CHROMIUM SUPPLEMENTATION IN MANAGEMENT OF TYPE 2 DIABETES AMONG PATIENTS ATTENDING THIKA LEVEL 5 HOSPITAL, KENYA: A RANDOMIZED PLACEBO CONTROLLED STUDY

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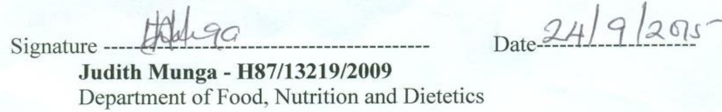
DECLARATION

This Thesis is my original work and has not been presented for a degree in any other University or award.



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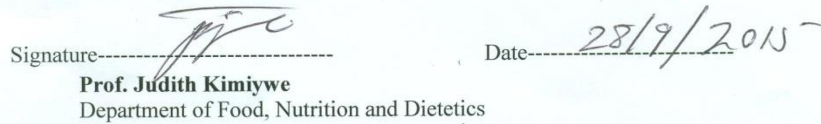

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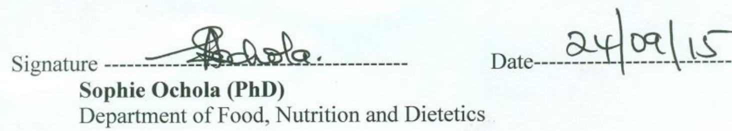

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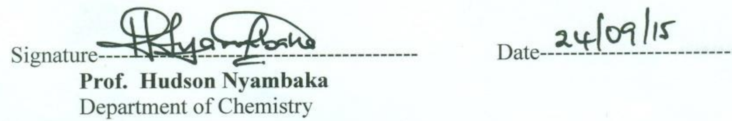

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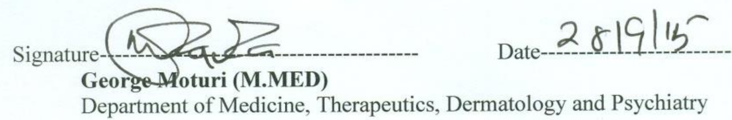

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DEDICATION

This work is dedicated to my late parents Emanuel Munga and Merceline Adhiambo Munga for their commitment to give me a foundation that would prepare me for the academic arena.

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OPERATIONAL DEFINITIONS OF TERMS

Blood glucose control: Blood glucose levels within the determined range of good control depending on when the last meal was consumed.

Chromium status: Quantities of the trivalent chromium circulating in the blood.

Dietary patterns: Nutrition assessment on type of foods eaten in the previous 24hours including snacks in addition to 7 day food frequency.

Physical activity: Any form of exercise an individual may be involved in formally/ informally on a regular basis

Socio-economic status: Individuals education level, occupation and property ownership

DEFINITION OF TERMS

Arteriosclerosis:	A gradual loss of elasticity in the walls of arteries due to thickening and calcification.
Cataracts:	Opacity of the crystalline lens of the eye causing partial or complete blindness.
Cholesterol:	A sterol found in nervous tissue, red blood corpuscles animal fat and bile
Glycated haemoglobin:	A measure of the average plasma glucose concentration over prolonged period of time
Hypoglycaemia:	Circulating blood glucose lower than normal
Hyperglycaemia:	Circulating blood glucose higher than normal
Neuropathy:	A disease process of nerve degeneration and loss of function
Phlebotomy:	The puncture of a vain for the withdrawal of blood
Triglycerides:	Human fat- an ester of glycerol and three fatty acids
Venipucture:	The insertion of a needle into a vein for the withdrawal of blood

ABBREVIATIONS AND ACRONYMS

AAS	Atomic Absorption Spectrometry
ADA	American Diabetes Association
BMI	Body Mass Index
CVD	Cardiovascular Disease
DM	Diabetes Mellitus
DMI	Diabetes Mellitus Information
EV	Extraneous Variable
FBS	Fasting blood sugar
FDA	Food and Drug Administration
GI	Glycemic Index
GPAQ	Global Physical Activity Questionnaire
HbAlc	Glycated Haemoglobin
HDL	High Density Lipoproteins
LDL	Low Density Lipoproteins
LMWcr	Low Molecular Weight Chromium Binding Substance
RDA	Recommended Dietary Allowance
RR	Relative Risks
SAAS	Statistical Analysis System
SPSS	Statistical Package for Social Sciences
WC	Waist Circumference
WHO	World Health Organization
WHR	Waist to Hip Ratio

ABSTRACT

Diabetes Mellitus is a condition with common elements of hyperglycaemia and glucose intolerance. It is the fourth leading cause of death in high-income countries and evidently epidemic in newly industrialized countries as well as economically developing nations such as Kenya. Type 2 Diabetes is characterized by insulin resistance and relative insulin deficiency. Studies have reported low serum chromium levels in Type 2 diabetics compared to non-diabetic population. Chromium has been reported to potentiate insulin action in muscle cells. Studies show contradictory findings on beneficial effects of chromium on; blood sugar (FBS and HbAlc), blood lipids (LDL, HDL, triglycerides and total cholesterol), adiposity measures (BMI, WC and W/H ratio), fat mass and lean body mass. Consequently similar studies have been recommended to investigate further the benefits of chromium with a view of coming up with conclusive evidence. The purpose of this study therefore was to investigate the contribution of chromium supplementation in the management of fasting blood sugar, HbAlc, LDL, HDL, triglycerides, BMI, W/H ratio and WC in Type 2 Diabetes mellitus patients in Thika level 5 Hospital, Kenya. This was a double blind randomized controlled trial with a sample size of 180 participants on hypoglycaemic drugs. The sample was randomized into two study groups at a ratio of 1:1 (control group and intervention group). Participants in the intervention group received chromium picolinate (500mcg/day) while the control group received placebo for a period of 4 months. Data was collected at baseline, monthly and end of month 4, using semi-structured questionnaires. Information collected included: demographic characteristics, medical history, physical activity and dietary patterns. Additionally, anthropometric measurements (weight, height and waist and hip circumferences) were taken. Blood samples were analysed for chromium levels, HbAlc, serum ferritin and serum lipids (LDL, HDL, total cholesterol and triglycerides). Descriptive statistics such as mean and standard deviation were used to analyze demographic, socioeconomic, blood sugar, blood lipids and anthropometry and nutrient adequacy from 24 hour recall; mode was used to describe the 7 day food frequencies. Principle component analysis was used to determine the wealth index/socioeconomic categories. T-test was used to determine differences in continuous data between the study groups and Chi-square test used to determine associations in categorized variables between the study groups. Wilcoxon test was used to determine differences in food frequencies between the study groups while GPAQ was used to analyze physical activity levels. Multiple regression was used to determine predictors of elevated HbAlc. On the whole, at baseline, the participants had high fasting blood sugar with low serum chromium levels. Chromium supplementation significantly reduced HbAlc (DID: intervention 1.44% versus control -0.79%; p=0.001) and LDL elevation (DID: intervention -0.09 mmol/l versus control -0.91 mmol/l; t-test; p=0.010). Chromium supplementation had no effect on BMI, WC and W/H ratio; no effect on chromium status (t-test; p=0.241), triglycerides (t-test; p=0.648) and HDL (t-test; p=0.648). The predictors for elevated (above 9%) HbAlc were age and fasting blood sugar; the higher the fasting blood sugar and the older the patients, the higher the likelihood of elevated HbAlc. Since chromium supplementation was effective in reducing HbAlc and LDL among Type 2 diabetics in this study; it is recommended that chromium is used in management of blood sugar in Type 2 Diabetes.

