you had childhood obesity (95 % CI of 1.2–6.3 and1.6–4.7 respectively) [46]. Furthermore, a cross sectional study in 2009 in Brazil among 6-14 years students found that the odds of having MetS was 32.7 and 6.1 if a student was obese or overweight respectively (p value < 0.0001)[47].

1.3.3 Body composition predictors of MetS

1.3.3.1 Definitions

Body composition is an assessment tool for nutritional status. It refers to the amount of fat relative to muscle in the body. Total body mass is composed of fat mass (FM)which includes the entire content of chemical fat and lipids in the body and fat free mass (FFM) which includes the rest of the body apart from fat. FFM is also known as lean tissue or muscle. FFM comprises of bone, water and mineral [48]).

1.3.3.2 Body composition assessment

Several methods exist for the assessment of body composition. They are divided into 4 groups depending on how many compartments they divide the total body mass into during assessment. There are 1) two-compartment models (2C); 2) three compartment models(3C) 3) four compartment models (4C) and 4) multi-compartment models [49]. 2C models are the most

commonly used models. They divide the body into FM and FFM. They include densitometry (under water weighing and air displacement)) and skinfold thickness assessment. [50]. Densitometry methods assess body composition from the density of water or air displaced after placing a human being under water or in an air chamber [50]. Skin fold thickness assessment is the most commonly used, inexpensive and easy to use method. Measurements are performed by callipers in the following areas: 1) biceps, 2) triceps,3) subscapular and 4) supra-iliac area. The sum of the of the average measurements from the four areas is used in different equations to estimate body fat percentage [51]. The limitation of a 2C model. is that it assumes that total body water (TBW), bone and mineral do not suffer from variations like hydration status which later affect the body composition estimates. Furthermore, specifically for skinfold thickness assessment, body estimates are affected by the quality of the callipers used and the expertise of the individual doing the assessment.[48]

3C models further divide FFM into TBW and solids (bone mineral and body protein). They include dual energy Xray-absorptiometry (DXA), DDT and multi-frequency bio-impedance. The limitation of a 3C model is that it can be inaccurate in individuals with significantly depleted body protein mass. 4C models divide FFM into TBW, bone mineral and body protein. Multi-compartment models further divide FFM from the 4 components to measure either total hydrogen, carbon or bromide. The limitation of 4C and multi-compartment models is that they need more than one device to estimate body composition rendering them complex and expensive.

DDT is a 3C model reference method that has high precision in determining TBW to estimate FM and FFM. It uses deuterium oxide which is a stable isotope of hydrogen (^{2}H). Stable isotopes are non-radioactive variants of a specific element that have the same number of protons but different number of neutrons [49]. Deuterium exists naturally in water and soil. As such during the procedure, baseline saliva samples are taken to estimate the natural concentration of deuterium. During the procedure, a dose of water labelled with deuterium is given to a participant and, following equilibration after 3-4 hours, enrichment of the body water pool is measured using samples of either saliva, urine, or blood. Samples are generally analysed by isotope ratio mass spectrometry [51]. The figure 4 describes how deuterium administered in the body is used to calculate TBW.

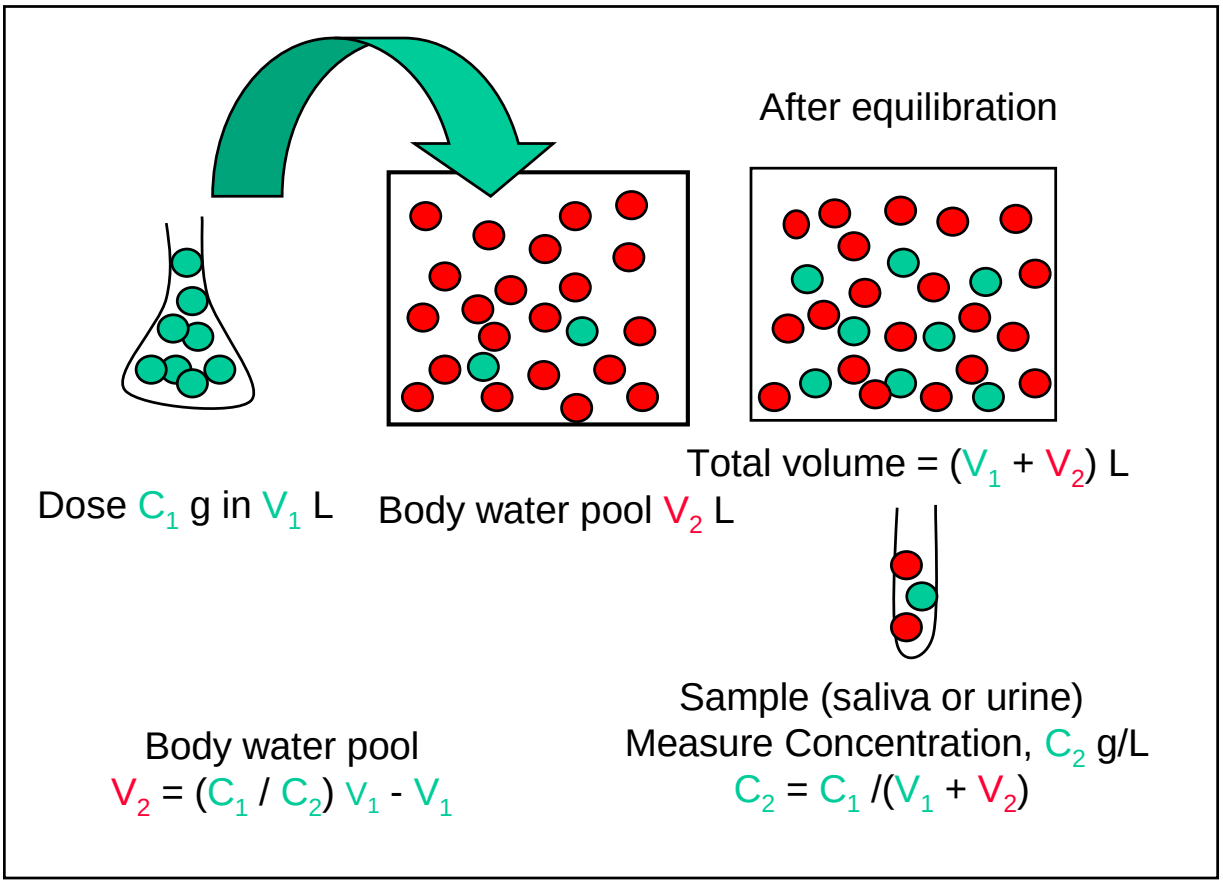


Figure 4. Schematic diagram of how deuterium is used to estimate total body water. A dose of deuterium labelled water is administered in the body. This dose mixes and dilutes with the total body water volume. After equilibration of 3-4 hours, a saliva or urine sample is taken to measure concentration of deuterium in the body. From the dose of deuterium given and the concentration of deuterium in the body measured from saliva, total body volume or water is calculated as V2=(C1/C2) V1-V2. Dose= deuterium labelled water; C1= concentration of deuterium administered; V1 = Volume of water in the administered labelled water; V2= total body water volume; C2 = concentration of deuterium in saliva or urine sample Adapted from Wells et, al 2005 [51].

The advantage of DDT is that it estimates body fat mass with high precision. The disadvantage is that it is expensive and not readily available for clinical use.

1.3.3.3 The role of nutrition as assessed by body composition in the development of MetS

The role of obesity in the development of MetS has been emphasised above. Body composition is a better too to assess obesity than anthropometry as it estimates the actual fat content unlike anthropometric indices like BMI, WC, WHR and WHtR. Few studies have been conducted to look at the association of body composition and MetS. For example, a cross sectional study in 14,807 Korean adults aged 18-65 in the year 2012 that used DXA found that a combination of high muscle and low fat was associated with significantly lower insulin resistance ($P < 0.001$) compared to (low muscle and low fat). In addition, a combination of (low muscle and high fat) and (high muscle and high fat) were significantly associated with the prevalence of MetS (p values < 0.001)[52]. Concurring with this, a study in Greece mong 5-15 years' children in 2011 found that body fat mas was positively associated with hsCRP, triglycerides and fasting insulin. HsCRP is an inflammatory marker of chronic inflammation that has been implicated in the development of MetS while fasting insulin has been shown to correlate positively with glucose [53][3]. Therefore, high insulin levels infer high blood glucose levels.

1.4 Justification of the study

MetS is a major public health concern as it is on the rise in developing countries including SSA. Adolescents with MetS grow up into adults with MetS that later develop CVD and T2DM. CVD and T2DM are one of the leading causes of deaths globally and in LMIC However, there is limited regional data specifically in adolescents that would help developing countries traditionally struggling with infectious diseases and undernutrition to now deal with the triple burden of infectious diseases, under- and over-nutrition including. The objective of this study was therefore to describe the burden and understand some risk factors for the development of MetS in Blantyre district.

1.5 Objectives

1.5.1 Broad Objective

The main objective of the study was to describe the prevalence and predictors of MetS in adolescents and young adults in Blantyre district, Southern Malawi.

1.5.2 Specific objectives

1. To describe the prevalence and severity of MetS in adolescents and young adults in Blantyre district.
2. To determine the predictors (demographic, anthropometric, body composition) of MetS in adolescents and young people in Blantyre District.

CHAPTER 2: METHODS

2.1 Type of research study

This was a cross sectional study that did secondary analysis of existing data collected as part of a prospective cohort study. The cohort study objective was to assess the medium-term (12 years' post-treatment) nutritional status, physical function, metabolic profile and body composition using nuclear techniques of children previously treated for moderate or severe acute malnutrition. The data used for the secondary analysis was collected as part of the 12 year follow up of the cohort members in 2018-2019.The design of the study is depicted in the figure 5.

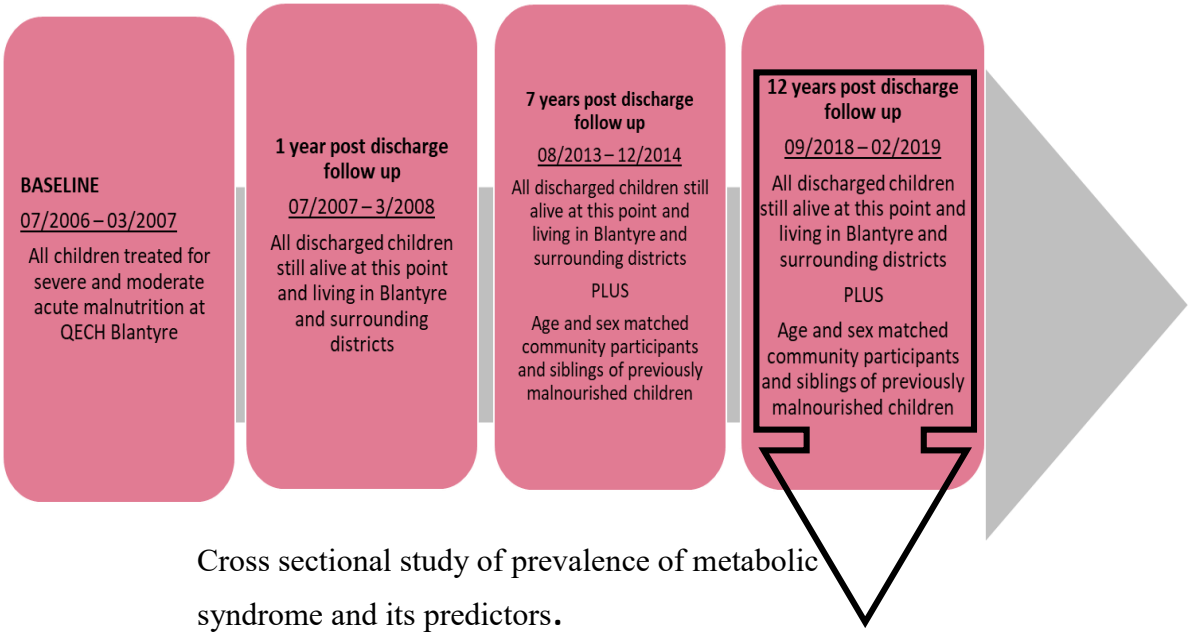


Figure 5: Schematic diagram of the study design indicating the relationship between the cross-sectional study and the parent cohort study.

2.2 Study place

The study was conducted in Blantyre district in Malawi. Malawi's population is relatively young with children below 15 years of age contributing to 48% of the whole population [43]. Blantyre district has a population of 1, 251,484 [54] of which 311,443 are adolescents aged 10-19 years representing about 25% of the whole Blantyre district population [54]. The prevalence of poverty, unemployment and self-employment in Blantyre are approximately at 24%, 8% and 36%

respectively. Approximately 65% of the Blantyre population live in informal settlements with poor living conditions [55].

In 2015-2016, 89.9% and 37.3% of the Blantyre population used improved sources of drinking water and improved sanitation respectively. In terms of education, 53.2% had completed primary school and 10% had completed secondary school. Most men were in manual work while most women were farmers [43]. Blantyre district had a fertility rate of 3.4 and an under-five mortality rate of 67 per 1000 live births. In under-five children, the prevalence of SAM was 1.3% and MAM was 3.1%. For women in the reproductive age, about 23.8% and 9.5% were overweight and obese respectively [43].

2.3 Study population

The study population included 3 different groups of adolescents and young adults resident in Blantyre district who participated in the CHROSAM study. CHROSAM study looked at chronic disease outcomes in children with previous history of acute malnutrition 7 years post-treatment Detailed description of the cohort has been published before [56][57][45]. The first group of the study population included adolescents and young adults who were previously treated for severe and moderate acute malnutrition in 2006-2007 at QECH and have remained in periodic follow-up until now. The second group included age, sex and community matched peers of the previously malnourished children who were added to the cohort in 2013-2014. The last group included siblings of the previously malnourished children aged ±5 years to the index malnourished child who were also added to the cohort follow up in 2013-2014. Of the original cohort members and those added in 2013-2014, only those still resident in and around Blantyre district at the time of the 2018-2019 survey were included.

The previously malnourished children were treated as in-patients at QECH with therapeutic milks (F75 and F100) and ready-to-use therapeutic food (RUTF) based on national guidelines in existence at the time adopted from community-based management of acute malnutrition and WHO guidelines[39][58]. Both the siblings and age, sex and community matched peers of the previously malnourished children had no history of treatment for acute malnutrition. During the 2018-2019 survey, all age, sex and community matched peers of the original cohort members who were not located were replaced with new members.

2.4 Study period

The secondary analysis was conducted from March 2018 to August 2019.

2.5 Sample size

There was no a priori set sample size. for this secondary analysis. All eligible adolescents and young adults who formed the cohort study sample as described above from whom data was available were included in the analysis. Accounting for drop outs, deaths and refusals, a total of 402 participants were surveyed in 2018-2019 comprising 152 previously malnourished children, 120 siblings and 130 age, sex and community matched peers of the previously malnourished adolescents and young adults and this represented about 70% of the cohort members resident in and around Blantyre.

There are no published reports of prevalence of MetS in adolescent Malawians but published reports from the nearby countries such as South Africa estimate MetS prevalence of 4.5% in black adolescents of this age group [59]. Diet and socio-economic factors are likely predictors of MetS[36][59] and different between the Malawian and South African populations which may result in differences in the prevalence of MetS components. For example, the prevalence of raised BP was 14.2% and raised FBG was at 2.1% in 2013 in Malawi [10] while in South Africa raised BP was at 49.5% and raised FBG at 18.6% in 2011[14]. Based on these assumptions we estimated the prevalence of MetS to be lower in Malawian adolescents. A prevalence of MetS of 3% was thus assumed for the Malawian adolescents and young people. A post hoc sample size estimate for a population proportion based on 90% power and 95% confidence (2 sided) yields a sample size of 50 based on the following formula:

$n= Z_{1-\alpha/2}^2 P (1-P)/d^2$

Where n = desired sample size

Z = Standard normal deviation (1.96 for a 95% confidence level)

P = the proportion of the population having the characteristic being measured. (The proportion is estimated at 3%, i.e. P = 0.03.

d=degree of accuracy will be set at 0.05

$n=1.96^2 \times 0.03(1-0.03) / 0.05^2 = 44.7 = 45 + (10\%$ non-response) $= 50$

2.6 Inclusion and exclusion Criteria

Inclusion criteria

1. All surviving adolescents and young adults who were treated for acute malnutrition at QECH Moyo ward clinic between 2006 and 2007 or their age, sex and community matched peers or their siblings aged ±5 years to the index malnourished adolescent or young adult included in the CHROSAM cohort study and resident in the Blantyre district area.

2. Signed or thumb printed informed consent from the young adults or signed or thumb printed informed consent from a legally acceptable guardian and assent from adolescents aged less than 18 years.

Exclusion criteria

1. Participants with evidence of severe or moderate acute malnutrition or severe chronic illness at the time of data collection.