CHAPTER ONE: INTRODUCTION

1.1 Background to the study

Diabetes mellitus (DM) refers to a group of heterogeneous disorders associated with elements of hyperglycaemia and glucose intolerance, due to insulin deficiency, impaired effectiveness of insulin action or both (IDF Diabetes Atlas, 2013). DM is classified on the basis of aetiology and clinical presentation into Type 1, Type 2, genetic defects in beta cells, genetic defects in insulin action, exopancrease diseases, endocrinopathies, drug or chemical induced, infection induced and gestational Diabetes (Swindale, 2013).

Type 2 Diabetes also referred to as adult-onset Diabetes is recognized as a disorder whose pathophysiology consists of insulin secretory abnormalities, hepatic glucose overproduction and insulin resistance in peripheral tissues such as liver and skeletal muscles (Zhong *et al.,* 2010). When there is insulin resistance, the muscle, fat, and liver cells do not respond properly to insulin. As a result, the body requires more insulin to maintain normal glycaemia. Initially, the pancreas increases insulin production to counterbalance the insulin resistance and maintain normal glucose levels (Sushi *et al.,* 2012). However, in many individuals the pancreas eventually fails to keep up with the body's demand for insulin leading to hyperglycaemia and setting the stage for the development of Diabetes and cardiovascular disease (Sushi *et al.,* 2012).

Type 2 Diabetes accounts for 85-95% cases of Diabetes in high income countries and may be higher in low and middle income countries (IDF Diabetes Atlas, 2013).Type 2 Diabetes often goes undiagnosed for many years due to gradual development of hyperglycaemia that is initially asymptomatic, though patient remains at risk of

developing macro and micro-vascular complications. The diagnosis of Type 2 Diabetes majorly occurs after the age of 40 years but could also occur earlier (ADA, 2010; IDF Diabetes Atlas, 2013).

Type 2 Diabetes is often associated with obesity or high percent body fat predominantly distributed in the abdominal region, which can cause insulin resistance and lead to elevated blood glucose levels. Other risk factors include metabolic syndrome, stress, unhealthy dietary habits, physical inactivity, increasing age, insulin resistance, family history of Diabetes, less than optimum intrauterine environment, ethnicity and in women with prior gestational Diabetes (ADA, 2010; Alberti, 2006)

Globally, it is estimated that 382 million people or 8.3% of adults in the age group 20-79 have Type 2 Diabetes of whom, the greatest number is between the ages 40 and 59 years. Out of the diabetic population, 80% live in low- and middle-income countries where Kenya is classified. This number is expected to increase by more than 50% in the next 20 years if preventive programmes are not put in place (IDF Diabetes Atlas, 2013).

The prevalence of Type 2 Diabetes in the African Region has been estimated to 19.8 million people or 4.9% of the adult population, and has been projected to 23.9 million by 2030. According to IDF Diabetes Atlas, (2013), the highest prevalence of Diabetes in Africa region is on the island of Reunion (15.4%), followed by Seychelles 12.1%), Gabon (10.7%) and Zimbabwe (9.7%). Some of Africa's most populous countries have the highest numbers of people with Diabetes, including: Nigeria (3.9 million), South Africa (2.6 million), Ethiopia (1.9 million), and the United Republic of Tanzania (1.7 million). A study done by Motala *et al.,* (2008), indicated the highest prevalence among

the ethnic Indian population of Tanzania and South Africa as well as a marked high prevalence in urban than rural areas.

In Kenya, according to Government of Kenya (GOK) (2010) health report, the then ministry of Medical Services estimated prevalence of Diabetes Mellitus at 3.3% and this figure has been projected to rise to 4.5% of the population by the year 2025. In the urban areas, the prevalence is estimated at 10%. Type 2 Diabetes is the most prevalent (80%) and Kenyans are developing it at a younger age compared to developed countries (GOK 2010; Mario and Sridevi, 2008).

Diabetes Mellitus is now one of the most common non-communicable diseases globally. It is the fourth leading cause of death in most high-income countries. There is substantial evidence that it is epidemic in many economically developing nations like Kenya and newly industrialized nations. This is aggravated by high levels of under-nutrition in infants, low birth weight and infections that contribute to metabolic disorders in the later life. An estimated 522,600 people in Africa died from Diabetes-related causes in 2013. This represents 8.6% of deaths from all causes in adults, where 76.4% of those deaths occurred in people under the age of 60. There were more than 50% more deaths from Diabetes in women compared to men (IDF Diabetes Atlas, 2013).

Diabetes is associated with complications such as coronary artery and peripheral vascular disease, stroke, diabetic neuropathy, limb amputations, renal failure and blindness. These result to increased disability, reduced life expectancy and enormous health costs for virtually every society (IDF Diabetes Atlas, 2013; Yaping *et al.,* 2012).

Globally, healthcare expenditures on Diabetes account for 11.6% of the total healthcare expenditure and about 80% of the countries spend between 5% and 13% of their total healthcare finances on Diabetes (Diabetes Care, 2005). In Africa, at least USD 4 billion was spent in 2013 towards Diabetes health care and this cost is expected to increase by 58% in 2035 (IDF Diabetes Atlas, 2013). Diabetes also imposes large economic burdens in the form of lost productivity and foregone economic growth, as a result of lost earnings due to lost work days, restricted activity days, lower productivity at work, mortality and permanent disability. Such losses are relatively larger in poorer countries due to premature death (Diabetes Care, 2005). Studies done in Kenya report a challenge with management of tight blood sugars resulting to amputation of the lower limbs among other complications related to hyperglycaemia (Otieno *et al*., 2003; Motala *et al*., 2008). These implications of Diabetes on individuals and society at large warrant further research in form of preventive and management programs. Contribution of chromium in management of blood sugar among Diabetics in Kenya is not known.

Nutrition therapy is an essential component of Diabetes management regardless of the client weight, blood glucose levels or use of medication. People with Diabetes generally have the same nutritional requirements as the general population that promote health and well-being; that is loss of weight if overweight, consumption of less saturated fat and cholesterol, more fibre and less sodium (Franz *et al.,* 2008; Mendosa, 2003). Dietary deficiency of chromium is believed to be positively associated with risk of Diabetes and its complications (Nair *et al.,* 2008; Shilpi *et al.,* 2011). Diets from refined carbohydrates are not only low in chromium, but lead to enhanced chromium losses. Consumption of refined carbohydrates lead to rapid rise in blood sugar that results to mobilization of

chromium from body stores as a corrective mechanism to restore insulin sensitivity in the muscle cells. The mobilized chromium is not reabsorbed but lost via urine leading to decreased chromium stores. Persistent hyperglycaemia, coupled with polyuria in poorly controlled Diabetes, worsen chromium losses. This increases the likelihood of deficiencies in Type 2 diabetics (Anderson, 2007; Hamid *et al.,* 2012).

Studies have reported that adequate chromium levels potentiate activity of insulin that increases cell sensitivity leading to uptake of glucose circulating in blood to be utilized for respiration purposes or if in excess, stored as glycogen and fats thus reducing amount of circulating glucose (Barbara and Donald, 2014).

Lower serum levels of chromium have been observed in Type 2 diabetics compared to non-diabetic population (Nouramonammadi and Underwood, 2007). A study done by Ekmecioglu and Anderson, (2007), demonstrated lower levels of chromium in the lymphocytes of diabetics and no difference in other blood components with the non-diabetic control group. The low serum levels of chromium in diabetics have been attributed to insulin resistance, hyperglycaemia and osmotic dieresis resulting from glycosuria which increases urine chromium excretion (Hamid *et al.,* 2012).

The effect of mineral chromium in improvement of hyperglycaemia, LDL cholesterol, triglycerols, HDL cholesterol, fat mass and lean body mass has been studied both on animals and humans (Richard, 2007). However, not all studies have reported beneficial effects associated with improved chromium status. Gosh *et al.,* (2010) supplied Indian subjects with chromium (400 micrograms/day) and reported significant reduction in fasting blood glucose and HbAlc. A meta- analysis by Perpetua *et al.,* (2010) on

chromium supplementation trials reported effect of chromium supplementation on lowering of HbAlc, fasting blood sugar, total cholesterol and triglycerides among Type 2 diabetics.