2. Participants less than 10.0 years of age.

2.7 Data collection

2.7.1 Data collection

Data collection for the primary study analysis was conducted between September 2018 and February 2019. Locator maps and phone numbers collated during the 2013-14 survey were used to locate participants. In addition, health surveillance assistants (HSAs) from specific health centres also helped to trace participants. Participants were enrolled in their homes where socio-demographic characteristics and at times anthropometry were conducted and participants were booked to come to the central site (QECH) in the following two weeks for additional procedures. Phone call reminders to participants were made a day before the visit. Upon arrival at QECH, briefings for the clinic activities were done followed by identity confirmation then participants' bladder emptying. The following procedures were done in the clinic in this order: 1) DDT. 2) FBG assessment 3) blood pressure 4) blood sample collection 5) anthropometry and 6) Bioelectrical

impedance (BIA) All variables collected in the clinic were recorded on participants' clinic data collection forms (paper based) except for BIA where the data was stored in the BIA machine. The paper-based data was later transferred to the tablets with engraved data collection forms from a research electronic data capture (REDCap) application. Data collectors were trained on the use of REDCap prior to data collection and the application was pilot tested before use. Table 2 below summarises the variables collected from the participants for the primary study and the secondary analysis.

Table 2. Description of variables collected at the 12 year follow up indicating which variables were collected for the primary analysis and those that were included in secondary analysis

Variable	**Primary study**	**Secondary analysis**
Socio-demographic data	√	√
Weight	√	√
Height	√	√
Hip circumference	√	√
Waist circumference	√	√
Mid-upper arm circumference	√	X
Body composition from BIA	√	X
Body composition from DDT	√	√
FBG	√	√
Blood pressure	√	√
HbA1C	√	X
HDL	√	√

LDL	√	X
Trig	√	√
CRP	√	X
Leptin	√	X

BIA= bio-electrical impedance; DDT = deuterium dilution technique; FBG = fasting blood glucose; HbA1c = glycated haemoglobin; HDL = high density lipoprotein; LDL =low density lipoprotein; Trig = Triglycerides, CRP =C-reactive protein

2.7.2 Interviewer administered questionnaires

Questionnaires engraved in REDCap tablets were used to collect socio-demographic variables of interest. These included age, sex, household location, human immunodeficiency virus (HIV) status, anti-retroviral therapy (ART) use, level of educational attainment and occupation status of parents and marital status of caregiver.

2.7.3 Assessment of body composition by DDT

Body composition was estimated by DDT following an overnight fasting upon request. The examination was done in an adolescent or young adult who was well hydrated and had an empty bladder. Firstly, a baseline saliva sample was collected using a cotton wool ball as illustrated in the standard operating in appendix 3 and recorded in a data collection form. Secondly, a dose of 99.8%, deuterium (Cambridge Isotope Laboratories (CIL)) diluted in drinking water at a ratio of 1:10 was administered to participants in 60 ml plastic bottles using a straw. This dose was calculated at 0.1mg/kg body weight and prepared beforehand based on weight measured in the participant's home and stored in the administration dose bottles. Immediately after dose administration, the participants consumed 50ml of water twice to rinse the residual deuterium from the administration bottle. Post deuterium dose saliva samples were collected at 3 hours and 4 hours after taking the dose. Participants were instructed to avoid eating, drinking and any physical activity until the final saliva samples had been taken. To keep them occupied in between dose administration and saliva sample collection, the participants participated in other study procedures

and played games such as chess, *bawo* and cards. The saliva samples were stored in a cooler box with ice packs and later stored in a –20ºC freezer within 6 hours of collection.

The analysis of deuterium enrichment in the collected saliva samples was measured by an Agilent 4500 Fourier Transform Infrared spectrometer (FTIR) at the College of Medicine (COM) Mangochi campus. The enrichment of deuterium was used to calculate body composition as fat mass and fat free mass by trained FTIR technicians using standardised and validated excel spreadsheets with engraved formulas. The excel spreadsheet that was used is attached in appendix 5. Fat mass which was described as excess fat if body fat% calculated as (fat mass/ total body weight * 100) was ≥25 in males and ≥ 30 in females.

2.7.4 Fasting blood glucose assessment

Participants were requested not to consume anything from the dinner before the day of assessment. In clinic, before the procedure, participants were asked to confirm whether they fasted or not. A finger prick blood spot was done using cuvettes and the sample was analysed using Hemocue Glucose 201 RT.

2.7.5 Blood pressure measurement

BP was measured using sphygmomanometers and stethoscopes by trained nurses. Participants abstained from any vigorous physical activity and fluid intake before measurements. Participants sat quietly for about five minutes before measurement. Measurements were done in duplicate and were recorded to the nearest 2mmHg. The difference between two measurements did not exceed 5 mmHg, if it did the measurements were repeated by a second nurse [60].

2.7.6 Blood sample collection and laboratory analysis for Trig and HDL

Venous blood sample was collected using disposable sterile butterfly needles and intravenous cannulas (20 and 22 gauge) by trained nurses from the paediatric accident and emergency unit at QECH. A 10 mL venous blood sample was collected and distributed to one Ethylene Diamine Tetra-acetic Acid (EDTA) tube (5mL) and one anticoagulant tube (5mL). Samples were transferred twice per day from the clinic to the laboratory which was approximately 1.5 km away. Immediately after collection, samples were stored in a cooler box with ice packs for a maximum of 3 hours before being transferred to the COM laboratory. At the laboratory, on the same day of

sample collection and within 2 hours of sample arrival, the samples were processed into clear plasma and serum using a centrifuge that ran at 3000rpm for 15 minutes. A pipette was used to aliquot the plasma and serum from the EDTA tube and the anti-coagulant tube respectively into 2 ml cryovials for storage. All cryovials were stored in a -20^{0}C freezer for 4 weeks before being transferred to a -80^{0}C freezer [61]. This was for quality control to avoid sample degradation. Temperature readings and signs of refrigerator failure were always monitored on the freezers.

Serum samples for HDL and Trig were analysed using Mindray BS-120 Biochemistry analyser at Mangochi District Hospital by trained laboratory technicians. Quality control checks were done on a daily basis using multi-control sera. The technicians used prescribed reagent inserts per specific test to run the samples using spectrophotometry as described below.

HDL was measured in two phases. The first phase involved adding dextran sulphate to form water-soluble complexes with non-HDL cholesterol fractions. This stabilised the non HDL fractions, preventing them from reacting with the second reagent. The second reagent contained PEG modified enzymes that selectively reacted with the cholesterol present in the HDL particles. In the presence of hydrogen peroxide, the second reaction gave a purple/blue pigment which was measured photo-metrically at or near 600nm. The intensity of the coloured complex formed was directly proportional to the HDL concentration in the sample giving a measurement at the end [62].

For Trig, firstly a reagent containing lipase was added to the serum to hydrolyse triglycerides to glycerol and FFA. Glycerol was later converted to glycerol-1-phosphate by glycerol kinase. The next step involved oxidation of glycerol-1-phosphate by glycerol phosphate oxidase. Oxidised glycerol- 1-phosphate in the presence of hydrogen peroxide gave a red coloured quinonimine dye which was measured photo-metrically at or near 500nm. The intensity of the coloured complex formed was directly proportional to the triglycerides concentration of the sample giving a measurement at the end[61].

2.7.7 Anthropometry measurements

For all anthropometric measurements, the inter-observer technical error of measurement (TEM) was tested prior to the beginning of the study against a reference anthropometrist. All anthropometry equipment were calibrated before the start of the study and at regular intervals as per standard operating procedures.

Height: This was measured in duplicate using a high-quality height board (Harpenden stadiometer, manufacturer; Holtain Limited, Crosswell, Crymych, UK) and recorded to the nearest 1 mm. The stadiometer was placed on a levelled hard surface. Participants stood with the back of their head, buttocks, calves and heels touching the stadiometer. They stood with feet 0.5 cm apart looking straight ahead. Measurements were taken in deep inspiration [63]

Weight: This was measured in duplicate using an electronic weighing scale (SECA,874, manufacturer; Seca GmbH & Co., Hamburg, Germany) with a precision of measurement of 100g. The scale was placed on a perfectly horizontal, flat hard surface with no obstruction. Participants wore minimal, light clothing. Participants stood still on the scale with feet 0.5 cm apart after the scale registered 0.00 [63].

Waist circumference: Waist circumference was measured at the level midway between the lower rib margin and the iliac crest (bony prominence of the hip bone). Measurements were done in duplicate using a non-elastic tape measure. Participants stood with their feet fairly close together (12-15 cm apart) wearing only light underwear. The readings were taken at the end of gentle expiration to the nearest millimetre [64].

Hip circumference: Measurements were done in duplicate using a non-elastic tape measure. Participants stood with their feet fairly close together (12-15 cm apart) wearing only light underwear. The tape was positioned around the buttocks and recorded the maximum circumference around the buttocks. Measurements were recorded to the nearest millimetre [64].

Anthropometric indices: From the anthropometric measurements of weight, height, waist and hip circumference, body mass index (BMI), waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR) were calculated. BMI was calculated by dividing weight in kilograms by height in metres^2. WHR was calculated as waist circumference in cm divide by hip circumference in cm. WHtR was calculated as waist circumference in cm divide by height in cm. BMI Z-scores were also calculated using WHO AnthroPlus [65] based on the 2007 WHO reference-standards [65]. The table 3 describes the cut off points for the anthropometric indices for the mentioned anthropometry variables.

Table 3. Anthropometric indices cut-off points

Anthropometry Index	**Cut-off points**
Body mass index for age Z-score	
Normal weight	≥-2.0 &≤1.0 Z-score
Severe malnutrition	<-3.0 Z -scores)
Moderate malnutrition	≥-3.0 & <-2 Z-scores)
Overweight	>1.0 &≤2.0 Z-score
Obese	>2 Z- score
Height for Age (Z score)	
Normal	> -2.0 & <2.0 Z -scores
Stunted	(<-2.0 Z -scores
Tall	(> 2.0 Z-scores)
Waist to hip ratio (males)	
Normal	≤ 0.9
Abnormal	>0.9
Waist to hip ratio (females)	
Normal	≤0.85
Abnormal	>0.85
Waist to height ratio	
Normal	≤0.5

Abnormal	>0.5

2.7.8 Assessment of MetS

MetS was assessed using the harmonised IDF criteria and MSSS. Assessment focussed on 1) prevalence of each of the different components of MetS, 2) prevalence of MetS itself and 3) Severity of MetS.

Prevalence of MetS components: The IDF- criteria was used to determine the prevalence of each of the MetS components: 1. Higher WC, 2. raised triglycerides, 3. raised blood pressure, 4. raised FBG and 5. reduced HDL. The cut off points for all these components are described in Table 4.

Prevalence of Mets: A participant was classified as having MetS if they had 3 or more MetS components.

Table 4: The IDF consensus definition of metabolic syndrome components' cut off points in children and adults[66][1]

Age group (years)	Waist circumference	Triglycerides	HDL	Blood pressure	Glucose
10≤16	≥ 90th percentile or adult cut- off if lower	≥1.7mmo/L (≥150mg/dL)	<1.03 mmol/L (< 40mg/dL)	Systolic ≥130 mmHg diastolic≥85 mmHg	≥ 5.6 mmol/L (100 mg /dL) (if ≥ 5.6 mmol/L [or known T2DM] recommend an OGTT
16+	≥ 94cm (male) ≥ 80cm (female) *	≥1.7mmo/L	<1.03 mmol/L (< 40mg/dL) (male) <1.29mmol/ L (<50mg/dL) (female) or on high lipids treatment	Systolic ≥130/mmHg daistolic≥85 mmHg or on antihypertensive drugs	≥ 5.6 mmol/L (100 mg /dL) (if ≥ 5.6 mmol/L [or known T2DM] recommend an OGTT

HDL = high-density lipoprotein; T2DM = Type 2 diabetes mellitus; OGTT = oral glucose tolerance test; IDF = international diabetes federation. [1]Values which are not qualified by gender apply for both males and females*****The IDF Consensus group recognises that there are ethnic, gender and age differences but research is still needed on outcomes to establish risk. Currently the European cut offs are used for Africans and they were used for this analysis Adapted from George et, al 2007[67]

Presence and severity of MetS using MSSS: MSSS was calculated using an online calculator accessed on http://metscalc.org using the following variables: weight, height, birth date, FBG, systolic blood pressure, HDL, patient's race/ ethnicity, patient's gender. The score is a sum of the five Z-scores from the five components of Mets. MSSS uses BMI- Z score which has been standardized in different races unlike waist circumference [25]. For each of the 5 components, the calculator uses different weightings while considering the interactions that occur across the MetS components themselves and the possible differential influences of individual MetS components on the specific score derived. [25]. The equations for the development of MSSS have already been described in figure 2.

All individuals with a Z-score of < 1 were categorised as having no MetS while all individuals with a Z-score of > 1 were categorised as having MetS. Severity of MetS from the score was classified according to the following scheme. Table 5 describes the classification of MetS severity by MSSS.

Table 5. Categorisation of severity of MetS derived from MSSS calculator[25]

MetS score	MetS severity
≥plus 3 Z-score	Severe
Plus 2 to plus 3 Z-score	Moderate
Plus 1 to plus 2 Z-score	Mild
Below plus 1 Z-score	No MetS

2.8 Data Management

2.8.1 Data quality in the field and clinic

Two experienced and well-trained nurses in anthropometry took all anthropometry measurements. Inter-observer and intra-observer technical error of measurements were determined before the start of data collection. For the remaining study variables of interest, each data collector specialised in a specific measurement and collected the data on all study

participants to minimise data collector variation. In addition, written standard operating procedures and additional instructions for specific data collection processes were available for reference both in the field and in the clinic.

2.8.2 Data processing and cleaning

Data was exported from the main study database, cleaned and analysed in Stata version 14. The database that was used to capture data was REDCap. REDCap is a secure web application for building and managing online surveys and databases. It is specifically geared to support online or offline data capture for research studies and operations [68]. REDCap was downloaded on tablets which data collectors used to enter data in the field and clinic. In the primary study, data was collected offline and uploaded online to the server weekly.

Drop-outs were individuals that were interviewed in the field but did not come to clinic for assessments. The dropouts were analysed to see if there was a significant difference in the number of dropouts between previously malnourished and community participants. The result of this analysis was that there was no significant difference between the two groups. Descriptive analysis was run to have an overall picture of the data and to help identify errors, missing variables and outliers. The anomalies were corrected using source documents like clinic data collection forms and booking diaries. If the variables could not be corrected, specific participants affected were dropped for that specific analysis. A Normality test was undertaken on all variables of interest.

2.8.3 Statistical analysis

2.8.3.1 Statistical principles

All our statistical tests and confidence intervals were two-sided at 95% significance level.

2.8.3.2 Descriptive analysis

Data for continuous variables with normal distribution was summarized as means and standard deviations while data for continuous variables which was not normally distributed data was summarized as medians and percentiles. All categorical variables were summarized as percentages.

2.8.3.3 Inferential statistics

Independent T-test was used for all normally distributed continuous data to determine if there was any difference in the means between the two groups. Chi-square test was used to assess differences in percentages of all categorical variables. Associations between categorical variables was done using Chi-square test. Stepwise logistic regression model was used with all possible independent co-variates and they were all adjusted for age and sex.

2.8.3.4 Bivariate analysis

First, Chi-square test was used to determine if there was any association between an individual predictor and MetS prevalence. The following variables were included in the bivariate analysis: 1) socio-demographic features age (age group), sex, household location, HIV status, ART use, level of education and occupation of mother, marital status of caregiver, 2) WHR, WHtR, BMI-Z score, Height for age Z-score and 3) excess fat%.

2.8.3.5 Multivariate predictors of MetS

All variables that showed significant association with MetS at bivariate analysis level were included in the model for multivariate predictors of MetS. Additional variables included were those that are biologically expected to influence MetS according to literature such as age and sex. These were included even if they were not significant in bivariate testing.

2.8.3 Results presentation

Results for this research study have been presented in tables and graphs.

2.8.4 Dissemination of results

Results on NCDs risk among previously malnourished children will been shared with QECH nutrition rehabilitation unit and the College of Medicine Public Health Nutrition Group. The results will also be shared with the Ministry of Health, participants, COMREC, supporting institutions and submitted for publication in a peer reviewed journal.

2.9 Study Limitations

It was a challenge to choose a single cut-off point for the prevalence of MetS components that represented all participants since most participants were adolescents in the growing phase and in different pubertal stages. Another limitation was data on predictors of MetS that was not collected

and thus not included in the analysis for example hereditary factors, nutrition (current dietary practices) and physical activity. There was no data on puberty to categorize participants in different pubertal stages as puberty affects anthropometry and body composition which were some of the possible predictors of Mets. The last limitation was the cross- sectional design of the study that limited us 1) to ascertain the temporal relationship of MetS and its predictors, 2) explore if the changes in anthropometry and body composition that happened over time could influence MetS and 3) calculate prevalence rate because the controls were identified 7 years after the previously malnourished and data from the first 2 follow ups did not have all components of Mets, therefore prevalence was used.