Clinical reviews by Balk *et al.,* (2007) and kleefstra *et al.,* (2007) indicated that chromium supplementation gave positive results in some populations like Indian and Chinese and not others like western populations. This was supported by a study done by Yinan *et al.,* (2011) that reported ethnic or genetic factors as significantly contributed to the differences in blood sugar findings after supplementation with chromium. There is paucity of literature on the contribution of chromium supplementation in the management of blood sugar among diabetics in Africa especially Kenya.

1.2 Problem statement

In Kenya the Ministry of Health's goal is to improve the quality of life and reduce complications and premature mortality in people with Diabetes (GOK, 2010). The interventions that have been put in place by the Kenya government on management of Type 2 Diabetes mellitus include provision of insulin and oral hypoglycaemic drugs at the Sub-county and County government hospitals at subsidized costs. However, achievement of tight glucose control is quite a challenge for many patients (Otieno *et al.,* 2003).

Studies on chromium picolinate supplementation in addition to anti-diabetic medication have demonstrated ability of chromium to increase insulin sensitivity, reduce blood sugar levels as well as amount of insulin needed by people with Diabetes (Anderson, 2007; Aviva and Peter, 2011; Cefalu *et al.,* 1999; Gosh *et al.,* 2002; Martin *et al.,* 2006 and

Mieebi *et al.,* 2014). Similarly, a study done by Shilpi *et al.,* (2011) indicated significant improvements in fasting blood glucose, HbAlc, total cholesterol, triglycerides and LDL levels There is paucity of literature on similar studies in Africa and Kenya hence the contribution of chromium supplementation on blood sugar remains unknown.

In contrast, some studies have reported no beneficial effect of chromium supplementation on the biomarkers of Type 2 Diabetes (Kleefstra *et al.,* 2006; Sushi *et al.,* 2012; Yinan *et al.,* 2011).The different findings in these studies were attributed to short study period of less than three months, unknown dietary micronutrient profile and genetic factors. The difference in genetic factors makes it unknown whether chromium is effective in management of blood sugar among Africans living in Africa. Moreover, chromium is not part of management of hyperglycaemia among Type 2 diabetics in Kenya.

Recommendations have been made for studies lasting more than 3 months in well-defined, at-risk populations where dietary intakes are known to determine the effects of chromium on markers of Diabetes (Anderson, 2007; Chrystalleni*et al.,* 2012; Onakpoya*et al.,* 2013 and Sridhar 2013). The prevalence of diabetes and related deaths has gained an upward trend in Africa and Kenya with higher rates in the urban areas. There is therefore need to conduct studies that would contribute to management of tight blood sugars and reduce the premature deaths among diabetics.

1.3 Purpose of the study

The purpose of this study was to determine the contribution of chromium picolinate supplementation in addition to anti-diabetic drugs on fasting blood sugar, HbAlc and Lipid profile among Type 2 diabetics attending Thika level 5 Hospital Diabetes clinic.

1.4 Objectives of the study

The objectives of the study were to:

1. Determine characteristics of Type 2diabetics attending Thika level 5 hospital Diabetes clinic in terms of; demographics, socio-economic status, dietary practices, physical activity levels and medication profile
2. Establish baseline biochemical characteristics as determined by serum chromium levels, fasting blood glucose HbAlc, total cholesterol, triglycerides, HDL, LDL and ferritin among Type 2diabetics in study site
3. Assess the effect of chromium supplementation on BMI, waist to hip ratio and waist circumference among individuals with Type 2Diabetes in study site
4. Determine the effect of chromium supplementation on fasting blood glucose, HbAlc, total cholesterol, triglycerides, HDL, LDL, serum chromium levels and ferritin levels among Type 2diabetics in study site
5. Establish the predictors of elevated HbAlc among Type 2diabetics in study site

1.5 Hypotheses of the study

Ho_1. There is no significant relationship between serum chromium levels and HbAlc

Ho_2. There is no significant relationship between serum chromium levels and the lipid profile (Triglycerides, total cholesterol, HDL and LDL)

Ho_3. There is no significant relationship between serum chromium levels and body anthropometric measurements (WC and BMI)

1.6 Significance of the study and expected outputs

The findings of this study made a contribution on the effect of chromium supplementation in addition to routine care (hypoglycaemic therapy, physical activity and diet counselling) in management of blood sugar among Type 2diabetics. The results from this study can be used to make recommendations for practice by health care providers and for policies on management of Type 2 Diabetes by the Ministry of Health and Kenya Diabetes Association to include chromium as a nutrient supplement in the management of Type 2 Diabetes. The study findings have also contributed to on-going research on management of Type 2 Diabetes.

1.7 Delimitations of the study

The study was conducted among patients aged 20-64 years with Type 2 Diabetes since; the onset of Type 2 Diabetes is at the later years and there are various medical challenges present other than Diabetes that the elderly who are above 64 years face. The patients attending Thika level 5 hospital Diabetes clinic were from middle and low socio-economic status thus the study findings can only be generalized to Type 2 diabetics with similar characteristics.

1.8 Limitations of the study

Compliance to oral intake of chromium supplements was a likely challenge to the study. To minimize this challenge, chromium supplement and placebo were provided at the clinic as part of the regularly provided drugs by the health personnel at the clinic and participants followed up by phone calls. A monthly questionnaire was used to determine adherence to chromium intake based on participants' recall basis.

1.9 Conceptual framework

The conceptual framework explains the relationship between dependent (blood glucose, serum lipids and adiposity) and the independent variables (serum chromium levels). The extraneous variables that also affected the dependent variables were; dietary intake, physical activity, socio-demographics and medication.

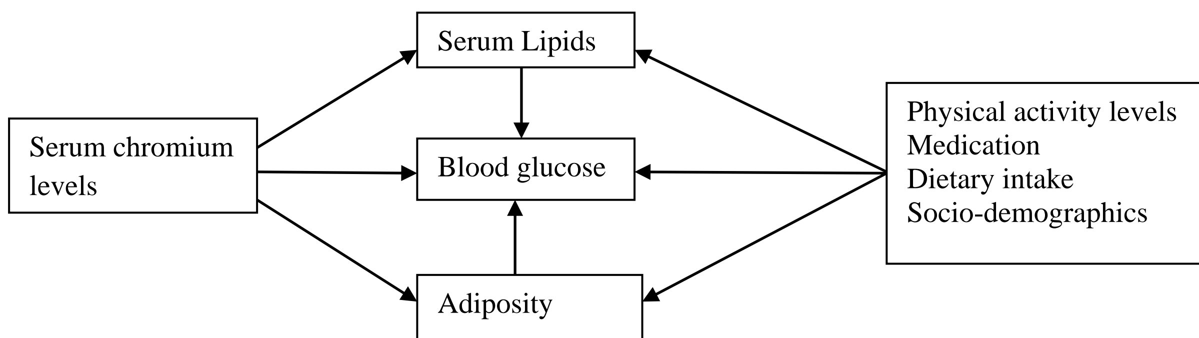

Figure 1.1: Factors influencing blood sugar. Modified from Oso and Onen, (2009)

The dependent variables in this study were blood sugar (FBS, HbAlc), blood lipids and adiposity measures (BMI, WC and W/H ratio). The independent variable was serum chromium levels. Chromium potentiates insulin activity by increasing cell sensitivity to insulin and consequently uptake of glucose by the cells for respiration and glycogen or fat

stores. This reduces amount of blood glucose. This study investigated the serum chromium levels of the Type 2 diabetics at baseline and end of study. Associations were determined between serum chromium levels and blood sugar among the Type 2 diabetics.