2.10 Ethical Considerations

2.10.1 Research approval

Approval for primary data was provided by the College of Medicine Research Ethics Committee, approval number P.09/17/2281. Secondary data was anonymized before being shared with the investigator. The data was not shared with any other sources upon receipt. The secondary analysis was given a waiver by the College of Medicine Research Ethics Committee.

2.10.2 Protection of human subjects

The primary study was structured in accordance with the International recommendations guiding doctors in biomedical research involving human participants. All procedures of the study inflicted no harm on the participants including the following procedures:

1. *Blood samples* – This posed no risk to the child. A 10 mL venous blood sample was collected from the children at enrolment. The collected volume represented less than 1% of the participant's total blood volume and it was replaceable from the participant's own blood production within a day.

2. Deuterium dilution assessment – This also posed no risk to the child. It involved simply drinking of doubly labelled water and collecting saliva with an oral swab. This has been done many thousands of times worldwide without complications. This has been performed by our research team previously in rural Malawian villages in years past without any adverse events or community objections.

2.10.3 Informed consent

On the day of enrolment, participants were informed about the study processes, the duration and requirements of participation in the study, and the benefits of participation in the study, through an interactive oral presentation. Illiterate guardians and participants were read the informed consent forms in the presence of literate family members or neighbours. Furthermore, forms with information about the study was left with guardians and participants to read again in their free time before coming to the central site. Only participants and guardians who expressed continued interest in the study after being fully informed were enrolled. Details of informed participant information and informed consent are provided in appendix 2 and 3.

2.10.4 Respect for autonomy

Adolescents, young adults and caretakers were repeatedly told that participation was voluntary, and that they could quit at any time.

2.10.5 Confidentiality

All procedures were done in confidence and all data was anonymized.

2.10.6 Benefits

At the conclusion of the study, participants were compensated for their study participation. Participants and guardians were given lunch and transport reimbursements. In addition, a token of appreciation was given in the form of zitenje, mathematical instruments, exercise books and pens.

CHAPTER 3: RESULTS

3.1 Socio-demographic, anthropometric and body composition characteristics

Based on information curated from the CHROSAM follow up, a total of 159 previously malnourished adolescents and young adults were identified as living in and around Blantyre. Similarly, a total of.126 age, sex and community matched peers were identified from the CHROSAM follow up and 121 siblings aged ±5 years to a previously malnourished adolescent or young adult were identified. For adolescents and young adults who no longer had a sex, age and community matched peer from the previous follow up, a replacement was identified. A total of 30 replacement peers with no previous follow up were thus included in the study. In total, 159, 156, and 121 previously malnourished, age and sex matched peers and malnourished adolescents and young adults' siblings respectively were approached to participate in the survey. Out of these, 142, 123 and 116 previously malnourished, age and sex matched peers and malnourished adolescents and young adults siblings respectively accepted and provided consent to take part in the study, making a total of 381. Of these 10 participants were excluded from the analysis because they were below 10.0 years of age which is the lower limit of adolescent age definition. Ultimately data was analysed from 371 participants.

Table 6A describes socio-demographic characteristics of the participants and Table 6B describes anthropometric and body composition characteristics of the population. There were almost equal number of males (186) and females (185), age range 10-28 years. Most participants (78.7%) were in early adolescence, aged 10-14 years. The mothers of the participants had on average attended some primary education (66%) and the majority were self-employed (71 %). Overall underweight and obesity were rare but a fifth of the participants were stunted. Prevalence of stunting, underweight and obesity were 23.5%, 1.3% and 5.8% respectively. In terms of body composition, 14% of the population had excess fat.

Table 6A: Socio-demographic characteristics of adolescents and young adults in Blantyre district.

Characteristic	N (%)	TOTAL
Sex		371
Female	185 (.49.9)	
Male	186 (50.1)	
Age group (years)		371
10-14 years	291 (78.7)	
15-19 years	64 (17.3)	
>19 years	15 (4.0)	
Location		371
rural	197 (53.1)	
urban	174 (46.9)	
Participant HIV status		371
Unknown	6 (2.4)	
Negative	192 (77.4)	
Positive	50 (20.2)	
Missing	123.(33.1)	
Participant on ART		50[2]
No	2 (4.0)	
Yes	48 (96)	
Missing	0	
Maternal education		371
No schooling	56 (18.0)	
Primary school	207 (66.6)	
Secondary school	48 (15.4)	
missing	60 (16.2)	
Maternal occupation[1]		371

Paid/salaried work	28(10.0)	
Self- employed	198(71.0)	
Unemployed	53(19.0)	
Missing	92 (24.8)	
Marital status of caregiver		371
Divorced/separated	62 (21.0)	
Married	175 (59.3)	
Single (never married)	8 (2.7)	
Widow	50 (17.0)	
Missing	76 (20.5)	

[1]34 mothers of participants were deceased.[2]50 individuals were HIV positive and all of them indicated whether they were on ART or not. HIV =. human immunodeficiency virus; ART = anti-retroviral therapy.

Table 6B: Anthropometric and body composition characteristics of adolescents and young adults in Blantyre district

Characteristic	N (%)	Total
Height for Age (Z score)		371
Normal (> -2.0 & <2.0 Z -scores)	235 (63.3)	
Stunted (<-2.0 Z -scores)	87 (23.5)	
Tall (> 2.0 Z-scores)	49 (13.2)	
Body Mass Index for Age Z score (BMIZ)		371
Severe malnutrition (<-3.0 Z -scores)	4 (1.3)	
Moderate malnutrition (≥-3.0 & <-2 Z-scores)	22 (5.9)	
Normal weight (≥-2.0 &≤1.0 Z-score)	277 (74.9)	
Overweight (>1.0 &≤2.0 Z-score)	50 (13.1)	
Obese(>2)	18 (5.8)	
Waist to Hip Ratio (ABBR) (Units)[1]		371
Normal (<0.9 & <0.85)	197 (53.1)	
Abnormal ((≥0.9 & ≥0.85)	174 (46.9)	

Waist to height ratio		
Normal (<0.5)	326 (87.9)	371
Abnormal (≥0.5)	45 (12.1)	
Body Fat percent (Measure and Units)[2]		371
Excess fat% (≥25 & ≥30)	41 (14.0)	
No excess fat% (<25 & <30)	251 (86.0)	
Missing	9 (2.4)	
History of acute malnutrition		371
Previously treated for acute malnutrition	150 (40.4)	
Never treated for acute malnutrition	221 (59.6)	

[1]Waist to Hip Ratio (ABBR); normal for males = <0.9 and females = <0.85. Abnormal for males = ≥0.9 & abnormal for females = ≥0.85. [2] Body fat percent from deuterium dilution technique was calculated by dividing fat mass by total body weight * 100; Excess fat for males = ≥25 & for females = ≥30, No excess fat in males = <25 & for females = <30.

3.2 Prevalence of components of Mets

The prevalence of MetS components are shown in Table 7A and 7B below. In general, participants resident in rural locations had higher prevalence of all MetS components except for blood pressure as compared to the urban resident adolescents and young adults. The most prevalent MetS components in the total sample were raised FBG and reduced HDL while the least common component was raised blood pressure. 92.5 % of individuals with raised WHtR also had had higher waist circumference. Individuals with excess fat % had higher prevalence of all MetS components compared to those with no excess fat% except for reduced HDL where the prevalence was almost the same; 29.5% versus 30.1%.

The prevalence of all MetS components were comparable between those who had history of treatment for acute malnutrition and those who had no history of treatment for acute malnutrition except for waist circumference which was higher in participants with no history of acute malnutrition treatment, with a proportion difference of 8.4%, p value =< 0.05 as shown in figure 7.

Table 7A. Prevalence of MetS components by socio-demographic characteristics of adolescents and young adults in Blantyre district.

Prevalence of Mets components, n (%)

Characteristic	↑FBG	↑RBP	↑HDL	↑Triglycerides	Central obesity
Sex					
Female	49 (27.2)	4(2.2)	53 (29.4)	22 (11.3)	33 (16.9)
Male	62 (35.2)	7 (4.0)	56 (31.8)	25(14.7)	25 (12.8)
Age group (years)					
10-14 years	79 (29.9)	5 (1.9)	76 (28.8)	37 (12.7)	43(14.8)
15-19 years	26 (42.6)	6 (9.8	23 (37.7)	6 (9.4)	6 (9.4
>19 years	3 (21.4)	0 (0.0)	4 (28.6)	1 (6.7)	3 (20.0)
Household location					
Rural	63 (36.6)	2 (1.2)	73 (42.4)	37 (18.8)	32 (16.2)
Urban	42 (25.)	8 (4.8)	29 (17.6)	10 (5.7)	25 (14.3)
Participant HIV status					
Don't know	3(50.0	0 (0.0)	1 (16.7)	0 (0.0)	0.(0.0)
Negative	65 (34.8)	7 (3.7)	56 (30.0)	24 (11.7)	29(14.1)
Positive	15 (31.3)	0 (0.0)	13 (27.1)	4 (7.8)	6 (11.8)
Participant on ART					
No	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0
Yes	15 (31.3)	0 (0.0	12 (26.1)	3 (6.25)	5 (10.4)
Maternal education					
No schooling	17 (30.9	1 (1.7)	22 (39.2)	5 (8.5)	4 (6.8)
Primary school	63 (32.8)	6 (3.1)	59 (30.1)	36 (16.2)	39 (17.6)
Secondary school	14 (28.6	1 (1.9)	10 (19.2)	4 (10.7)	8 (14.5)
Maternal occupation					
Paid/salaried work	11 (36.7)	3 (10.0)	8 (26.7)	2 (6.5)	6 (19.4)
Self- employed	64 (34.0)	4 (2.1)	58 (30.8)	36 (16.5)	37 (17.1)

Unemployed	8 (14.5)	1 (1.8)	15 (27.3)	6 (10.7)	5 (8.9)
Marital status of caregiver					
Divorced/separated	17 (29.3)	3 (5.2)	24 (41.4)	8 (12.5)	7 (10.9)
Married	58 (34.1)	3 (1.8)	50 (29.4)	23 (12.3)	32 (17.2)
Single (never married)	5 (62.5)	1 (12.5)	1 (12.5)	1 (11.1)	1 (11.1)
Widow	10 (21.3)	1 (2.1)	14 (29.8)	7 (13.5)	8 (15.4)

MetS = metabolic syndrome; FBG = fasting blood glucose; RBP = raised blood pressure; HDL = high-density lipoprotein; HIV = human immunodeficiency virus; ART = anti- retroviral therapy

Table 7B. Prevalence of MetS components by anthropometric and body composition in adolescents and young adults in Blantyre district.

	Prevalence of Mets components, n(%)				
Characteristic	**↑FBG**	**↑RBP**	**↑HDL**	**↑Triglycerides**	**Central obesity**
Height for Age Z score					
Normal	80 (32.2)	10 (4.0)	70 (28.2)	9 (3.6)	19 (7.7)
Stunting	28 (29.5)	1 (1.0)	32 (33.7)	4 (4.2)	2 (2.1)
Tall	6 (25.0)	0 (0.0)	7 (33.3)	36(63.2)	37 (67.3)
BMI-Z-score					
Severe malnutrition	1 (20.0)	0.(0.0)	1 (20.0)	0.(0.0)	0 (0.0)
Moderate malnutrition	9.(39.1)	0 (0.0)	13 (56.5)	1 (4.3)	0.(0.0)
Normal weight	89 (30.5)	11 (3.8)	87 (29.8)	11 (3.8)	10 (3.3)
Overweight	8 (42.1)	0(0.0)	4 (21.1)	1 (5.3)	10 (52.6)
Obese	4 (23.5)	0 (0.0)	4 (23.5)	34 (66.7))	38 (74.5)
Waist to hip ratio					
Normal	57(31.1)	9 (4.9)	52 (28.4)	7 (3.8)	7 (3.8)
Abnormal	54 (31.2)	2 (1.2)	57 (32.9)	40 (23.1)	51 (29.5)
Waist to height ratio					
Normal	107 (31.0)	11 (3.1)	103 (29.9)	12 (3.5)	9 (2.6)

abnormal	7 (36.8)	0 (0.0)	6 (31.6)	37 (67.3)	49 (92.5)
Body composition					
Excess fat%	21 (47.7)	2 (4.5)	13 (29.5)	3 (6.8)	12 (27.3)
No excess fat%	78 (30.0)	8 (3.1)	81 (30.1)	8 (3.0)	12 (4.4)
History of acute malnutrition					
Previously treated for acute malnutrition	47 (32.6)	3 (2.1)	40 (28.4)	17 (11.2)	13 (8.6)
Never treated for acute malnutrition (community peers)	67 (30.0)	8 (3.6)	69 (30.9)	32 (14.3)	45 (18.2)

MetS = metabolic syndrome; BMI = body mass index; FBG = fasting blood glucose; RBP = raised blood pressure; HDL = high density lipoprotein

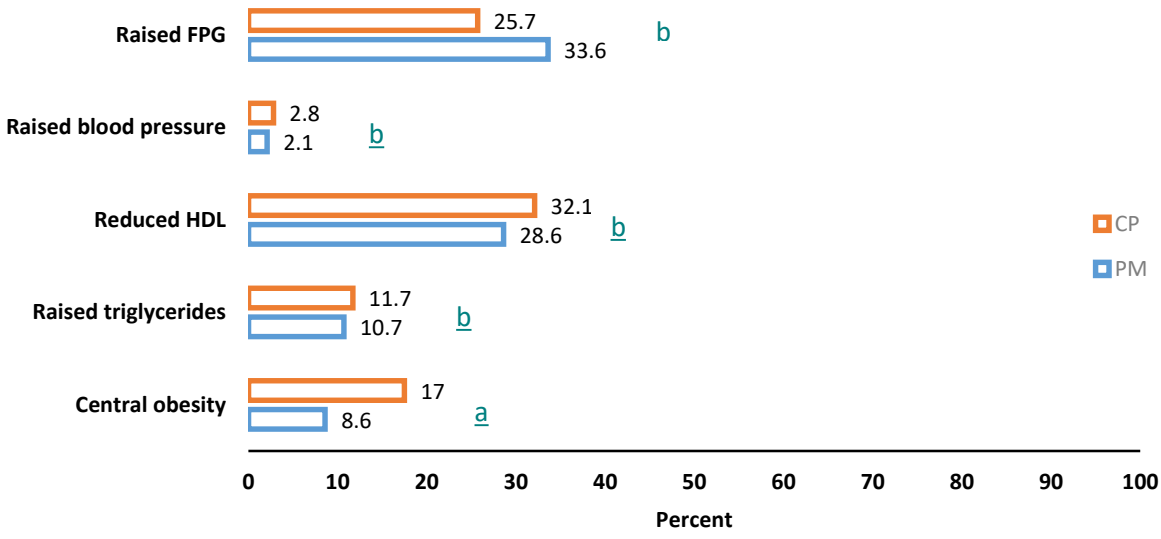


Figure 6. Prevalence of components of metabolic syndrome in children who were previously malnourished and age sex and community matched peers with no previous history of malnutrition.

PM= previously malnourished; CP = community peers; HDL = high-density lipoproteins; FBG= fasting blood glucose. P-values follow the prevalence in community peers. a = significantly different at 0.05 level; b = not significantly different at 0.05 level.

3.3 Prevalence and severity of MetS

The overall prevalence of MetS was 3.1% by MSSS and 2.5% by IDF criteria and all participants with MetS were classified as mild MetS by MSS criteria. Table 8A and 8B shows the prevalence of MetS in relation to various risk factors. There was no statistically significant difference in the prevalence of MetS by sex, age-group, household location, HIV status, history of taking ART and maternal level of education. Participants with caregivers who were single or never married and those participants whose mothers were on paid employment had significantly a higher MetS prevalence (13.8% versus 1.6%, p value 0.007) and (12.5% versus 3.4 versus 2.4, p value 0.01) compared to their counterparts respectively. No statistically significant differences were observed in the prevalence of MetS based on anthropometric indicators of stunting (Height for age Z-score), obesity (BMI and WHR). However, participants with high WHtR showed a higher MetS prevalence of 10.5% than those with normal WHtR at 2.1%, p value 0.03. Prevalence of MetS did not differ based on history of treatment for acute malnutrition. The prevalence of MetS however differed based on percentage of excess fat, with participants with excess fat% having higher prevalence of MetS (9.5%) compared to those with no excess fat 1.1% (p- value; 0.01)

Table 8A. Prevalence of MetS by socio-demographic characteristics amongst adolescents and young adults in Blantyre district.

	MetS status, n (%)		
Characteristic	**MetS**	**No MetS**	**p-value[1]**
Sex			
Female	3 (1.8)	177(98.2)	0.33
Male	6 (3.5)	170 (96.5)	
Age group (years)			
10-14 years	8 (3.0)	256 (97.0)	1.00
15-19 years	1 (1.6)	60(99.4)	
>19 years	0 (0.0)	14 (100.0)	
Household location			0.62

Rural	6(3.5)	166 (96.5)	
Urban	3 (1.8)	162 (98.2)	
Participant HIV status			1.00
Unknown	0 (0.0)	6 (100.0)	
Negative	5 (2.7)	182 (97.3)	
Positive	1 (2.1)	47 (97.9)	
Participant on ART			1.00
Yes	1 (2.2)	45 (97.8)	
No	0 (0.0)	2 (100.0)	
Maternal education			0.63
No schooling	3 (5.4)	52 (94.6)	
Primary school-	5 (2.6)	187 (97.4)	
Secondary school	0 (0.0)	49 (100.0)	
Maternal occupation			**0.01**
Paid/salaried work	4 (13.8)	25 (86.2)	
Self- employed	3 (1.6)	180 (98.4)	
Unemployed	0 (0.0)	53 (100.0)	
Marital status of caregiver			**0.01**
Divorced/separated	2 (3.4)	56 (96.6)	
Married	4 (2.4)	164 (97.6)	
Single (never married)	1 (12.5)	7 (87.5)	
Widow	0 (0.0)	47 (100.0)	

[1] =exact test, p values corrected to decimal places; MetS = metabolic syndrome; HIV = human immunodeficiency virus; ART = anti-retroviral therapy

Table 8B. Prevalence of MetS by anthropometric and body composition characteristics amongst adolescents and young adults in Blantyre district

	MetS status, n (%)		
Characteristic	**MetS**	**No MetS**	**P value[1]**

Height for Age (Z score)			0.15
Normal	9(3.4)	235(96.6)	
Stunting	0(0.0)	93 (100.0)	
Tall	0 (0.0)	19 (100)	
BMI-Z-score			0.53
Severe malnutrition	0(0.0)	5(100.0)	
Moderate malnutrition	1 (3.6)	27 (96.4)	
Normal weight	7 (2.4)	285(97.6)	
Overweight	1 (5.3)	18 (94.7)	
Obesity	0 (0.0)	3 (100.0)	
Waist to hip ratio			1.00
Normal	4 (2.3)	169(97.7)	
Abnormal	5 (2.7)	178 (97.3)	
Waist to height ratio			**0.03**
Normal	7(2.1)	330 (97.9)	
abnormal	2.(10.5)	17.(89.5)	
Body composition factors			
Excess fat	4 (9.5)	38 (90.5)	**0.01**
No excess fat	3 (1.1)	261 (98.9)	
Treatment of acute malnutrition[2]			1.00
Previously treated for acute malnutrition	4 (2.9)	136 (97.1	
Never treated for acute malnutrition (community peers)	3 (2.8)	113 (97.2)	

[1]exact test, p value corrected to decimal places; [2]only age, sex and community peers were compared to previously malnourished children. BMI = body mass index; MetS = metabolic syndrome.

3.4 Unadjusted association of MetS with socio-demographic, anthropometric and body composition predictors

Table 9A and 9B shows the unadjusted analysis of the association between MetS and the predictors. As indicated in the table, there was no association between all sociodemographic characteristics and MetS. The odds of having MetS was 18% in individuals with raised WHtR than those with normal WHtR. Individuals with excess fat were 9 times more likely to have MetS than those with no excess fat.

Table 9A. Unadjusted association between socio-demographic predictors and prevalence of MetS amongst adolescents and young adults in Blantyre district

	MetS status, n(%)				
Characteristic	**MetS**	**No MetS**	**Odds ratio**	**95% CI**	**p-value[1]**
Sex					
Female	3 (1.8)	177(98.2)			
Male	6 (3.5)	170 (96.5)	0.48	0.11, 1.95	0.36
Age group (years)					
10-14 years	8 (3.0)	256 (97.0)			
15-19 years	1 (1.6)	60(99.4)	3.79	0.47, 30.76	0.21
>19 years	0 (0.0)	14 (100.0)			
Household location					
Rural	6(3.5)	166 (96.5)	0.5	0.12, 2.06	0.34
Urban	3 (1.8)	162 (98.2)			
Participant HIV status					
Unknown	0 (0.0)	6 (100.0)			
Negative	5 (2.7)	182 (97.3)	0.79	0.09, 6.9	0.8
Positive	1 (2.1)	47 (97.9)	collinearity		
Participant on ART[2]					
Yes	1 (2.2)	45 (97.8)			
No	0 (0.0)	2 (100.0)			
Maternal education[3]					
No schooling	3 (5.4)	52 (94.6)	Ref		
Primary school-	5 (2.6)	187 (97.4)	0.84	0.09, 7.72	0.87
Secondary school	0 (0.0)	49 (100.0)			
Maternal occupation[4]					
Paid/salaried work	4 (13.8)	25 (86.2)	0.17	0.02, 1.75	0.14
Self- employed	3 (1.6)	180 (98.4)	Ref		
Unemployed	0 (0.0)	53 (100.0)			

Marital status of caregiver[5]					
Divorced/separated	2 (3.4)	56 (96.6)	1.51	0.27, 8.46	0.64
Married	4 (2.4)	164 (97.6)	0.25	0.02, 3.07	0.28
Single	1 (12.5)	7 (87.5)	Ref		
Widow	0 (0.0)	47 (100.0)			

[1]logistic regression was used for the unadjusted association. [2,3,4,5] =There was no positive outcome to run the test. HIV = human immunodeficiency virus; ART = anti-retroviral therapy; MetS = metabolic syndrome

Table 9B. Unadjusted association between anthropometric and body composition predictors of MetS and MetS prevalence of MetS amongst adolescents and young adults in Blantyre district

	MetS status, n(%)				
Characteristic	**MetS**	**No MetS**	**Odds ratio**	**95% CI**	**p-value[1]**
Height for Age Z score [2]					
Normal	9(3.4)	235(96.6)			
Stunting	0(0.0)	93 (100.0)			
Tall	0 (0.0)	19 (100)			
BMI- Z score [3]					
Severe malnutrition	0(0.0)	5(100.0)			
Moderate malnutrition	1 (3.6)	27 (96.4)	1.22	0.07,20.94	0.74
Normal weight	7 (2.4)	285(97.6)	Ref		
Overweight	1 (5.3)	18 (94.7)	2.26	0.26,19.39	0.14
Obesity	0 (0.0)	3 (100.0)			
Waist to hip ratio					
Normal	2.(1.0)	164 (99.0)	Ref		
Abnormal	7(4.1)	191 (95.9)	4.08	0.84,19.9	0.08
Waist to height ratio					
Normal	7(2.1)	330 (97.9)	Ref		
abnormal	2.(10.5)	17.(89.5)	5.18	0.33,0.91	**0.04**
Body composition factors					
Excess fat%	4 (9.5)	38 (90.5)	8.87	1.91,41.08	**0.01**
No excess fat%	3 (1.1)	261 (98.9)	Ref		
Treatment of acute malnutrition					
Previously treated for acute malnutrition	4 (2.9)	136 (97.1	0.67	0.12,3.70	0.6

Never treated for acute malnutrition[4]	3 (2.8)	113 (97.2)	Ref

[1]Logistic regression, p values corrected to decimal places; C. I.= confidence interval; [2,3] logistic regression model was not done because there was no positive outcome; [4] only age, sex and community matched peers were analysed; BMI = body mass index, MetS = metabolic syndrome.

3.5 Multivariate association of MetS with socio-demographic, anthropometric and body composition factors

We modelled the association between MetS predictors and MetS. Risk factors included in the logistic regression model were those chosen a priori based on assumed biologic relationship between the predictor and MetS. In addition, we included predictors that were associated with MetS at 0.1 level in the bivariate analysis. In the multivariate analysis, only excess fat % was associated with MetS; OR 5.88, (95% CI of OR. 1.37,35.4) p = 0.04 All other variables were not statistically significantly associated with MetS. Table 10 shows the adjusted association between predictors of MetS and MetS prevalence using logistic regression model.

Table 10. Adjusted association between predictors of MetS and MetS prevalence

	MetS status		
Characteristic	**Odds ratio**	**95% CI**	**p-value[1]**
Age group(years)	1.01	0.69,1.48	0.95
10-14	Ref		
15-19			
>19[2]			
Sex			
Male	0.88	0.16,4.75	0.88
Female	Ref		
Waist to hip ratio	0.86	0.15,4.70	0.86
Abnormal	Ref		
Normal			
Body composition factors	5.88	1.37,35.4	**0.04**
	Ref		
Excess fat%			
No excess fat%			
Waist to height ratio	0.53	0.43,6.4	0.62
Abnormal	Ref		
Normal			
Maternal occupation	1.37	0.61,3.05	0.45
Paid/salaried work	Ref		

Self-employed	
Unemployed[3]	

[1]Logistic regression; p values corrected to two decimal places; CI= confidence interval;[2,3]no positive outcome was available for the model to run; MetS =. Metabolic syndrome

CHAPTER 4. DISCUSSION

The aim of this cross-sectional study was to analyse the prevalence and where present, the severity of MetS and describe its predictors in adolescents and young adults in Blantyre. In this population, the most prevalent MetS components were FBG (31.3%) and reduced HDL (30%), while the least was raised BP (3.0 %). The overall prevalence of MetS was 2.5 % by the IDF criteria and 3% by MSSS and all participants who had MetS were classified as having mild MetS. Amongst the collected socio-demographic characteristics, none were associated with the prevalence of MetS except for maternal occupation and marital status of the caregiver of the participant. Amongst anthropometric characteristics, only WHtR was associated with MetS. Previous treatment of acute malnutrition was not associated with MetS. Presence of excess fat, was the only body composition indicator assessed and it was found to be associated with prevalence of MetS. The odds of having MetS was 6 times in individuals with excess fat compared to those who did not have excess fat. This chapter discusses how these findings compare with the prevailing scientific knowledge around this topic.

4.1 Prevalence of MetS

The estimated low prevalence of MetS in these Malawian adolescents and young adults of 2.5% was expected as several studies report prevalence of less than 10% in adolescents [59][8][12][9][7]. For example, in South African black population, MetS prevalence was reported at 4.6% [59] in and in the USA it was at 9.8%.[12]. The prevalence of MetS seems to be higher in higher income than low income countries and part of the explanation for this difference could be related to differences in socio-economic status (SES). Higher SES guarantees ability to afford high dense calorie and high fat food and sedentary lifestyle. Consequently, individuals with higher SES stand a higher chance of developing MetS as excess energy in the body stimulates FFA metabolism that ignites MetS development cascade[3][10][11]. This possibly explains why USA, the richest of the three countries has the highest prevalence followed by South Africa (relatively poor) then Malawi (the poorest). Rich countries have the majority of its population with a higher economic status. Individuals with higher income tend to eat food with high calories and fats predisposing themselves to MetS as compared to those with lower economic status.

Overall the prevalence of hyper-triglyceridaemia and reduced HDL in the Malawian population was almost similar to that found in South African adolescents[59]. In the Malawian adolescents, hyper-triglyceridaemia was at 12.3% and reduced HDL was at 30% while in South African adolescents it was at 8.9% and 31.3% respectively. However, our Malawian population had remarkably raised FBG at 31.3% compared to 5.2 % in South African adolescents while the South African adolescents had remarkably more central obesity at 23% and hypertension at 32.7% as compared to Malawian adolescents who were at 14.6% and 3 % respectively [59].The prevalence of hyperglycaemia in our context was similar to that found in Caucasian Brazilian children 6-14 years of age which was at 35.7%[47]. Raised BP was the least prevalent component in our context. This is consistent with findings from a study that looked at prevalence of MetS in overweight Hispanic adolescents in California where the prevalence of raised blood pressure was at 4%[69] and a meta-analysis that showed that the pooled prevalence of raised BP was 5.5% in African adolescents [70].This is contrary to adult population studies where BP is usually one of the most common MetS components. For example, in South African black adult population raised blood pressure was at 49.5% in 2009 [14] and in Malawian population it was at 14.7% in 2013[10].

4.2 Socio-demographic predictors and MetS

There was no significant difference in the prevalence of MetS by age group (3% versus 1.6% versus 0%) and sex (1.8% versus 3.5%) with p values of > 0.05. This is consistent with findings in Brazilian adolescents between the ages of 14-19 years where they found no association between sex, age, and economic status with MetS prevalence [47]. However results from the two studies are not in keeping with other studies where MetS in adolescents was higher in males and increased with age .This was seen in USA adolescent population where it was 10.9% in males and 6.3% in females [12] . The same trend was seen in South African adolescents where the prevalence was 3.1% in females and 6% in males and it further increased with age (p-value 0.001). These contradictory findings could be explained physiologically. Normally the development of MetS comes from an interaction of biological and environmental factors. Older age and male sex in adolescence is a risk factor for MetS development. However, without enabling environmental factors like high calorie and fat diet, MetS development cannot be attained by biological factors alone. This could be the case in our study. In our population, there

was lack of heterogeneity in the social characteristics of the participants as evidenced from the lack of statistical significant association between all social characteristics and MetS in the un-adjusted analysis (p- values > 0.05). This homogeneity in social factors in our population can be inferred to other social factors like diet and physical activity which were not assessed. In the end, the lack of enabling social factors weakened the effect of biological factors of sex and age on the development of MetS leading to no differences in MetS prevalence by sex or age in our study.

There was no association between maternal level of education and marital status of caregiver with MetS prevalence in the un-adjusted analysis with p- values of >0.05. This is in keeping with a cross sectional study in Iran in 2014 among 3506 adults aged 30-70 years where there was no association between level of education and marital status among participants with MetS prevalence. However it is contradicting with findings from a Brazilian study among adolescents that showed that adolescents whose mothers had ≤ 8 years of schooling had reduced MetS prevalence (OR=0.56; 95%CI from 0.35 to 0.91)[35].

Our study findings did not support that setting affects the prevalence of MetS as we did not find any difference in MetS prevalence among the rural and urban adolescents, (p- value in un-adjusted analysis;0.34) unlike what has been reported in other studies[11]. In the Iranian study described above, the MetS prevalence in urban population was (41.4%) versus the rural population (32.6%), p value; 0.002. This discrepancy could be because in Malawi, the SES between urban and rural populations is not significantly different to render significant different type of diets and therefore different levels of risk for the development of MetS. This is supported by the findings in the Malawi STEPS survey where they found no difference in the prevalence of hypertension and diabetes between the urban and rural adult populations. In 2013, the prevalence of hypertension and diabetes was 14.1% and 1.5% in rural population while in urban it was at 15.5% and 2.5% respectively[10].

Contrary to what has been found in adult studies in individuals living with HIV, our study did not find any association between HIV and ART with MetS prevalence. However, HIV has been shown to be a risk factor for MetS as it leads to dysregulated inflammatory processes that lead to a state of chronic low-grade inflammation that later induces MetS development. [45] [46]. In addition, ART has been found to cause insulin resistance, hyperlipidaemia, and lipodystrophy

[47][71]. This contradictory finding could be because ART and HIV metabolic effects are time dependent. The longer you have HIV and the longer you take ART, the more chances of developing metabolic effects. Our participants being adolescents (younger in age) had not been exposed to HIV and ART for longer period to incur these metabolic effects hence no difference in MetS prevalence with those who are HIV negative[72]. In addition, lipodystrophy characterised by fat loss or fat accumulation or both which lead to dyslipidaemia and later MetS has shown to be of lower prevalence in children than adults. Only 25% of children manifest lipodystrophy as compared to adults [72]. Finally, the standard ART treatment for children in Malawi is a combination of lamivudine, zidovudine and niverapine. This combination does not contain a protease inhibitor. Protease inhibitors is a specific class of HIV drugs that have been more implicated in metabolic effects of ART contributing to 62% of all metabolic complications in people living with HIV [72].

4.3 Anthropometric predictors and MetS

Obesity which is accumulation of excess fat is directly linked to the development of MetS and later on CVD and T2DM. Anthropometric indices of BMI, WHR and WHtR were used to assess obesity in this study. However, only WHtR was associated with MetS in the unadjusted analysis (OR 5.18, p value 0.039). This is in keeping with a study that evaluated BMI, WHR and WHtR as indicators of impaired glucose tolerance, and found that WHtR was the best indicator of them all [73]. In addition, a cross sectional study done in Caucasians in Spain amongst adult workers assessing the use of body adiposity index in predicting cardiovascular and metabolic health risks found WHtR to be the best index in correlating with MetS components and the Framingham risk score (p value <0.01). The Framingham score estimates the risk of developing coronary heart disease in the next 10 years [72].WHtR was also shown to predict MetS significantly well in an Egyptian study of women as described below .

However, BMI was not associated with MetS in our study. This could be because BMI measures generalised obesity which does not always infer central obesity and central obesity is one of the components of MetS. In addition, BMI does not differentiate between adipose and muscle tissue [31]. Furthermore, BMI is not able to differentiate between fat compartments, an essential issue because visceral adipose tissue has been shown to be more associated with cardiovascular risk than subcutaneous adipose tissue [72]. Studies have shown that BMI is not a strong predictor of

body fat. When compared to reference methods of estimating body fat such as underwater weighing and DDT, the amount of variance (R2) in body fat explained by BMI is about 70–80% in adults (.14,15) Thus BMI is limited in predicting those at risk of MetS [73]. In a cross sectional study among Egyptian women aged 25-35 years to predict the validity of body adiposity indices in predicting MetS and its components, BMI had the least area under the curve (61.3%) as compared to WHR (67.3%) and WHtR (65.9%). In addition, BMI had the least correlation coefficients with all MetS components [73].