The intervening variables in this study were physical activity levels, medication, dietary intake and socio-demographics. These factors also affect the dependent variables hence likely confounders to the study outcome. These variables were measured and compared across the study groups at baseline to determine if randomization was successful.

Type 2 Diabetes has been associated with the developed nations but studies have reported high prevalence in the developing and newly industrialized nations as well. Studies have also reported positive associations between hyperglycaemia and age especially 40 years and above. Overconsumption of refined energy sources has been cited as a contributing factor to hyperglycaemia, overweight and obesity that increase insulin resistance. This study investigated the socio-demographic characteristics of the Type 2 diabetics and determined any associations with elevated blood sugar levels.

Physical activity reduces insulin resistance, LDL cholesterol while increasing HDL cholesterol which is a risk factor to cardiovascular complications among Type 2 diabetics. Low physical activity level has been associated with insulin resistance and hyperglycaemia among Type 2 diabetics. This study investigated the physical activity levels of participants and associations with blood sugar levels.

BMI, waist to hip ratio (W/H) and waist circumference (WC) are adiposity measures that determine the degree of overweight in an individual and the distribution of body fat stores. Studies have reported that Overweight and obesity increase insulin resistance

leading to hyperglycaemia among Type 2 diabetics. This study investigated on BMI, W/H and WC of participants and determined their associations with elevated blood sugar.

Various hypoglycaemic drugs are prescribed to Type 2 diabetics by physicians to help regulate blood sugar levels. Different individuals used different drugs or combinations in different doses to achieve glucose control. The physicians may also change drug type and dosage as they deem necessary. This study investigated the type and dosage of hypoglycaemic drugs used by the participants during the study and monitored for 4 months. Medication intake was used to test any differences between the study groups on a monthly basis. There were changes in drug intake and dosage that was similar across the study groups in all the 4 months of the intervention.

CHAPTER TWO: LITERATURE REVIEW

2.1 Overview of pathophysiology of Type 2 Diabetes Mellitus

Diabetes occurs when the body cannot produce enough of the hormone insulin or cannot use insulin effectively. Insulin acts as a key that lets the body's cells take in glucose and use it as energy. Diabetes imposes unacceptably high human, social and economic costs on countries at all income levels. Global estimates indicate that 8.3% of adults (382 million people) have Diabetes, and the number of people with the disease is set to rise beyond 592 million in less than 25 years. Despite the array of tools currently in use to tackle the disease such as, effective drug therapies, advanced technology, ever-improving education and preventive strategies, the battle to protect people from Diabetes and its disabling, life-threatening complications is being lost (IDF Diabetes Atlas, 2013).

Chromium III is the principal form of chromium in foods as well as the form utilized by the body. Chromium participates in glucose metabolism by enhancing the effects of insulin (a hormone critical to the metabolism and storage of carbohydrate, fat, and protein in the body). Insulin is secreted in response to increased blood glucose levels after a meal and it binds to insulin receptors on the surface of the cells, which activates the receptors and stimulates the glucose uptake by the cells (Cronin et al., 2004, Yinan et al., 2011). In skeletal muscles, insulin promotes glucose uptake by stimulating a cascade of signalling processes initiated by the binding of the insulin to extra cellular alpha-subunit of the insulin receptor on the cellular membrane. This interaction provides cell with glucose for energy while preventing elevation of blood glucose (Cronin et al., 2004, Yinan et al., 2011)

Chromium influences glucose metabolism by potentiating insulin action by taking part in its signal amplification mechanism (Cronin *et al.,* 2004, Yinan *et al.,* 2011 and Shilpi *et al.,* 2011). Due to different signalling in liver cells, insulin does not regulate their glucose uptake, but stimulates glycogen synthesis and blocks glucose release into the blood, which is otherwise required for maintaining normal blood glucose levels (Saltiel, 2001).

Literature in this chapter has been reviewed on socio demographic characteristics, management practices (dietary patterns, physical activity and medication), chromium as a micronutrient in management of Type 2 diabetics and its effect on chromium status, blood sugar, lipid profile, waist circumference (WC) and Body mass Index (BMI).

2.2 Socio-demographic characteristics of Type 2 Diabetics

The majority of the 382 million people with Diabetes are aged between 40 and 59, and 80% of them live in low- and middle-income countries (IDF Diabetes Atlas, 2013). A study done by Yaping *et al.,*(2012) in China indicated that women with Type 2 Diabetes were more likely to be in post-menopausal than their non-diabetic counterparts in the control group. A study done in Scotland by Brian *et al.,* (2012) indicated the mean age for Type 2 diabetics was 62.7 years while a study done by Seth *et al.,* (2014) indicated 58.9 years as the mean age of the Type 2diabetics in the study. Dympna *et al.,* (2014) study on Type 2 diabetics had mean age of 57.8 among females and 58.8 among the male participants

Another study in Kenya by Otieno *et al.,* (2005), found mean age of 54.45 for women and that of men was 55.8 years while a study by Richard *et al.,* (2013) in an urban slum in Nairobi-Kenya found Type 2 Diabetes to be more prevalent in the 45-54 year category. In

addition, a study by Masemiano (2010) in a rural hospital in Kenya reported a mean age of Type 2 diabetics to be 61.8 years.

Despite the predominantly urban impact of the epidemic, Type 2 Diabetes is fast becoming a major health concern in rural communities with little gender differences (Masemiano, 2010; Richard *et al.,* 2013).Globally, there are about 14 million more men than women with Diabetes (198 million men versus 184 million women). However, this difference is expected to increase to 15 million (303 million men versus 288 million women) by 2035 (IDF Diabetes atlas, 2013). A study by Seth *et al.,* (2014) indicated more females (70.4%) than the males with above 50% having completed 8th grade..

2.3Diet and Type 2 Diabetes

Nutrition therapy is an essential component of Diabetes management regardless of the client weight, blood glucose levels or use of medication. The aim of nutritional intervention for management of Diabetes is maintenance of; blood glucose to normal levels (<8mmols), healthy weight (BMI between 20-25), normal blood lipids levels (HDL >4.2 for males and >1.68 for females; LDL <4.1; triglycerides, 1.81 and total cholesterol <5.2) and normal arterial pressure levels of 120/80 (Franz *et al.,* 2008). People with Diabetes generally have the same nutritional requirements as the general population that promote health and well-being; that is loss of weight if overweight, eat less saturated fat and cholesterol, more fibre and less sodium (Mendosa, 2003).

According to systematic review by Chrystalleni *et al.,* (2012), diets rich in vegetables, fruit, and whole cereals have a lot of potential in controlling Diabetes and attenuating its progression. Eating a consistent amount of food everyday improves blood sugar control

as well as weight control. Eating at the same time every day is important especially for those taking long acting insulin and oral hypoglycaemic drugs. Skipping a meal may result in hypoglycaemia (Dek, 2006).

Kant and Graubard, (2005) examined correlation of the dietary diversity score with biochemical indices related to Diabetes and found an inverse relationship between individual index-score and glucose levels, plasma haemoglobin A1c, and serum C-peptide (but not with insulin levels). A study done by Seth *et al.,* (2014) indicated that Food insecurity was associated with lower overall dietary quality and lower consumption of plant-based foods, which was associated with poor longitudinal glycemic control. A study done by Leila *et al.,* (2011) on Type 2 diabetics indicated beneficial effects on Type 2diabetic patients' cardio-metabolic parameters from; a diet rich in fruits, vegetables, whole grains, low-fat dairy products and, low in saturated fat, total fat, cholesterol, refined grains and sweets. This study agrees with the findings of Fung *et al.,* (2004; 2007) who reported an inverse relationship between insulin levels and the healthy-type diet.