The contradictory finding where WHR was not associated with MetS in our study as compared to the Egyptian study describe above [73] could be due to physiological differences of the two study populations. Our study was done in female and male adolescents, where the majority (78.7%) were aged 10-14 years while the Egyptian study was done in women 25-35 years of age. The majority of our study population were still in the pubertal development stages and not fully developed as compared to the Egyptian population. As such WHR was not the best measure of obesity in our population hence no difference in MetS prevalence amongst individuals with high WHR and individuals with normal WHR.

4.4 Body composition predictors and MetS

The significant association (OR 5.88, p, value, 0.04) between presence of excess fat% and MetS in the adjusted analysis was expected physiologically and epidemiologically. Physiologically excess body fat ignites a chain of different metabolic pathways that lead to the development of MetS. Presence of fat around abdominal viscera increases the metabolism of FFA which lead to dyslipidaemia. In addition, FFA in the blood lead to atherosclerosis as the FFA get deposited in the endothelium of blood vessels leading to hypertension which is a MetS component too Furthermore, FFA inhibit glucose uptake in skeletal cells and impairs pancreatic β cells function.[3]. Studies have reported an association between body fat and MetS markers. For example, a study in Greece among children and adolescents aged 5-15 years showed an association between body composition factors and MetS. Body fat mass in kilograms and as fat percentage showed a positive correlation with raised Trig and insulin resistance while showing a negative correlation with HDL(reduced HDL is a MetS component, while raised HDL is protective for MetS[53].

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

MetS was relatively rare in this population and there were no specific socio-demographic and anthropometric indices that explained the prevalence of MetS. Body composition, especially presence of excess fat% increases the odds of getting MetS. However, the lack of association between socio-demographic characteristics and MetS could be attributed to the lack of heterogeneity in the socio-demographic characteristics. Limitations of anthropometric indices such as BMI to detect excess fat, inappropriateness of WHR assessment in our context and shorter duration post-acute malnutrition treatment for assessment of MetS probably explains the lack of association between anthropometry and MetS.

5.2 Recommendations

WHtR could be used as proxy index for assessment of obesity in adolescents and young adults where body composition assessment tools are not available. There is need for further studies to describe the prevalence and risk factors of MetS in adolescents and young adults in Malawi with a larger sample size. There is also need for further studies to describe the prevalence and incidence of MetS in children who suffered from acute malnutrition 20- 30 years post treatment in our African region. These study findings would help inform previously malnourished children, care providers, nutritional health policymakers and other stakeholders on possible interventions that could help reduce the prevalence of MetS by addressing the risk factors of MetS in our context

References

[1] Ford ES. The metabolic syndrome and mortality from cardiovascular disease and all-causes: Findings from the National Health and Nutrition Examination Survey II Mortality Study. *Atherosclerosis* 2004; 173: 307–312.

[2] Han TS, Lean ME. A clinical perspective of obesity, metabolic syndrome and cardiovascular disease. *JRSM Cardiovasc Dis* 2016; 5: 204800401663337.

[3] Retracted: A Comprehensive Review on Metabolic Syndrome. *Cardiol Res Pract* 2019; 2019: 1–1.

[4] World Health Organization. Non communicable disease. *Factsheet*, http://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases (2018, accessed 16 August 2018).

[5] De GN, Olmos M, Calleja A, et al. Nutrición Hospitalaria Trabajo Original. *Nutr Hosp* 2019; 34: 15–18.

[6] Agirbasli M, Tanrikulu AM, Berenson GS. Metabolic Syndrome: Bridging the Gap from Childhood to Adulthood. *Cardiovasc Ther* 2016; 34: 30–36.

[7] DeBoer MD, Gurka MJ, Woo JG, et al. Severity of the metabolic syndrome as a predictor of type 2 diabetes between childhood and adulthood: the Princeton Lipid Research Cohort Study. *Diabetologia* 2015; 58: 2745–2752.

[8] Okafor C. The metabolic syndrome in Africa: Current trends. *Indian J Endocrinol Metab* 2012; 16: 56.

[9] Eisenmann JC. On the use of a continuous metabolic syndrome score in pediatric research. *Cardiovasc Diabetol* 2008; 7: 1–6.

[10] Mudie K, Lawlor DA, Pearce N, et al. How does the association of general and central adiposity with glycaemia and blood pressure differ by gender and area of residence in a Malawian population: A cross-sectional study. *Int J Epidemiol* 2018; 47: 887–898.

[11] Jahangiry L, Khosravi-Far L, Sarbakhsh P, et al. Prevalence of metabolic syndrome and its determinants among iranian adults: Evidence of IraPEN survey on a bi-ethnic population. *Sci Rep* 2019; 9: 1–7.

[12] Aguilar M, Bhuket T, Torres S, et al. Prevalence of the metabolic syndrome in the United States, 2003-2012. *Jama* 2015; 313: 1973–1974.

[13] Kaushal S, Gupta V, Prakash G, et al. Correlates of metabolic syndrome and prevalence among urban population of Agra, Uttar Pradesh, India. *Int J Community Med Public Heal* 2016; 3: 3570–3575.

[14] Peer N, Lombard C, Steyn K, et al. High prevalence of metabolic syndrome in the Black population of Cape Town: The Cardiovascular Risk in Black South Africans (CRIBSA) study. *Eur J Prev Cardiol* 2015; 22: 1036–1042.

[15] S R. Early Postnatal Stunting Increases Risk of Hypertension among Young Rural Adults from India- Pune Rural Cohort. *J Nutr Heal Sci* 2016; 3: 1–8.

[16] Bonora BM, Marescotti M, Marcuzzo G, et al. Synergistic interactions among metabolic syndrome components and homeostasis model assessment of insulin resistance in a middle-aged general population over time. *Metab Syndr Relat Disord* 2015; 13: 171–178.

[17] Deboer MD, Gurka MJ. Clinical utility of metabolic syndrome severity scores: Considerations for practitioners. *Diabetes, Metab Syndr Obes Targets Ther* 2017; 10: 65–72.

[18] Lemieux I. Metabolic Syndrome Diagnosis: The Sooner the Better. *Metab Syndr Relat Disord* 2018; 16: 205–207.

[19] Lee AM, Gurka MJ, Deboer MD, et al. Current Evidence and Future Potential. 2016; 14: 411–413.

[20] Brage S, WEDDERKOPP, NIELS EKELUND U, FRANKS PW, et al. Features of the Metabolic Syndrome Are Associated With Objectively Measured Physical Activity and Fitness in Danish Children. *Diabetes Care* 2004; 27: 2141–2148.

[21] Heshmat R, Heidari M, Ejtahed HS, et al. Validity of a continuous metabolic syndrome score as an index for modeling metabolic syndrome in children and adolescents: The CASPIAN-V study. *Diabetol Metab Syndr* 2017; 9: 1–8.

[22] GN H, K W, DW D, et al. Objectively measured sedentary time, physical activity, and metabolic risk: the Australian Diabetes, Obesity and Lifestyle Study (AusDiab). *Diabetes Care* 2008; 31: 369–371.

[23] Deboer MD, Gurka MJ. Ability among adolescents for the metabolic syndrome to predict elevations in factors associated with type 2 diabetes and cardiovascular disease: Data from the national health and nutrition examination survey 1999-2006. *Metab Syndr Relat Disord* 2010; 8: 343–353.

[24] Wijndaele K, Duvigneaud N, Matton L, et al. Sedentary behaviour, physical activity and a continuous metabolic syndrome risk score in adults. *Eur J Clin Nutr* 2009; 63: 421–429.

[25] Gurka MJ, Ice CL, Sun SS, et al. A confirmatory factor analysis of the metabolic syndrome in adolescents: an examination of sex and racial/ethnic differences. *Cardiovasc Diabetol* 2012; 11: 1–10.

[26] Gurka MJ, Golden SH, Musani SK, et al. Independent associations between a metabolic syndrome severity score and future diabetes by sex and race: the Atherosclerosis Risk In Communities Study and Jackson Heart Study. *Diabetologia* 2017; 60: 1261–1270.

[27] Lee AM, Gurka MJ, DeBoer MD. Trends in Metabolic Syndrome Severity and Lifestyle Factors Among Adolescents. *Pediatrics* 2016; 137: e20153177–e20153177.

[28] Price AJ, Crampin AC, Amberbir A, et al. Prevalence of obesity, hypertension, and diabetes, and cascade of care in sub-Saharan Africa: a cross-sectional, population-based study in rural and urban Malawi. *Lancet Diabetes Endocrinol* 2018; 6: 208–222.

[29] Francis-Emmanuel PM, Thompson DS, Barnett AT, et al. Glucose metabolism in adult survivors of severe acute malnutrition. *J Clin Endocrinol Metab* 2014; 99: 2233–2240.

[30] de Rooij SR. *Metabolic consequences of prenatal exposure to the Dutch famine*. 2007.

[31] Uauy R, Kain J, Corvalan C. How can the Developmental Origins of Health and Disease (DOHaD) hypothesis contribute to improving health in developing countries? *Am J Clin Nutr* 2011; 94: 1759–1764.

[32] Itoh H, Kanayama N. Nutritional conditions in early life and risk of non-communicable diseases (NCDs) from the perspective of preemptive medicine in perinatal care. *Hypertens Res Pregnancy* 2015; 3: 1–12.

[33] Black RE, Victora CG, Walker SP, et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. *Lancet* 2013; 382: 427–451.

[34] Adair LS. Long-term consequences of nutrition and growth in early childhood and possible preventive interventions. *Nestle Nutr Inst Workshop Ser* 2014; 78: 111–120.

[35] Castro JAC, Nunes HEG, Silva DAS. Prevalence of abdominal obesity in adolescents: association between sociodemographic factors and lifestyle. *Rev Paul Pediatr (English Ed* 2016; 34: 343–351.

[36] Cho KI, Kim BH, Je HG, et al. Gender-specific associations between socioeconomic status and psychological factors and metabolic syndrome in the Korean population: Findings from the 2013 Korean national health and nutrition examination survey. *Biomed Res Int*; 2016. Epub ahead of print 2016. DOI: 10.1155/2016/3973197.

[37] Bendech MA. Nutrition Terminology and Hunger Situation Analysis. *Mainstreaming Nutr Natl Agric Food Secur Invest Plans Africa South AFrica Work* 2013; 13.

[38] NACS. MODULE 2. Nutrition Assessment and Classification. *Nutr Assessment, Couns Support*.

[39] Gross JG, Wendel PL. Well synchronised triggered clock. *Electron Eng* 1974; 46: 9.

[40] Organisation WH. Malnutrition. *Factsheet*, http://www.who.int/en/news-room/fact-sheets/detail/malnutrition (2018, accessed 1 April 2018).

[41] Vonaesch P, Tondeur L, Breurec S, et al. Factors associated with stunting in healthy

children aged 5 years and less living in Bangui (RCA). *PLoS One*; 12. Epub ahead of print 2017. DOI: 10.1371/journal.pone.0182363.

[42] Charles A, Medisch A, Universiteit C, et al. Obesity in Sub-Saharan Africa. *Metab Syndr* 2017; 1–13.

[43] Malawi DHS. Malawi 2015-16 Demographic and Health Survey. *DHS Progr*, https://dhsprogram.com/pubs/pdf/FR319/FR319.pdf (2015).

[44] Wang N, Wang X, Li Q, et al. The famine exposure in early life and metabolic syndrome in adulthood. *Clin Nutr* 2017; 36: 253–259.

[45] Lelijveld N, Seal A, Wells JC, et al. Chronic disease outcomes after severe acute malnutrition in Malawian children (ChroSAM): a cohort study. *Lancet Glob Heal* 2016; 4: e654–e662.

[46] Liang Y, Hou D, Zhao X, et al. Childhood obesity affects adult metabolic syndrome and diabetes. *Endocrine* 2015; 50: 87–92.

[47] Rosini N, Moura SAZO, Rosini RD, et al. Metabolic Syndrome and Importance of Associated Variables in Children and Adolescents in Guabiruba - SC, Brazil. *Arq Bras Cardiol* 2015; 37–44.

[48] Durnin BYJVG a, Womersley J. and Its Estimation From Skinfold Thickness : Measurements on. *Br J Nutr* 1973; 32: 77–97.

[49] Ellis KJ. Human body composition: In vivo methods. *Physiol Rev* 2000; 80: 649–680.

[50] Body_comp_repeatabilityvalidity_CFP-2018-0093R1.

[51] Wells JCK, Fewtrell MS. Measuring body composition. *Arch Dis Child* 2006; 91: 612–617.

[52] Kim K, Park SM. Association of muscle mass and fat mass with insulin resistance and the prevalence of metabolic syndrome in Korean adults: a cross-sectional study. *Sci Rep* 2018; 8: 2703.

[53] Padidela R, Nilsson O, Linglart A, et al. X-Linked Hypophosphatemia Registry-an international prospective patient registry. 2018; 2: 3252.

[54] Office NS. 2018 Malawi population and housing census Main report. *Genet Med* 2018; 8: 12S-252S.

[55] UN-HABITAT. *Malawi : Blantyre Urban Profile insert Picture*. 2011.

[56] Kerac M, Bunn J, Seal A, et al. Probiotics and prebiotics for severe acute malnutrition (PRONUT study): a double-blind efficacy randomised controlled trial in Malawi. *Lancet* 2009; 374: 136–144.

[57] Kerac M, Bunn J, Chagaluka G, et al. Follow-up of post-discharge growth and mortality after treatment for severe acute malnutrition (FuSAM study): A prospective cohort study. *PLoS One*; 9. Epub ahead of print 2014. DOI: 10.1371/journal.pone.0096030.

[58] Dent N, Guerrero S, Hallam A, et al. Community-based Therapeutic Care (CTC).

[59] Sekokotla MA, Goswami N, Sewani-Rusike CR, et al. Prevalence of metabolic syndrome in adolescents living in Mthatha, South Africa. *Ther Clin Risk Manag* 2017; 13: 131–137.

[60] Pickering TG, Hall JE, Appel LJ, et al. Recommendations for Blood Pressure Measurement in Humans and Experimental Animals. *Hypertension* 2005; 45: 142–161.

[61] CDC. Centers for Disease Control and Prevention Laboratory procedure manual: total cholesterol, HDL-cholesterol, triglycerides, and LDL-cholesterol. 2004; 1–23.

[62] Studies C. Laboratory procedure manual, 1972. *Abstr Hosp Manag Stud*; 13.

[63] Fetal TI, Consortium NG. International Fetal and Newborn Growth Standards for the 21 st Century The International Fetal and Newborn Growth Consortium. 2009; 1–59.

[64] Tolonen H, Kuulasmaa K, Laatikainen T, et al. Recommendation for indicators, international collaboration, protocol and manual of operations for chronic disease risk factor surveys. *WWW-publications from Eur Risk Monit Proj Finnish Natl Public Heal Inst*. Epub ahead of print 2002. DOI: 10.1007/SpringerReference_11722.

[65] Kilombero District. District Profile. 2013; 20–58.

[66] Povel CM, Beulens JW, Van Der Schouw YT, et al. Metabolic syndrome model definitions predicting type 2 diabetes and cardiovascular disease. *Diabetes Care* 2013; 36: 362–368.

[67] Alberti SG, Zimmet P. The IDF Consensus definition of the Metablic Syndrome in Children and Adolescents. *Int Diabetes Fed* 2007; 24.

[68] Patridge EF, Bardyn TP. Research electronic data capture (REDCap). *J Med Libr Assoc* 2018; 106: 142–144.

[69] Cruz ML, Weigensberg MJ, Huang TTK, et al. The Metabolic Syndrome in Overweight Hispanic Youth and the Role of Insulin Sensitivity. *J Clin Endocrinol Metab* 2004; 89: 108–113.

[70] Noubiap JJ, Essouma M, Bigna JJ, et al. Prevalence of elevated blood pressure in children and adolescents in Africa: a systematic review and meta-analysis. *Lancet Public Heal* 2017; 2: e375–e386.

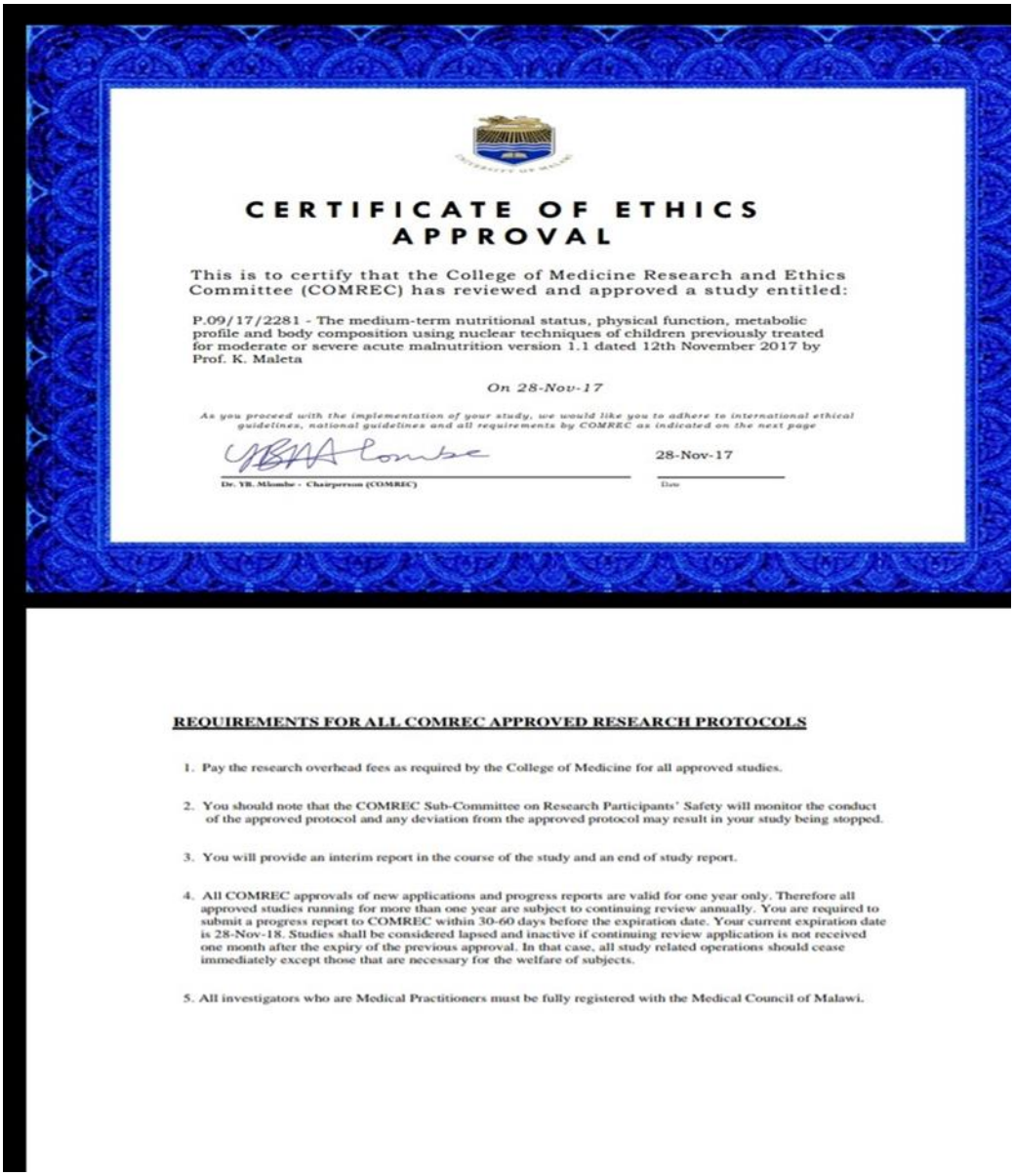
[71] Costa CRB, Melo ES, Antonini M, et al. Associação entre fatores sociodemográficos e comportamentais com a síndrome metabólica em pessoas vivendo com HIV. *Rev Gauch Enferm* 2019; 40: e20180379.

[72] Bennasar-Veny M, Lopez-Gonzalez AA, Tauler P, et al. Body Adiposity Index and Cardiovascular Health Risk Factors in Caucasians: A Comparison with the Body Mass Index and Others. *PLoS One* 2013; 8: 1–8.

[73] Zaki ME, Kamal S, Reyad H, et al. The validity of body adiposity indices in predicting metabolic syndrome and its components among Egyptian women. *Open Access Maced J Med Sci* 2016; 4: 25–30.

Appendices

Appendix 1: COMREC approval for primary data collection



CERTIFICATE OF ETHICS APPROVAL

This is to certify that the College of Medicine Research and Ethics Committee (COMREC) has reviewed and approved a study entitled:

P.09/17/2281 - The medium-term nutritional status, physical function, metabolic profile and body composition using nuclear techniques of children previously treated for moderate or severe acute malnutrition version 1.1 dated 12th November 2017 by Prof. K. Maleta

On 28-Nov-17

As you proceed with the implementation of your study, we would like you to adhere to international ethical guidelines, national guidelines and all requirements by COMREC as indicated on the next page

28-Nov-17

Dr. YR. Mlombe - Chairperson (COMREC) — Date

REQUIREMENTS FOR ALL COMREC APPROVED RESEARCH PROTOCOLS

1. Pay the research overhead fees as required by the College of Medicine for all approved studies.
2. You should note that the COMREC Sub-Committee on Research Participants' Safety will monitor the conduct of the approved protocol and any deviation from the approved protocol may result in your study being stopped.
3. You will provide an interim report in the course of the study and an end of study report.
4. All COMREC approvals of new applications and progress reports are valid for one year only. Therefore all approved studies running for more than one year are subject to continuing review annually. You are required to submit a progress report to COMREC within 30-60 days before the expiration date. Your current expiration date is 28-Nov-18. Studies shall be considered lapsed and inactive if continuing review application is not received one month after the expiry of the previous approval. In that case, all study related operations should cease immediately except those that are necessary for the welfare of subjects.
5. All investigators who are Medical Practitioners must be fully registered with the Medical Council of Malawi.

Appendix 2: Permission to use secondary data

COLLEGE OF MEDICINE

TO: IRB Administrator

FROM: Dr Kenneth M Maleta

DATE: 7th October 2018

RE: Permission to use secondary data collected as part of **P.09/17/2281 -** *The medium-term nutritional status, physical function, metabolic profile and body composition using nuclear techniques of children previously treated for moderate or severe acute malnutrition version 1.1 dated 12th November 2017* by Prof. K. Maleta

I write to certify that the study team for the above-named research study has granted Ms. Queen Mwakhwawa Kapito permission to use part of the study data for her Master's in Public Health dissertation. Ms. Mwakhwawa has been granted permission to use study participants background data as well as cardiometabolic profile data collected as part of this study for her own analysis. De-identified data will be made available to her as soon as it has been cleaned after collection.

Kenneth M Maleta
PI RAF6052 Research Project and
Group Lead, Public Health & Nutrition Research group.

Appendix 3: English Consent forms

(English version, to be translated into Chichewa and read aloud to all participants and guardians)

Dear participant, father or guardian,

WHAT IS THE RESEARCH ABOUT?

You are being asked to participate in a study that the University of Malawi, College of Medicine is doing in Malawi, together with the Lilongwe University of Agriculture and Natural Resources and the Ministry of Health and University College London in the UK. The purpose of the study is to explore the long-term effect of severe acute malnutrition on body composition, function and risk of chronic diseases. In Malawi, severe acute malnutrition has been a public health problem for a long time. We hope that the results of the study will help the Ministry of Health and stakeholders to improve care of patients being treated for acute malnutrition, to strike the balance between regain of body composition and function and risk of chronic diseases. The findings from this study are also expected to be used by health experts across the world. We have planned to compare children who were previously malnourished with those who have not (siblings and neighbors). Your contribution to this research and the body of evidence generated is very valuable.

RESEARCH PROCEDURES:

First, we will first ask you questions on socio-demographic variables which will help explain the results obtained under each objective. Secondly, we will measure you by taking your weight, height, and circumferences (head, waist and hip). Thirdly, we will assess your body composition a machine which will involve putting some gel on you and attaching some wires and reading from a machine. This procedure is entirely harmless and will not cause you any discomfort and will only last 3-4 minutes. Fourthly, we will collect some blood from your arm. We will collect 7.5 mls of blood which we will use to measure your hemoglobin, glucose, and fats. We will provide you results of the hemoglobin immediately but the other tests will require to be done in a laboratory far away hence will not be immediately available. Any left-over samples will be stored for 5 years initially and we may extend the storage period for another 5 years. However, any future extension of storage period will only be done after approval from College of Medicine Research and Ethics Committee. Fifth, we will invite you to come to a central site where we will

collect some saliva before and after giving you a specially made solution of heavy water. The procedure will require that you drink a 30 mls of the heavy water and wait for 3-4 hours during which time we will collect some saliva. This will help us calculate how much fat and lean tissue you have. Because it will take you time to get to the site and also require transport we will provide you with money for transport and reimburse you for the time you spend with us. Any left-over samples will be stored for 5 years initially and we may extend the storage period for another 5 years. However, any future extension of storage period will only be done after approval from College of Medicine Research and Ethics Committee.

ABOUT TAKING PART IN RESEARCH

Your participation in this study is voluntary; taking part in the study will not in any way disadvantage you. Make sure that you have understood the objectives and procedures of the study, if you have questions please do ask the members of the study team. If you decide to participate, you are still free to withdraw at any time and without giving a reason. Furthermore, you are also free to decline responding to certain questions in this study.

How will the information I give in the study be kept private / who will see my information?

All your information will be kept confidential. Information will be stored in password protected computers. To protect your privacy, we will use a code number to identify you and all information about you. We will keep records and samples securely locked. Your name, or any other facts that might point to you, will not appear when we present this study or publish its results. Your data may be shared with other researchers only in securely anonymised form.

After reading or hearing this information, we ask your permission to enroll you and your child into the trial. Please feel free to ask clarifications to any unclear issues or to consult your family members or friends about the decision. If thereafter you are willing to give your informed consent, please sign the attached form.

Please feel free to contact us or people that supervise research in the College of Medicine at any point if you have further questions. We sincerely thank you for your time and consideration.

Blantyre, _____ 2017

Dr. Kenneth Maleta Secretariat

Principal investigator

0888 232 202

COMREC

P/ bag 360, Chichiri Blantyre 3,

Tel: 08711911 Ext 334

Appendix 4: Chichewa consent forms

APPENDIX 1A: PARTICIPANT INFORMATION (CHICHEWA VERSION)

Wokondendwa otenga nawo mbali pakafukufukuyi, bambo kapena osunga mwanayu.

CHOLINGA CHA KAFUKUFUKUYU

Tikukupemphani kutenga nawo mbali pa kafukufuku amene sukulu ya Ukachenjede ya College of Medicine, ikupanga mogwirizana ndi sukulu ya Ukachenjede ya Agriculture and Natural Resources, unduna wa zaumoyo komanso sukulu ya Ukachenjede ya College London yaku UK. Cholinga cha kafukufukuyi ndi kufufuza zotsatira za kunyentchera nthawi yomwe munthu anali mwana ndi m`mene thupi lingaonekere, lingagwirire ntchito ndi chiopysezo ku matenda osapatsirana ngati matenda a sugar, mtima, kuthamanaga magazi.

Ku Malawi, kunyentchera lakhala vuto lalikulu kwa nthawi yaitali. Tikukhulupirira kuti zotsatira zakafukufukuyi zithandiza Unduna wa Zaumoyo ndi ogwira nawo ntchito kuti anthu onyentchera athandizidwe moyenera, kugwirizana ndi kubwezeretsa kwa thupi ndi magwiridwe ake antchito komaso chiopysezo ku matenda osapatsirana. Zotsatira za kafukufukuyi zikuyembekezeka kuzagwiritsidwa ntchito ndi a katswiri a za umoyo padziko lonse lapansi.

Takonza kuti tizasiyanitse ana amene anali onyetchera kale ndi amene sananyetcherepo (amene ali achibale kapena oyandikana nawo nyumba). Kutenga nawo mbali kwanu pakafukufukuyi ndi chinthu cha mtengo wa patali ndipo izo tizipeze zidzakhala zothandiza kwambiri.

MACHITIDWE A KAFUKUFUKU

Choyamba, tikufunsani mafunso okhudzana ndi moyo wanu zimene zithandize kufotokoza zotsatira zomwe tidzazipeze pa cholinga chilichonse. Chachiwiri, tidzakuyezani kulemera kwathupi lanu, kutalika kwanu ndi kukula kwa chiuno chanu. Chachitatu, tidzakuyesani mmene mbali zosiyanasiyana zathupi lanu zilili ndi makina. Izi tizazichita pokupakani mafuta ndikulumikiza mawaya ndi kuwerenga zomwe makina akunena za inu. Kuyesa uku sikopweteka ndipo sizidzayambitsa kusamva bwino kulikonse. Izi zidzachitika kwa mphindi pakati pa zitatu ndi zinayi.

Chachinayi, tizakutengani magazi kuchoka pamkono wanu. Tizakutengani magazi okwana 7mls (kukwana spoon yayikulu) amene azagwiritsidwe ntchito kuyeza kuchuluka kwa magazi, shuga

ndi mafuta mu thupi lanu. Tizakupatsani zotsatira zakuchuluka kwa magazi pompopompo, koma zoyesa zina zidzakachitira ku ma office a sukulu yophunzisirako a za chipatala (College of Medicine). Choncho, zotsatira za zoyesa zina sizidzapezeka pompopompo. Magazi otsalira tidzawasunga kwa zaka zisanu ndipo mwinanso tidzaonjezera nthawi yousunga magaziwo mpaka zaka zina zisanu. Kusunga magazi kwa nthawi yowonjezerayi kudzachitika pokha-pokha a bungwe la kafukufuku la COMREC atatipasa chilolezo.

Chachisanu, tizakuitanani ku likulu la kafukufukuyu kumene tidzatenga malovu anu pa nthawi zosiyana-siyana komanso muzamwamadzi a kafukufuku olemera kukwana 30mls . Izi zizatithandiza kuti tipeze kuchuluka kwa mafuta ndi mnofu wanu. Tizakupatsani ndalama zoti muyendere ndiponso kukupatsani kena kake chifukwa tidzakutengerani nthawi kuti mufike ku likulu la kafukufukuyu. Malovu otsalira tidzawasunga kwa zaka zisanu ndipo mwinanso tidzaonjezera nthawi yosunga malovuwa mpaka zaka zina zisanu. Kusunga malovu kwa nthawi yowonjezerayi kudzachitika pokha-pokha a bungwe la kafukufuku la COMREC atatipasa chilolezo.

ZOKHUDZANA NDI KUTENGA NAWO MBALI PA KAFUKUFUKUYI

Kutenga nawo mbali pakafukufukuyi zitengera kufuna kwanu; kutenga nawo mbali pakafukufukuyi sizidzakupatsani mavuto aliwonse

Onetsetsani kuti mwamvetsetsa zolinga ndi dongosolo la kafukufukuyi. Ngati Muli ndi mafunso, funsani oyendetsa kafukufukuyi.

Ngati musankhe kutenga nawo mbali pakafukufukuyi, muli ndi ufulu kusiya panthawi iliyonse osapereka chifukwa. Mulinso ndi ufulu okana kuyankha mafunso ena mu kafukufukuyi

Kodi zomwe ndinene(/kupereka) mukafukufuku ameneyu zidzasungidwa bwanji mwa chinsinsi/adzaone zaineyo ndindani?

Zonse zokhudzana ndi inu tidzazisunga mwa chinsisi. Zokhudzana ndi inu zidzasungidwa mu makina a kompyuta otetezedwa. Pofuna kusunga chinsinsi, tizagwiritsa ntchito nambala yapaderadera imene idzasunge zokhudzana ndi inu ndiponso kukuzindikiritsani. Ndondomeko ya zomwe muli komanso zotengedwa kuchoka kwa inu zidzakhomeredwa mumabokosi.

Dzina lanu, kapena chichilichonse chomwe chingakuzindikiritseni inu sizidzasindikizidwa muzotsatira zakafukufukuyu. Tikhoza kuzagawana zokhudzana ndinu koma osakutchulani dzina.

Mutatha kuwerenga zalembedwazi, tikupemphani kuti inu ndi mwana wanu mutenge nawo mbali pakafukufukuyu. Khalani omasuka kufunsa mafunso ngati simunamvetse kapena kukambirani ndi a pabanja anu kapena anzanu zokhudzana ndi chisankho chanu. Mukapanga chisankho chofuna kutenga nawo mbali, chonde sainani pa pepala ili.

Panthawi iliyonse imene muli ndi mafunso, chonde khalani omasuka kulumikizana nafe kapena oyang`anira zakafukufuku ku sukulu ya College of Medicine.

Tathokoza chifukwa cha nthawi yanu ndi kumvetsetsa kwanu.

Blantyre, _____, 2017

_____	_____
Dr. Kenneth Maleta	Secretariat
Principal investigator	COMREC
0888 232 202	P/ bag 360, Chichiri Blantyre 3,
	Tel: 08711911 Ext 334

Date of visit: |___|___| |___|___| 20 |___|___|

Number (code) of visit: |___|___|

Chidziwitso cha opanga kafukufuku (IAEA number) |___|___|___|___|

Dzina la mwana _____

Mudzi ochokela _____

Dzina la mtsogoleri wa banja _____

Chidziwitso cha banja lanu cha ku chipatala _____

Njira ya kunyumba kwanu _____:

A _____ andiuza za cholinga ndikufunikila kwa kupanga kafukufuku was kunyetchera munthu ali mwana ndi momwe thupi lake limagwirira ntchito atakula.. Ndili ndi mpata ndi ufulu ofunsa pa zomwe andifotokozela nthawi iliyonse. Ndikudziwanso kuti kutenga nawo gawo kapenanso ayi sikukhudzana ndi mbali ina iliyonse ya m'mene ndimakhalira.