2.3.1Carbohydrates and Type 2 Diabetes

According to reviews by Sheard *et al.,* (2004), a consistent carbohydrate and calorie intake is fundamental to glycaemic control. Clinical studies by Colditz *et al.,* (1997) that glycaemic index and glycaemic loads of carbohydrates determine the short term glycaemic response. Studies prove that low glycaemic foods improve glucose levels, dyslipidemia and HbAlc (Villegas *et al.,* 2007; Jenkins *et al.,* 2008). Cross sectional study by Buyken *et al.,* (2001) indicated positive relationship between GI and HbAlc and inverse relationship to HDL levels. Carbohydrates should be derived from unrefined

cereals, fruits and vegetables as well as low fat dairy products. Carbohydrates should take up 45-65% of energy intake where, one third is composed of simple carbohydrates (Leila *et al.,* 2011; Wheeler and Pi-Sunyer, 2008). Highly processed food and foods rich in simple carbohydrate increase the risk of appearance of Diabetes and forerunner situations (Chrystalleni *et al.,* (2012). Carbohydrates sources can also be substituted for each other depending on their calorie/carbohydrate loads such that one can have mixed carbohydrates meals to serve their preferences (Dek, 2006).

2.3.2 Proteins and Type 2 Diabetes

The substitution of some dietary carbohydrate for protein in a low-fat (30% total energy) diet may improve body composition and cardiovascular disease (CVD)risk factors including insulin sensitivity, glycemic control, and blood lipids in (Kriegen *et al.,* 2006; Parker *et al.,* 2002; Samaha *et al.,* 2003). A study by Thomas *et al.,* (2010) indicated substantial improvements in CVD risk factors and glycemic control following a high protein-calorie restricted diet.

Karma *et al.,* (2011) found that a high-protein, low-saturated fat energy-restricted diet where two eggs per day was consumed by Type 2 diabetics in the study did not adversely affect blood lipid profiles in individuals with HDL and folate more effectively than a diet containing iso-energetic alternative animal sources. The study concluded that a high-protein energy-restricted diet from eggs may have nutritional benefits and assist in metabolic control in individuals with Type 2 Diabetes. A review by Ann*et al.,* (2011) indicated that red meat consumption was negatively associated with physical activity while positively associated with BMI among Type 2 diabetics. Recommendations were hence made on reduction of red meat consumption, particularly processed red meat and

replacing it with other healthy dietary components such as nuts, dairy products, and whole grains.

A cohort study conducted on American Indians by Amanda *et al.,* (2012) indicated higher prevalence of Diabetes Mellitus with consumption of processed meat than unprocessed red meat. A study conducted in a sample of Greeks showed that meat and meat products, among all food groups, were the ones mostly associated with insulin resistance and hyperinsulinemia and recommended consumption of red meat in small quantities (Panagiotakos *et al.,*2005). On the contrary, a study by Larsen *et al.,* (2011) found no superior long-term metabolic benefit of a high-protein diet over a high-carbohydrate diet in glycaemic control among Type 2 Diabetes.

2.3.3 Dietary fats and Type 2 Diabetes

Fat is crucial in maintaining adequate hormonal balance, stabilizing blood sugar, increasing immunity, supplying energy and controlling hunger. Fat slows down the processing of food in the digestive tract hence lowering the overall glycaemic load. This makes steadier blood sugar levels over time and nice long lasting satisfaction after eating (ADA, 2003). A cross sectional study by Wirfält et al. (2001), indicated that fat intake from milk by women had 40% lower possibility of developing hyperinsulinemia. A case-control randomized trial among 99 Type 2 diabetics by Barnard *et al.,* (2009), proved that a vegetarian type diet of low fat content was more effective in improving glycaemia and plasma lipids than the conventional diet recommendations for Diabetes.

Intake of trans- fatty acids may cause insulin resistance as proved by Lefevre *et al.,* (2005) where, insulin was higher in those who consumed trans-fatty acids compared to

the cis- fatty acids. A study done by Summers *et al.,* (2002), indicated inverse association between unsaturated fatty acids and progression of Type 2 Diabetes. In order to achieve a reduction of LDL levels, ADA, (2008) recommends limited intake of saturated fats at <7% of energy intake, of dietary cholesterol at <200 mg, and to increase intake of soluble fibre at 10–25 g/µ and of sterols and stanols at 2 g/µ.

2.3.4 Fibre and Type 2 Diabetes

Non-starchy vegetables contain fibre and important vitamins, minerals, and micronutrients that enhance proper functioning of the body cells including the endocrine system whose main players are insulin and glucagon. A diet that is high in fibre (25-30 grams per day) may help to control blood sugar levels and haemoglobin (Bantle *et al.,* 2008). Studies have indicated that high fibre diet revealed remarkable decrease in pre-prandial plasma glucose, insulin concentrations, total plasma cholesterol concentrations and the very low Density lipoprotein cholesterol among Type 2 diabetics(Chandalia *et al.,* 2000; Qureshi *et al.,* 2002; Kabir *et al.,* 2002; Aller *et al.,*2004). Wirfält et al. (2001), in a cross-sectional study, observed that the group with an increased consumption of fibre from bread had 40% less likelihood of developing central obesity in male Type 2 diabetics. Overweight, especially central obesity is responsible for insulin resistance and dyslipedemia which are observed in Type 2which further negatively affects lipid metabolism (Kahn *et al.,*2001). ADA, (2008) recommends daily intake of 14g/1000kcal of fibre intake.

2.3.5 Alcohol and stimulants and Type 2 Diabetes

Alcohol intake is toxic to the nerves and may aggravate neuropathy. Alcohol increases triglycerides and poses risk to hypoglycaemia and nerve damage (Dek, 2006). High

amounts of alcohol are also related to arteriosclerosis. Alcohol may cause vomiting and sweating in a person under diabetic medication hence should be avoided (ADA, 2003). Coffee raises blood sugar level. It enhances the effect of adrenaline and glucagon which both release stored sugar from the liver increasing sugar level in blood. Both tea and coffee contain caffeine that affects the nervous system (IDF, 2005). Frequent stress due to regular coffee intake may lead to increased build-up of fat in the abdominal area leading to weight gain which can further worsen insulin resistance (glucose intolerance), worsening hyperglycaemia (IDF, 2005). ADA, (2008) recommends that daily intake of alcohol by adults with Diabetes should be limited to a moderate amount (one drink per day or less for women and two drinks per day or less for men).

2.4 Physical activity and Type 2Diabetes

Studies have indicated that physical activity to improves insulin sensitivity through changes in body fat mass as well as through fat mass loss-independent mechanisms such as: increased GLUT4 translocation and subsequent glucose utilization in skeletal muscle, improved capacity of skeletal muscle to oxidize fat, increased intra-myocellular lipid turnover and decreased quantity of lipid metabolites (Holloszy, 2005; Loprinzi *et al.,* 2011 and Shojaee-Moradie *et al.,* 2007).

Physical activity has been established to improve insulin sensitivity in a dose-dependent manner and that total duration of activity is a more important determinant of insulin sensitivity than activity intensity (Balkau *et al.,* 2008 and Ekelund *et al.,* 2009). Insulin Resistance Atherosclerosis Studies done by Kavouras *et al.,* (2007) and Esteghamati *et*

al., (2009) reported that increased participation in vigorous as well as non-vigorous physical activity is associated with significantly higher insulin sensitivity.

Exercise improves cardiovascular fitness, promotes bone strength and enhances the sense of well-being and also maintains long-term weight reduction (Woods, 2003; Zinman, 2004). Exercise burns calories which helps in weight loss and maintain a healthy weight and also improves circulation in arms and legs where people with Diabetes have complications. Exercise provides relief from stress, which is the major contributing factor in raising blood sugar levels (Diabetes Mellitus Information, 2006).

A randomized study done by Warden *et al.,* (2012) revealed better performance in weight reduction through brisk walking than those in the control group. A systematic review and meta-analysis done by Boule *et al.,* (2001) and Kraus *et al.,* (2002) on effects of structured exercise interventions in clinical trials in Type 2 diabetics exhibited significant decrease in HbAlc and decreased plasma triglycerides while increased HDL cholesterol. This study considered physical activity levels of the participants.

A study done in Kenya urban slum by Richard *et al.,* (2013) indicated high levels of physical activity among the Type 2 Diabetics that was work and travel related. Most participants were either walking or cycling as the main method of transport to and from places. The levels of overweight, obesity and elevated WC were high despite the high levels of physical activity.

A study by Tsvetan *et al.,* (2012) indicated that individuals with low activity had a significantly higher BMI and waist circumference when compared with individuals with medium and high activity. Hip circumference was also significantly higher in participants

with low and medium physical activity in comparison with participants with high physical activity. In addition, total weekly physical activity in the range of 500-1000 MET minutes was found to have considerable beneficial effect on insulin sensitivity. Physical activity guidelines for Americans classify physical activity levels as; low activity (<500 MET-min/week), medium activity (500-1000 MET-min/week) and high activity (>1000 MET-min/week). The guidelines further recommend engagement in weekly physical activity in the range 500-1000 MET-minutes for substantial health benefits (Tsvetan *et al.*, 2012).

2.5 Medications for Type 2Diabetes

Current therapeutic goals are to maintain glycaemic control (fasting blood glucose levels <6.5 mmol/l and glycated haemoglobin levels <7.0%) and to preserve the pancreatic insulin-producing b-cells using a combination of oral drugs and injectables (Marcheti *et al.,* 2009). Current medications used towards achievement of good control of blood sugar include;

There are oral agents that include Sulfonylureas that stimulate insulin secretion; Meglitinides that stimulate insulin secretion (short-acting); Biguanides that majorly decrease hepatic glucose production like metformin (1,1-dimethylbiguanide) hydrochloride, the most widely used oral anti-diabetic that enhances glucose metabolism in the liver through the insulin-independent AMPK pathway (Papanus and Maltezos, 2009). Other oral drugs include Alpha-Glucosidase inhibitors that slow absorption of starches; Thiazolidinediones that decrease peripheral insulin resistance; Dipeptidyl peptidase inhibitors that majorly increase incretin levels; Combinations such as glyburide and metformin (Ann, 2013). There are also the injectables that include Insulin (Long-

acting, Intermediate, Short-acting); Mixes (NPH/regular: 70/30, 50/50 and NPH/lispro: (Humalog Mix *Lilly*) 75/25); incretin mimetics and amylin analogues (Swindale, 2013).

2.6 Chromium and Type 2 Diabetes

2.6.1 Chemistry and importance of chromium

Chromium is a mineral element found primarily in two forms: 1) trivalent (chromium III), which is biologically active and found in food, and 2) hexavalent (chromium VI), a toxic form that results from industrial pollution. Trivalent chromium occurs in trace amounts in food and waters and appears benign. Healthy human population requires trivalent chromium in trace amounts for sugar and lipid metabolism although; its mechanisms of action in the body and the amounts needed for optimal health are not well defined (Cronin and Joseph, 2004).

Trivalent chromium is commercially available in several forms including chromium nicotinate, chromium histidinate, chromium picolinate, chromium-enriched yeast, chromium chloride, and glucose tolerance factor chromium (GTF). Chromium is available as part of many multivitamins or alone in tablet and capsule forms. Chromium picolinate is a formulation that was designed to improve absorption and may be least affected by nutritional and environmental factors (Zhong *et al.,* 2010)

Chromium is an active ingredient in a glucose tolerant factor where a low molecular weight chromium-binding substance (LMWcr) may enhance the response of the insulin receptor to insulin action. The ability of the LMWcr to activate the insulin receptor is dependent on it chromium content (Cronin *et al.,* 2004). Studies have also indicated that chromium enhances insulin action by increasing the insulin stimulated translocation of

glucose transporters to the cell membrane (Eastmond *et al.,* 2008). Other studies have also indicated that chromium reduces oxidative stress in the muscle cells that contribute to insulin resistance in Type 2 diabetics with poorly controlled blood sugar (Aviva and Peter 2011).

2.6.2 Recommended dietary allowances for chromium

There is no conclusive scientific evidence on chromium requirement to set recommended dietary allowances (RDA's). The RDA is the average daily intake that meets a nutrient requirement of nearly all (97 to 98%) healthy individuals. An adequate intake (AI) is established when there is insufficient research to establish an RDA; it is generally set at a level that healthy people typically consume. Adequate intakes for chromium have been set by institute of medicine staff to regulate safe intake limits for different age groups where adult women between 18-50 years should take 35ug/day while Men of the same age to receive 25ug/day (Institute of medicine staff, 2001). Based on Dietary guidelines for Americans (2010), for disease prevention and treatment, chromium supplement doses in adults are typically 200ug taken 1-3 times daily taken in 3-6 months. Meta-analysis done by Babra and Donald, (2014) on chromium studies found that, doses of 400-1000 mcg/dl had greater effect than dose of 200mcg/dl and recommended higher doses for diabetics than the non-diabetic population. Few adverse effects have been linked to high intakes of chromium, so the Institute of Medicine has not established a Tolerable Upper Intake Level (UL) for this mineral. This study used a daily dose of 500ug for a period of 4 months since this was within the safe dose range and minimum recommended duration in researches (Anderson 2007).

2.6.3 Chromium deficiency and Type 2 Diabetes

Trivalent chromium has specific transport mechanism hence only limited amounts enter the cells while the rest is excreted in the faecal matter. An absorption rate for a daily dose of 1000ug chromium picolinate is reported to range from 0.4% to 2.8% (Zhong *et al.,* 2010). Studies have reported significant age-related decreases in the chromium concentrations of hair, sweat and blood (Gibson, 2005) which has been further supported by recommendations of chromium intake by institute of medicine, food and nutrition board what suggest vulnerability to depletion of chromium stores to increase with increase in age (Institute of medicine, 2001). Rajpathak *et al.,* (2004) study indicated that subclinical chromium deficiency may contribute to insulin resistance and cardiovascular disease, particularly in aging and diabetic populations.

2.6.4 Chromium Toxicity and Type 2 Diabetes Studies

Several studies have demonstrated the safety of daily doses of up to 1000 ug/day for a maximum of 6 months (Cefalu *et al.,* 1999; Institute of Medicine 2004; Yuk Martin *et al.,* 2006). Few isolated reports of serious adverse reactions to chromium picolinate that involves Kidney failure and impaired liver functions were reported in a woman after use of 1200-2400 ug/day for 5 months for weight loss (Althius, 2002). The woman was also under active treatment with antipsychotic medication which makes the sole contribution of chromium in the reactions inconclusive (Cathy, 2012). This study used a dose of 500ug/day for 4 months and also excluded patients on any non-hypoglycaemic medications.

Yazaki *et al.,* (2010) supplemented 80 obese subjects with 1000ug chromium daily for a period of 6months. One subject out of the 80 experienced urticaria after consumption of

chromium picolinate for 35 days. The subject was discontinued from chromium intake and the condition resolved within 4 days. Liver and kidney functions remained normal for the six months in the study.

Safety of chromium picolinate in pregnant and nursing women has not been established. However, chromium picolinate administered to pregnant mice was found to cause skeletal birth defects in the developing foetus (Bailey *et al.,* 2006; Golubnitschaja and Yeghiazaryan, 2012). This study excluded pregnant and lactating women.

There are concerns that chromium picolinate may affect levels of neurotransmitters but is a potential concern for people with conditions such as depression, bipolar disorder and schizophrenia (Yeghiazaryan, 2012). Such individuals were excluded from this study. Laboratory studies using cell cultures and animals have suggested that high doses of chromium picolinate cause oxidative stress and DNA damage (Anderson 2007). This is supported by a systematic review by David *et al.,* (2008) which indicated that in vitro Cr (III) has the potential to react with DNA and to cause DNA damage in cell culture systems, but under normal circumstances, restricted access of Cr (III) to cells in vivo limits or prevents genotoxicity in biological systems.