Pakutha kulingalira pa uthengawu ndaloleza kuti mwana wanga otchedwa _____ alowe nawo mu gulu la ana a kafukufuku ameneyu. Koma ndili ndi ufulu woti ndikhoza kumuchotsa mwana wangayu mu kafukufukuyu nthawi iliyonse popanda kufotokoza chifukwa chilichonse.

Dzina la kholo: _____

Kusayina kwa kholo: _____

Kusayina kwa mwana _____

Date: |___|___| |___|___| 20 |___|___|

Blantyre.

Dzina la ofotokoza: _____.

Kusayina kwa ofotokoza: _____

Date: |___|___| |___|___| 20 |___|___| Blantyre

Appendix 5: Standard Operating Procedures for deuterium dilution technique Procedure for assessing body composition in children and adults with analysis of deuterium enrichment using the Agilent 4500 Series Tumbl-IR spectrometer

1) Introduction

This protocol describes the procedure for assessing body composition by deuterium dilution in children and adolescents, and also adults, with collection of saliva specimens that will be analysed using an Agilent 4500 Series Tumbl-IR portable Fourier transform infrared (FTIR) spectrometer.

When assessing body composition by deuterium dilution, the amount of deuterium consumed is used in the calculation of total body water (TBW). Therefore dose preparation and dose consumption are critical parts of the procedure, and need to be done with care, as errors at this stage will affect the accuracy of the body composition assessment.

The dose of 99.8 atom % deuterium oxide (D_2O) required for children and adults is 0.1 g per kg body weight. Based on 85th centile (+1 SD) weight-for-age (WHO 2007 Growth Reference) and 0.1 g D_2O per kg body weight, the dose for children aged 3-11 years is shown in Table 1.

Age	Girls Weight (kg)	Boys Weight (kg)	Dose D_2O (g)
3-4	16-19	16-19	1.9
5-6	21.3	21.1	2.1
6-7	23.7	23.6	2.4
7-8	28.1	28.1	2.8

8-9	31.8	31.4	3.2
9-10	36.1	35.2	3.6
10-11			4.0

TABLE 1. Amount of D_2O required to assess body composition of children aged 3-11 years.

Because the volume required is small (approximately 2-4 mL), it is advisable to make a 1 in 10 dilution of the 99.8 atom % D_2O, in a large container, and then dispense the required amount into individual dose bottles (Nalgene, wide mouth, leak-proof autoclaveable bottles). An equipment list is included in Annex 1.

In large studies, it is easier to make the doses in batches, based on the expected body weight of the participants. In the case of children, aged 3-11, three doses can be used containing 2, 3 or 4 g D_2O depending on the age and size of the children as shown in Table 2. A dose of 5 to 6 g D_2O is recommended for adolescents, depending on their body weight. The target enrichment is 100-250 mg/kg above the baseline.

The expected enrichment (mg/kg)can be calculated by dividing the dose in milligrammes by a prediction of total body water (TBW) in kilogrammes using the equation of Slater & Preston (2005).

For example,

Predicted TBW = 7.4 x height^3

Height = 165 cm = 1.65 m

Dose = 6 g D_2O = 6 000 mg

Estimated TBW = 7.4 x $(1.65)^3$ = 33.24 kg

Expected enrichment = 6 000 mg / 33.24 kg = 180.5 mg/kg (ppm)

Age (years)	Dose D_2O (g)	Amount of 1:10 dilution (g)

3-6	2	20
7-9	3	30
10-12	4	40
13-18	5-6	50-60
Adults	6	60

TABLE 2. Recommended amount of 1in 10 dilution of D_2O required to assess body composition of children and adults.

2) Preparation of deuterium oxide doses

Doses need to be carefully weighed on a balance reserved for this purpose, and should be prepared in a food preparation area, not in a laboratory. The doses are prepared in two stages. Firstly a large volume of 10% deuterium oxide in drinking water (the 'dose stock') is prepared in a large container with a cap, then this is dispensed into individual doses, based on the expected average body weight of the participants (see Table 2). All equipment used for preparing doses must be completely dry to avoid contamination by water.

2.1 Preparation of the 1 in 10 dilution of deuterium oxide (Dose stock)

- Make 1:10 dilution of 99.8% D_2O in large bottle: enough for the whole study
- For example, for 150 children with 4 g dose D_2O, 150 x 4 g D_2O = 600 g
- 600 g 99.8% D_2O + 5400 g bottled water = 6000 g. A container with a capacity of at least 7 litres is required, since the density of D_2O is higher than the density of H_2O. 1 g deuterium has a volume of 1.1 mL.

- A balance capable of weighing the container AND the contents to 0.1 g is required. In the example above, the balance needs a capacity of at least 10 kg and precision of 0.1 g.
-

Proceed as follows:

1. Tare a large bottle with its cap (e.g. Nalgene Carboy, low density polyethylene narrow neck bottle with handles and polypropylene cap, capacity 10 L, part number 2210-0020, or borosilicate glass (Pyrex or Duran) reagent bottle) on a digital top loading balance capable of weighing at least 10 kg to 0.1 g.
2. Add the required amount of deuterium oxide for the whole study (600 g D_2O), replace the cap and weigh again. Note the weight to 0.1 g.
3. Add 5400 g bottled drinking water, replace the cap, and weigh again to 0.1 g.
4. This 10% solution of deuterium oxide can be stored in a dark place at ambient temperature until the individual doses are prepared.

2.2 Preparation of individual doses

- Dose bottles must be screw capped and leak proof (e.g. 60 mL or 120 mL Nalgene wide mouth, leak proof, autoclavable, polypropylene bottles) to avoid losses during storage and contamination by moisture from the atmosphere. It is not necessary to autoclave the bottles, but these bottles will not crack or leak if stored in a freezer.
- The balance used to weigh the dose must have a weighing range that is adequate for the amount AND the container to be weighed. A balance weighing to 0.001 g is recommended.
- Keep a small amount of the dose water (10% D_2O in drinking water) to send to the analytical laboratory for analysis with the saliva specimens.
- Doses can be stored in a fridge, if they will be used within one week, or in a freezer at -20 degrees Celsius until required.

Proceed as follows:

1. Tare the dose bottle plus lid on an electronic balance with a precision of 0.001 g.
2. Pour some of the 10% D_2O into a large, clean container e.g. 600 mL beaker or a glass jug (recommended because it is hard to pour directly from the Carboy into a 100 mL measuring cylinder).
3. Transfer the approximate required amount (20 to 60 mL) of the dose stock (10% D_2O) to a 100 mL glass measuring cylinder.
4. Add the contents of the measuring cylinder to the tared bottle and replace the cap.
5. Record of the weight.
6. Label the dose bottle with the dose number, date of preparation and initials of the person who prepared the dose.

If the staff at the analytical laboratory will be calculating the body composition, they will need to know the weight of deuterium oxide in the 10% solution (Dose stock), the weight of water added, and the weight of the dose stock in the dose bottle.

2.3 Dose storage

The doses can be stored in a refrigerator or freezer at -20 degrees until required.

To ensure good hygiene and avoid cross contamination, doses should not be stored in the same place as saliva or other clinical specimens. The dose contains approximately 500 times as much deuterium as the biological samples. In addition, doses should not be stored with saliva specimens to avoid microbial cross contamination.

When transporting doses to and from the field, use separate boxes for doses and saliva samples.

2.4 Preparation of the diluted dose for analysis with the samples.

An aliquot (1-2 mL) of the dose stock solution (10% D_2O) should be sent to the analytical laboratory for analysis with the saliva specimens. The cryovials used to store saliva specimens are suitable containers. A dilution of the dose should be prepared as follows. This can be done using the same balance that was used to weigh the doses or at the analytical laboratory, if the samples are to be transferred.

1. Tare a 100 mL volumetric flask with its cap on a digital top loading balance weighing to 0.001 g.
2. Add 200 microlitres of the dose stock (10% D_2O) using a pipette. Replace the cap and note the weight
3. Make up to the mark using the same bottled water that was used to make the dose, replace the cap and weigh again.
4. Transfer to several cryovials (10-20) for immediate use, and keep the remainder in a 100 mL screw cap bottle for storage.
5. Keep 100 mL of the water used to make the dilution as a natural abundance sample, stored in crovials and a screw-cap bottle. Vials and bottles should be stored in a cool dark, place (cupboard or refrigerator, depending on the ambient temperature) until required.
6. Analyse the diluted dose and water used to make the dilution with each batch of samples. New vials should be used each day.

Note: It is good practice not to open the main bottle too often, to avoid fractionation. Small amounts should be stored in cryovials for daily use., and further aliquots can be prepared at intervals.

3) Procedure for measuring total body water (TBW)

The participant should have normal fluid and food intake on the day before the TBW estimation and avoid vigorous exercise and consumption of alcohol to avoid dehydration and depletion of glycogen stores.

For accurate measurements of TBW, participants should be asked to empty their bladder before starting. This will ensure that body weight is measured under the same conditions each time in longitudinal studies, and that water in urine is not included in TBW.

The procedure is summarised in Fig. 1.

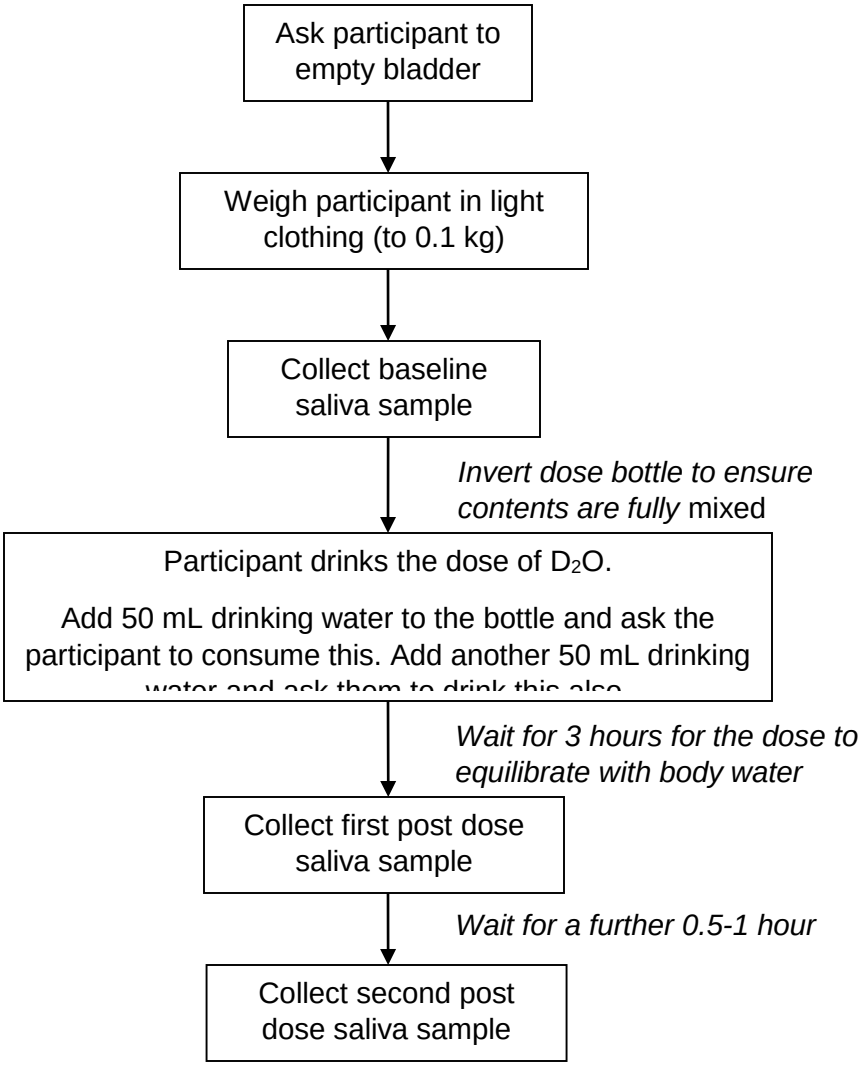


FIG. 1. Flow chart describing the procedure for measuring total body water by deuterium dilution.

3.1 Anthropometry

An accurate measure of body weight is required because body fat is estimated by difference of fat free mass (FFM) from body weight. Participants should be asked to empty their bladder (and if possible bowels) before being weighed, and should be weighed in light clothing. Standardising conditions in this way is particularly important in longitudinal studies. The accuracy of scales used for measuring body weight should be checked daily using a calibration weight of known mass.

Much care is taken to ensure the accuracy and precision of isotopic data. Body composition results will be compromised if equal care is not taken to ensure the accuracy of anthropometric measurements.

Measuring weight

- The participant's weight must be measured to the nearest 0.1 kg using electronic scales or any balance with adequate precision.
- The balance must be placed on a level surface. Check using a spirit level, if possible.
- Participants should wear minimal clothing and no shoes (Fig. 2). If they do not wish to wear minimal clothing during the weighing procedure, their clothes should be weighed separately afterwards, and the weight of their clothes subtracted to obtain an accurate measure of body weight.
- Record the weight on the participant's information sheet to 0.1 kg.
- In longitudinal studies measuring changes in body composition over relatively short periods of time, an accurate measurement of body weight is essential. Account must be taken of the weight of any clothing worn during the measurement.
- The accuracy of the scale should be checked regularly using a calibration weight of known mass.

FIG. 2. Measuring weight. Weight is measured in light clothing without shoes.

Measuring height

- Height must be measured to the nearest 0.1 cm using a stadiometer.
- The stadiometer must be placed on a level surface. Check using a spirit level, if possible. The accuracy of the stadiometer should be checked periodically using measure rods of known length.
- Height is measured without shoes.
- The participant should stand upright with their heels to the wall or touching the vertical post on the stadiometer. Their knees should be straight.
- Ask them to look straight ahead. Make sure the eyes are the same level as the ears (Fig. 3).

- The beam is lowered until it just touches the top of the head. Elaborate hair arrangements must be undone. Record the height in centimetres (to the nearest 0.1 cm) on the participant's information sheet. Repeat the measurement. Record both measurements and calculate the mean.

Fig. 3. Measuring height

For more information on measuring weight and height, see the IAEA Human Health Report on Improving Quality Assurance in Field Implementation of Stable Isotope Techniques in Nutrition: Part 1 Anthropometry (in preparation).

3.2 Dose administration

In adults and children, the dose should be consumed at least 2 hours after the last meal, preferably after an overnight fast. If this is not possible, a small meal may be given one hour after the dose was taken. The meal should be simple and less than 1250 kJ (300 kcal). This allows the dose to empty from the stomach before the meal, but water in the meal to equilibrate with body water before the post-dose saliva samples are collected. In infants and young children, the dose is usually given with a meal.

- Baseline saliva samples must be obtained before the dose is consumed.

- If the dose has been frozen, it should be completely thawed before use.
- Whether stored in a fridge or thawed following freezer storage, the bottle should be inverted several times to mix any condensation on the cap into the bulk of the liquid. This should be done immediately before the dose is consumed. This is because the condensation is fractionated relative to the bulk liquid. See the IAEA eLearning module on Assessing Body Composition by Deuterium Dilution technique for more information on fractionation.
- Do not open the bottle until it is time for the dose to be consumed.

When talking to participants, it is often better to use the term 'heavy water' or 'special water' rather than 'deuterium-labelled water' or 'stable isotope labelled water', as there can be confusion over the word 'isotope', which is often associated with radioactivity.

There is no radiation hazard associated with the use of deuterium oxide

ws:

1. Note the bottle number and the time the dose was taken on the participant's data sheet.
2. Participants should drink the dose through a straw to avoid spillage (Fig. 4).
3. Add about 50 mL drinking water to the dose bottle and ask the participant to drink this through the same straw. Repeat with another 50 mL drinking water. This will ensure that no labelled water is left in the bottle.

FIG. 4. Dose administration. The participant drinks the dose through a straw to avoid spillage.

3.3 Food and drink intake, and physical activity during the equilibration period

If possible participants should not eat or drink during the equilibration period, but if this is not possible e.g. in children and lactating women, a small meal can be given 1 hour after the dose. Keep a note of the volume of all drinks taken during the equilibration period, including the 100 mL used to rinse the dose bottle. This volume should be subtracted from the calculated TBW. If no additional fluid is consumed during the equilibration period, the 100 mL water used to rinse the dose water is usually ignored. Participants should not drink and eat between the 3 and 4 h saliva specimens to minimise any short-term effect of water intake on deuterium enrichment in saliva.

Participants should avoid physical activity during the equilibration period to minimise water loss in breath and evaporation from the skin (insensible water loss). There is less deuterium in water vapour than in body water due to isotopic fractionation. Therefore, an increase in insensible water loss will lead to an error in the calculation of TBW.

3.4 Saliva sampling

3.4.1 Preparing for saliva sampling

Good preparation before taking the samples and a clear understanding of the procedure are very important for accurate results. Clearly explain the procedure to the participant before sampling.