The available in vivo evidence suggests that genotoxic effects are very unlikely to occur in humans or animals exposed to nutritional or to moderate recommended supplemental levels of Cr (III) (David *et al.,* 2008). Review by Gregg, (2011) further supports this with the indication that Oral administration of chromium picolinate in living organisms has an average absorption of 2% compared to the 100% availability in the laboratory cell cultures. Acute oral toxicity has been reported to range from 1.5-3.3 mg/kg (Vincent *et*

al., 2006). Toxicity effect was not anticipated in this study with an oral dose of 500ug per day for a period of 4 months.

2.6.5 Chromium Interactions

2.6.5.1Chromium-nutrientInteractions

Chromium absorption may be enhanced or hindered by different nutrients. Chromium competes for one of the binding sites on the iron-transport protein transferin. However, studies have shown no significant effect on measures of iron nutritional status unless under hereditary hemochromatosis (Anderson, 2007). This study considered serum ferritin levels in the subjects at baseline and end of study to determine any influence of chromium supplementation on iron status.

Absorption of dietary chromium is low and only 1% is absorbed and retained (Barbara and Donald, 2014). Absorption of chromium may be enhanced by vitamin C, amino acids, B vitamins and oxalates; but reduced by high fibre, phytate and diets rich in simple sugars. The simple sugars increase urinary excretion of chromium (Zhong *et al.,* 2010). This study carried out dietary assessment on the participants to determine the nutrient component of their diets as well as the frequency of consumption of dietary sources of the nutrients of interest.

2.6.5.2 Chromium- drug Interactions

Chromium picolinate interaction with other drugs as reviewed by Steven *et al.,* (2009) in Diabetes medication revealed reduction of absorption of chromium in the human gut by antacids(particularly the antacids containing calcium carbonate); Corticosteroids; H2 blockers (such as cimetidine, famotidine, nizatidine, and rantidine) and Proton-pump

inhibitors (such as omeprazole, lansoprazole, rabeprazole, pantoprazole, and esomeprazole). These medications alter stomach acidity and may impair chromium absorption or enhance excretion (Davis *et al.,* 1995; Kamath *et al.,* 1997; Institute of medicine, 2001 and Natural medicines 2005).

Other medications may have their effects enhanced if taken together with chromium or they may increase chromium absorption as in the case of Beta-blockers (such as atenolol or propanolol); Corticosteroids; Insulin; Nicotinic acid; Nonsteroidal anti-inflammatory drugs (NSAIDS) and Prostaglandin inhibitors such as ibuprofen, indomethacin, naproxen, piroxicam, and aspirin (Davis *et al.,* 1995; Kamath *et al.,* 1997; Institute of medicine, 2001 and Natural medicines, 2005).

Clinical studies have demonstrated that Chromium supplements may actually enhance the effectiveness of certain Diabetes medications such as insulin, metformin (Glucophage), or sulfonylureas (Steven *et al.,* 2009). This study monitored drug use on a monthly basis and reported trends observed. The physician attending to the patients decided on the drugs and the doses independently based on the professional clinical practice. However, any changes in the drugs and the dose were observed and reported on a monthly basis.

2.6.6 Dietary sources of chromium

Dietary sources of trivalent chromium include brewer's yeast, lean meats (especially processed meats), cheeses, pork kidney, whole-grain breads and cereals, molasses, spices, and some bran cereals. Brewer's yeast (particularly yeast grown in chromium-rich soil) is a rich dietary source of chromium, as are organ meats, mushroom, oatmeal, prunes, nuts, asparagus, and whole grains and cereals. Vegetables, fruits, and most refined and

processed foods (except for processed meats) contain low amounts of chromium (Dietary guidelines for Americans, 2010)

2.7Chromium supplementation and fasting blood sugar in Type 2 Diabetes

The benefits of chromium picolinate supplements for Type 2 Diabetes has been studied and debated for a number of years. Some clinical studies have reported beneficial effects of chromium supplementation while others have not. Studies have reported that chromium picolinate supplement may reduce blood sugar levels as well as amount of insulin needed by people with Diabetes.

A study by Mohamed *et al.,* (2013) indicated that daily dose of 250mcg supplemental chromium for a period of three months significantly reduced fasting blood sugar among Type 2 diabetics. These findings are supported the earlier studies by Gosh *et al.,* (2010) who realized significant decrease in fasting blood sugar among diabetic Indian subjects following a 400mcg chromium supplementation for a period of 7 months. A meta-analysis by Perpetua *et al.,* 2010 on chromium supplementation studies indicates significant reduction of fasting blood sugar in the six trials analyzed.

Despite the beneficial results reported, Ali *et al.,* (2011) in a conducted study indicated no effect of 500mcg and 1000mcg chromium supplementation compared to the placebo group. These findings are further supported by a study done by Sushi *et al.,* (2012) that reported no significant difference in fasting blood sugar between chromium supplemented and placebo study groups. Additionally, a meta-analysis of nine randomized by Baily, (2014) reported that chromium at doses of 200-1,000 mcg/day for 8-16 weeks had no effect on fasting glucose concentrations. Suggestions have been made

to the effect that greater doses of chromium may be required to observe beneficial effects of chromium supplementation Vincent, (2013).

2.8Chromium Supplementation and Glycated Haemoglobin (HbAlc) in Type 2 Diabetes

Glycated haemoglobin provides a measure of long term glucose levels in the preceding 3 months usually maximised between 90-120 days (Althias e al., 20002). There are mixed findings on the beneficial effects of chromium on HbAlc. Gosh *et al.,* (2010) reported significant improvement in HbAlc after supplementing Indian subjects with 400mcg chromium daily for 7 months. Analysis of the findings of randomized trials by Perteua *et al.,* (2010) indicated significantly improved HbAlc after chromium supplementation. These findings were similar to those by Mohammed *et al.,* (2013) in a meta-analysis of randomized controlled trials with intake of chromium supplements of daily doses higher than 250mcg for at least 3 months.

Despite the findings on beneficial effects of chromium in Type 2 Diabetes, other studies have reported the contrary. A meta-analysis of seven randomized, placebo-controlled studies indicated that chromium intake of not less than 250 mcg/day for three months had no effect on the levels of HbA1c (Abdollahi *et al.,* 2013). Similarly, a study by Sushi *et al.,* (2012) reported no beneficial effects of chromium on HbAlc after supplementing 50 diabetic adults with 400mcg chromium daily for 3 months. These findings were attributed to short duration of study.

2.9 ChromiumSupplementation and Blood Lipids (Total Cholesterol, Triglycerides, HDL, LDL) in Type 2 Diabetes

Mixed study reports have been expressed on the contribution of chromium picolinate on lipid profile where some studies report significant improvements with supplementation while others report no improvement. According to Vincent *et al.,* (2004); Cefalu *et al.,* (2004); Balk *et al.,* (2007) and Humel *et al.,* (2007), chromium supplementation significantly reduced oxidative stress and improved lipid metabolism in Type 2 diabetics. These findings were supported by meta-analysis by Perpetua *et al.,* (2010) that reported significant reduction in LDL and triglycerides.

On the contrary, a meta-analysis by Mohamed *et al.,* (2013) on placebo controlled studies indicated no beneficial effect of chromium supplementation on HDL, LDL and triglycerides. These findings were attributed to short duration of the studies. Similarly, a study by Gosh *et al.,* (2010) on Type 2diabetic Indian subjects revealed no significant difference in total cholesterol, LDL, and triglycerides.