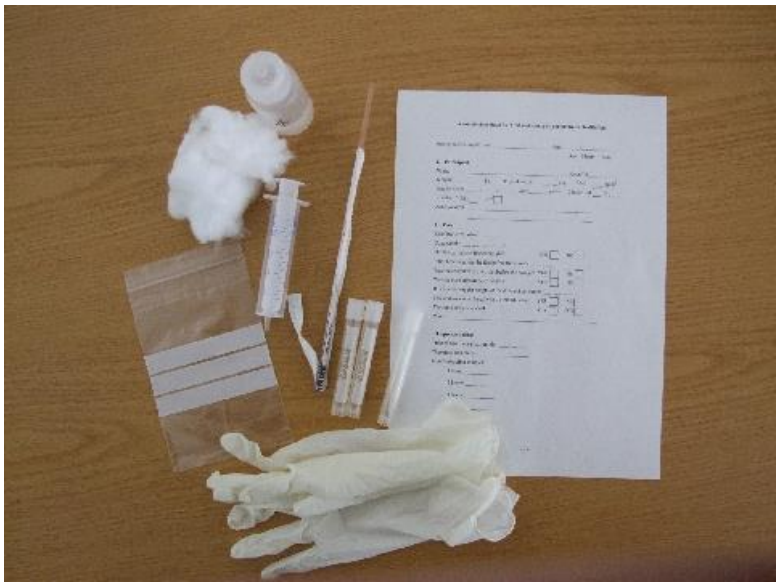


FIG. 5. Equipment required for saliva sampling.

Make sure the following items are available before starting (Fig 5).

Cotton wool balls or dental swabs:

- Cotton wool balls or dental swabs are used to collect saliva samples from adults and children (Fig 6).

Sample storage vials:

- Must be screw capped with a seal to prevent losses, fractionation and cross-contamination during storage e.g. 2 mL cryovials. It is a good idea to use different coloured caps for baseline and post dose samples e.g. blue caps for baseline samples and red caps for post dose samples, or to mark the cap of the baseline sample with a coloured pen.
- Must be completely dry before use.
- Must not be reused to prevent cross contamination between enriched (post dose) samples and unenriched (baseline) samples.

- Should be labelled with the participant's identification number, date and time the sample was taken. Names should not be written on sample vials to preserve confidentiality.

Disposable 20 mL syringes:

- Must be completely dry before use.
- Must not be reused to prevent cross contamination between enriched (post dose) samples and unenriched (baseline) samples.\
-

Gloves:

- New disposable gloves must be worn by the person taking the saliva sample.
- Gloves must be discarded before moving on to the next participant.
- Do not touch the dose bottle after putting on gloves to take the baseline saliva sample until after the sampling is complete.

Zip-lock bags:

- Two small ziplock bags are needed for each participant:
    - one for the baseline sample, and
    - one for the post dose samples.
- Another ziplock bag is needed to keep all the samples from the participant together.
- All bags must be labelled permanently with the participant's identification number.

Labels:

- Ensure labels are of good quality and cannot come off the containers.
- Use a permanent marker to write on the labels, to avoid the writing being smudged or removed, in particular when the samples are thawed.

Participant data sheets:

- Print outs of data sheets for each participant need to be available before the first sampling (baseline).
- To preserve confidentiality, do not write names on the data sheets. The names and corresponding participant IDs must be recorded separately.
- An example of a participant data sheet is shown in Annex 2.

3.4.2 Saliva sampling using cotton wool balls

FIG. 6. Saliva sampling using cotton wool balls.

Do not touch the dose bottle during sample collection, from the time you have put on gloves until the baseline saliva sampling procedure is completed.

1) Use clean gloves for each participant
2) When collecting samples ensure that the participant does not eat or drink anything for at least half an hour before saliva collection.
3) Give the participant a cotton wool ball to soak up saliva. Ask them to move it round their mouth for 2 minutes or until sodden, keeping their mouth closed while doing this. Asking them to think about their favourite food increases salivation.
4) Remove the plunger from a new 20 mL disposable syringe.

5) Ask the participant to transfer the cotton wool to the front of their mouth and transfer it directly from the mouth into the body of the syringe (Fig. 6).

6) Replace the plunger in the body of syringe.

7) Label a sample storage vial with Participant ID, Date and Time of collection.

8) Remove the lid from the vial, and use the syringe plunger to extract saliva from the cotton wool into the sample storage vial (Fig. 6). Replace the lid to avoid evaporation and subsequent isotope fractionation.

9) If there is not at least 1 mL of saliva repeat above steps with a new cotton wool ball or swab.

10) Discard syringe, cotton wool and gloves between participants. Do not reuse sample vials or syringes.

11) Record the participant's identification number, date and time the sample was taken on each vial. Record all dates and times of saliva collection on the participant's data sheet. Copy this information to a spreadsheet as soon as possible.

Participants should avoid physical activity until the final saliva samples have been taken.

3.5 Storage of saliva samples

A large study will generate hundreds of samples; therefore careful management and labelling of saliva samples is essential. Proceed as follows:

- Containers must be firmly closed to prevent loss of water by evaporation, and cross contamination between samples.
- Ziplock bags can be used to keep all samples for a single person together and prevent cross contamination between persons. Use a small bag for the baseline sample and another small bag for the post dose samples. Then place the two bags in a third, larger one, so that the samples from a single participant are kept together.
- Write the participant identification number on both the sample vials and the ziplock bags.
- Keep a log of samples in a spreadsheet.

Saliva samples should be stored frozen (-20ºC) until analysis to minimise bacterial growth. If this is not possible, samples should be stored in a refrigerator or a cool box until they can be transferred to a freezer (preferably within 24 hours).

To avoid contamination of samples:

- Never store samples and doses together;
- Always ensure that the cap of the sample bottles is tightly closed to avoid losses by evaporation and contamination by moisture from the atmosphere.

3.6 Transfer of samples to the analytical laboratory

Specimens must be packaged in accordance with IATA 650 Packaging Instruction. UN3373 Biological Substance Category B.

Briefly, specimens must be in primary containers (e.g. cryovials and zip lock bags), which are packed inside a secondary container and then in rigid outer packaging. Primary containers must be leak-proof and packed inside a secondary container in such a way that, under normal conditions of transport, they cannot break, be punctured or leak their contents into the secondary container. The secondary container must also be leak proof. Absorbent material must be placed between the primary and secondary containers. The absorbent material (e.g. cotton wool) must be in sufficient quantity to absorb the entire contents of the primary containers. Either the primary or the secondary container must be capable of withstanding, without leakage, an internal pressure of 95 kPa in the range of -40°C to 55°C (-40°F to 130°F). The outer packaging must not contain more than 4 L. The outer container must be labelled "Biological Substance – Category B packed in accordance with IATA 650. UN3373".

4) Sample analysis

The enrichment of deuterium is measured using a portable FTIR (Agilent 4500 Series Tumbl-IR portable FTIR spectrometer). This equipment is more robust and more sensitive than conventional transmission FTIRs with a liquid cell. The equipment is illustrated in Fig, 7. The Tumbl-IR is a mid infrared spectrometer, range 500-5000 $cm^{-1,}$, PC controlled (64-bit; Windows 7). It is portable with internal batteries and a toughened transport case. It has factory-aligned

optics, which make it robust, compared to models with moving mirrors. The path length is 100 mm. The sample size required for each analysis is only 20-30 microlitres. It can be used to analyse the deuterium concentration in water, saliva & serum. The equipment is calibrated against the reference method for deuterium analysis, which is isotope-ratio mass spectrometry, and gives the concentration of deuterium in the sample in mg/kg, without further processing of the data. Further discussion is beyond the scope of this protocol.

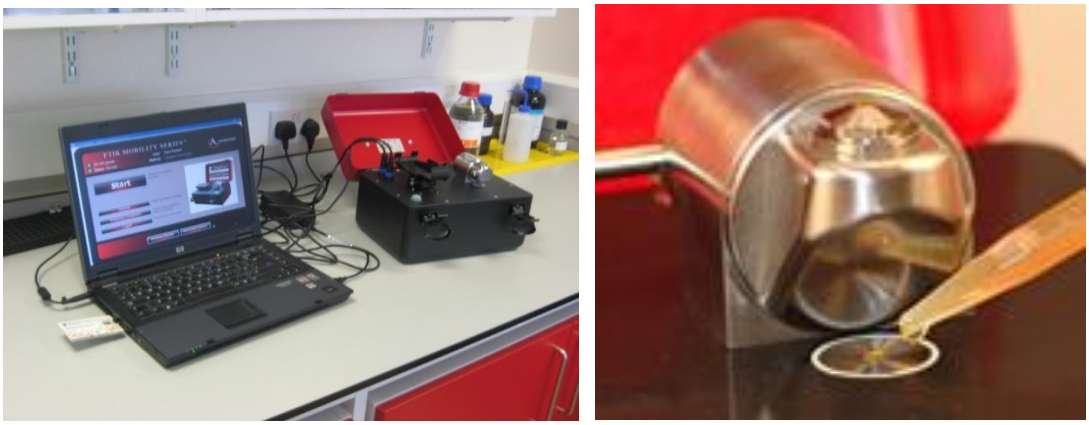


Fig. 7. Agilent 4500 Series Tumbl-IR portable FTIR spectrometer. The photo on the right shows a close-up of the head in the position for applying the sample to the window

5) Calculation of body composition

A spreadsheet template to calculate body composition is available from the IAEA. E-mail nahres@iaea.org for a copy.

Participant and Study	Input		
Study	Study name		
Department	Department of Human Nutrition		
Institute	Name of Institute		
Study ID	2		
Sex	Female		
Date of Birth (day/month/year)	17/11/1972		
Date of dosing (day/month/year)	25/04/2017		
Age (decimal years)	44.4		
Weight, kg	58.5		
Height, cm	161.7		
BMI-Z-Score (from Anthro)		For children and adolescents only	
Dose stock (10% dilution of 99 atom % D_2O)			
Weight of 99 atom % D_2O (g)	84.06		
Weight of drinking water added (g)	730		
Total weight of water + D_2O in the dose stock (g)	814.06		
Dose aliquot given to the participant			
Dose bottle number	2		
Weight 10% D_2O (g) in dose bottle	60.44	to 0.001 g (A)	
Time dose given (hr:min)	09:31		
Weight of 10% D_2O consumed per kg BW (g/kg body weight)	1.033	~1 g per kg body weight	
Saliva Sampling	**Time**	**Time since dosing (hours)**	
Post dose sample 1	12:30	02:59	
Post dose sample 2	13:30	03:59	
OUTPUTS			
Body Composition	**kg**	**% Body Weight**	**Normal Range**
TBW	27.62	47.2	40-70%
Fat-Free Mass	37.73	64.5	55-90%
Body fat mass	20.77	35.5	10-45%

Cell B10 on the first page of the workbook 'Field inputs+outputs', contains a pull-down menu for the sex of the participant. Click the mouse on the cell to reveal the arrows for the pull-down menu, then click the arrows to reveal the choices. Choose 'Male' or 'Female'.

Further information

IAEA publications and eLearning modules on stable isotope techniques in nutrition can be accessed via the Nutrition pages of the IAEA Human Health Campus http://humanhealth.iaea.org

History

Prepared by Christine Slater 7 November 2016 and revised to include younger children 30 January 2017.

Updated by CS 3 March 2017 to correct error in instructions for making the dose dilution, and on 4 May 2017 to include doses for adults, and update the information on the spreadsheet.

References

SLATER C, PRESTON T (2005) A simple prediction of total body water to aid quality control in isotope dilution studies in subjects from 3-87 years of age. *Isotopes in Environmental and Health Studies* **41**(2), 99-107.

Annex 1 List of equipment and consumables

For making deuterium oxide doses 10% D_2O

- 1 kg 99.8 at.% D_2O
- 6 L bottled drinking water
- 10 L Nalgene Carboy (LDPE bottle, PP cap), or similar large container
- Balance weighing up to approx. 10 kg to 0.1 g
- 500 mL glass beaker
- 100 mL measuring cylinder
- 150 x 60 mL Nalgene leak-proof bottles (or 150 x 120 mL bottles)
- Balance weighing to 0.001 g
- Labels
- Cryopens

For making the diluted dose for analysis of with the saliva samples

- 1 in 10 dilution of 99.8 at.% D_2O (aliquot of the dose)
- Aliquot of the water used to make the dose
- 1 mL automatic pipette + pipette tip
- 100 mL volumetric flask
- Analytical balance weighing approx. 200 g to 0.0001 g
- 2 x Screw cap borosilicate glass bottles (capacity 100 mL)
- Labels
- Permanent ink pens

For saliva sampling

- Cotton wool balls (local purchase)
- 200 mL disposable syringes with Luer tips
- 2-4 mL screw cap cryovials
- Clean test tube rack
- Permanent ink pens
- Zip-lock (or similar) sealable bags for storing cryovials

For dose consumption

- Pre-prepared dose 10% D_2O
- Straws (local purchase)
- Drinking water to rinse bottles (local purchase)

For anthropometry

- Non-stretchable tape measure e.g. SECA 201 measures to 205 cm x 1
- Portable Scales for weighing participants readable to 0.1 kg (e.g. SECA 876) x1
- Stadiometer/Shore Board x 1, plus bag x 1

For analysis of deuterium enrichment

- Agilent 4500 Series Tumbl-IR portable FTIR (Albania, Bosnia, Montenegro)
- 200 ul automatic pipette (Gilson or Eppendorf)
- Pipette tips for 200 uL pipette
- Ethanol or isopropyl alcohol for cleaning windows in small bottle
- Cotton buds/Q-tips

Appendix 6: Data collection forms for primary data collection

1. Anthropometry + Blood pressure

Study ID: _____

Date of assessment _____

2.1.Measurements

Weight (kg) - 1st measurement _____

Weight (kg) - 2nd measurement _____

Height (cm) - 1st measurement _____

Height (cm) - 2nd measurement _____

Mid-upper arm circumference (mm) - 1st measurement

_____

Mid-upper arm circumference (mm) - 2nd measurement_____

Hip circumference (cm) - 1st measurement _____

Hip circumference (cm) - 2nd measurement _____

Waist circumference (cm) - 1st measurement _____

Waist circumference (cm) - 2nd measurement _____

Head circumference (cm) - 1st measurement _____

Head circumference (cm) - 2nd measurement _____

2.2.Skinfolds

Triceps skinfold (mm) - 1st measurement _____

Triceps skinfold (mm) - 2nd measurement _____

Subscapular skinfold (mm) - 1st measurement _____

Subscapular skinfold (mm) - 2nd measurement

_____

Suprailiac skinfold (mm) - 1st measurement _____

Suprailiac skinfold (mm) - 2nd measurement _____

1.3. Additional measurements

Sitting height (cm) - 1st measurement _____

Sitting height (cm) - 2nd measurement _____

Knee-heel length (mm) - 1st measurement _____

Knee-heel length (mm) - 2nd measurement _____

Any comments about anthropometry assessment? _____

2.3.Blood Pressure

First measure of systolic blood pressure _____

First measure of diastolic blood pressure _____

Second measure of systolic blood pressure _____

Second measure of diastolic blood pressure _____

Any comment about blood pressure measurement? _____

2.Deuterium + Sample collection

Study ID: _____

Date of deuterium procedure _____

2.1.Check list before dosing Yes/No

Participant has fasted at least 2 hours?

Participant has been asked to urinate before test?

2-4 ml baseline saliva sample collected?

At what time was the pre-dose saliva taken? _____

2.2. Check list for dosing Yes/No

30 ml deuterium administered

2 x 50 ml water administered using the same container?

Participant consumed full dose?

Participant asked to wait while retaining from physical activity?

Weight of deuterium dose (g) _____

What was the weight of the dose not consumed by the_____
participant?

At what time was the dose taken? _____

At what time was the first post dose saliva sample collected? _____
(3h after dosing)

At what time was the second post dose saliva sample _____
collected? (4h after dosing)

Form 3 Deuterium sample collection

Did the participant take water between dosing and final saliva sample collection? Yes/No

How much water was taken? (dl) _____

Any comment for deuterium procedures? _____

3.Fasting blood glucose and Oral Glucose Tolerance Test (OGTT) Yes/No

Was fasting blood glucose measured?

Was Oral Glucose Tolerance Test done?

Any comment about blood glucose testing? _____

3.3.Check list for OGTT Yes/No

Has participant been fasting at least 8 hours?

Baseline blood sample collected?

75 g of glucose diluted in 250 ml water administered?

Participant asked to wait while refraining from physical exercise?

30 min blood sample collected?

2 hour blood sample collected?

Blood glucose at baseline (mmol/L) _____

Blood glucose at 30 min (mmol/L) _____

Blood glucose at 2 hours (mmol/L) _____

3.4.HbA1c

Was HbA1c measured? Yes/ No

Result of HbA1c (mmol/L) _____

Blood sample collected? Yes/No

Urine sample collected? Yes/No

Stool sample collected? Yes/No

Any comments about sample collection? _____