2.10 Chromium and Iron Status in Type 2 Diabetes

Chromium competes for one of the binding sites on the iron transport protein, transferrin. However, supplementation of older men with 925 mcg/day of chromium for 12 weeks did not significantly affect measures of iron status (Campbell *et al.,* 1997). A study of younger men found an insignificant decrease in transferrin saturation with iron after supplementation of 200 mcg/day of chromium for eight weeks, but no long-term studies have addressed this issue (Lukaski*et al.,* 1996). In a 12-week, randomized controlled trial, supplementation with chromium picolinate (200 mcg/day) did not affect iron status in premenopausal women when compared to placebo (Lukaski*et al.,*(2007). Iron overload

in hereditary hemochromatosis may interfere with chromium transport by competing for transferrin binding. It has been hypothesized that decreased chromium transport might contribute to the pathogenesis of Diabetes mellitus in patients with hereditary hemochromatosis (Food and nutrition board, institute of medicine, 2001).

2.11Chromium Supplementation and BMI in Type 2 Diabetes

Weight reduction is a primary therapeutic aim for people with Type 2 Diabetes (ADA, 2008). Studies have shown that weight reduction for people with Type 2 Diabetes was associated with reduced insulin resistance (Lee and Aronne, 2007; Nield *et al.,*2008).

Martin *et al.,* (2006) revealed improvement in insulin sensitivity and further attenuated body weight gain and visceral fat accumulation compared with placebo group after chromium supplementation. These findings were further supported by studies by Anton *et al.,* 2008; Brownley *et al.,* 2013 and Wing *et al.,* 2009 that also reported significant reduction in food craving and intake in overweight or obese women after supplementation with chromium picolinate. Onakpoya *et al.,* (2013) in a meta-analysis of randomized double blind placebo controlled studies on overweight and obese subjects found a significant reduction in weight after supplementing with chromium picolinate doses between 137mcg/day and 1000mcg/day for 8-24 weeks.

On the other hand, Cefalu *et al.,* (1999) assessed effect of chromium picolinate (1000mcg/day) on Type 2 diabetics and reported no significant change in body weight, abdominal fat and BMI.A study done by Kobla and Volpe, (2000) that found no beneficial effect of chromium picolinate supplementation on body composition. This finding is supported by a meta-analysis by Vincent *et al.,* (2003) on 12 placebo controlled

studies that compared the effect of chromium picolinate supplementation on lean body mass and found no beneficial effect.

2.12 Chromium Supplementation and Waist Circumference (WC) in Type 2 Diabetes

There is a growing recognition that central rather than general obesity is more contributory to and therefore better correlates with the risk of Type 2Diabetes (Depres *et al.,* 2008; Kim *et al.,* 2011; Korsic *et al.,* 2011).A study by He *et al.,* (2009) revealed a correlation of waist circumference (WC) with central obesity. Other studies have also found WC to be significantly associated to high fasting glucose, high serum insulin, high triglycerides and low HDL; which are all indicators of insulin resistance (Bardini *et al.,* 2011; Gonzalez *et al.,* 2011).A study by Mamtani *et al.,* (2013) indicated a cut-point of 94.65 cm to demonstrate predictive performance and further concluded that WC is strongly associated with the risk of both prevalent and incident Type 2 Diabetes and also an indicator of insulin resistance (irrespective of the presence of Type 2Diabetes).

Chromium supplementation studies however have not revealed any benefits with waist circumference reduction in Type 2 diabetics. Yuka Yazaki *et al.,* (2010) in a study where 1000mcg chromium supplementation was done on a randomized controlled trial for 24 weeks did not realize a significant reduction in waist circumference in the intervention group. On the contrary, an earlier study by Julie *et al.,* (2006) reported significant reduction in visceral fat accumulation in the group supplemented with chromium picolinate versus the control group.

2.13 Summary of literature review

The prevalence of Type 2 Diabetes in Africa and Kenya is high with projections to rise to higher levels in the year 2030. The major challenge to clients with Type 2 Diabetes is achieving or maintaining a tight blood sugar control. Blood sugar level is controlled through the diet, physical activity and use of hypoglycaemic drugs. Type 2 diabetics with poorly controlled blood sugar are at risk of chromium deficiency. Furthermore, the findings of some studies have demonstrated the positive effect of chromium supplementation in controlling fasting blood sugar levels, HDL, LDL, triglycerides, total cholesterol and HbAlc among Type 2 diabetics while some studies have not. The difference in the findings of these studies was attributed to supplementation period and dosage given. Most of these studies have not been conducted among Africans. This study therefore investigated the effect of chromium supplementation on blood glucose and lipids among Type 2 diabetics in Kenya.

CHAPTER THREE: METHODOLOGY

3.1 Research design

This was a double blind randomized controlled study that tested the effect of chromium supplementation in the management of blood glucose and lipids among Type 2 diabetics. This design reduced bias on adherence to chromium intake by the study participants and encouraged objective participation by the study assistants (Katzenellenbogen *et al.,* 2002).

3.2 Study variables

The study variables and expected outcomes are shown in Table 3.1.

Table 3.1: Dependent and Independent Variables

Dependent variables/Outcome	Independent Variables
Primary Outcome:	-Dietary patterns (number of meals, frequency of meals, type and amount of nutrient intake) -Physical activity level -Socio-economic status (Age, education level, occupation) -Anthropometry (BMI, waist circumference, waist to hip ratio) -Serum chromium levels
Blood glucose level (HbAlc and FBS)	
Secondary Outcomes:	
Serum chromium level	-Chromium supplementation -Dietary chromium intake
Serum lipid level- Total cholesterol, triglycerides, HDL and LDL	-Chromium status -Physical activity -Diet composition
Adiposity measures (BMI, WC &W/H ratio)	Serum chromium levels
Serum ferritin level	-Chromium supplementation -Dietary iron intake

FBS- Fasting blood sugar; BMI-Body mass index; HDL- High density lipoproteins; LDL- Low density lipoproteins

3.3 Study location

The study was done at Thika Level 5 Hospital that is a referral health facility located in Kiambu County. The Diabetes clinic located within the facility caters for diabetics from Thika town and its environs covering Kiambu, Machakos and Muranga Counties. The clinic served both male and female patients of all ages with glucose intolerance, Type 1 Diabetes and Type 2 Diabetes at the time of this study. The clients are mainly from the middle and low socio-economic brackets. The patients attended the clinic as regularly as deemed necessary by the physician at the health facility. The Diabetes clinic served about 1500 diabetics with Type 2 Diabetes accounting for 90% of the hospital patient attending Diabetes clinic. Follow-up visits at the clinic ranged from two weeks, one month and three months depending on the stability in the management of the client's blood sugar.

3.4 Target population

The target population was patients aged between20 and 64 years diagnosed with Type 2 Diabetes, attending Thika level 5 Hospital Diabetes Clinic.

3.4.1 Inclusion criteria

The study participants were patients diagnosed with Type 2 Diabetes aged between 20-64 years. The patients should have attended the clinic in the preceding six months and on hypoglycaemic therapy. Patients aged 65 years and above were excluded because of the heterogeneity in their health status that is characterized by multiple complications. They may also be on other medications not related to hyperglycaemia.

3.4.2 Exclusion criteria

Type 2 diabetics (20-64years) with a history or diagnosed and on medication related to heart diseases, cancer, renal failure, liver complications, HIV, pregnant and lactating women, alcoholics and clients on food supplements were excluded from the study (Figure 3.1). These were verified from the medical records at the clinic by the physician. Any participant that was diagnosed with these conditions during the study was immediately discontinued.

3.5 Sample size determination

The sample size was determined by the formula shown below (Kelsey *et al.,* 1996). The power of the test was at 80% with maximum allowable type I error of 5%.

$$n_1 = \frac{\left(Z_{n/2} + Z_{1-\beta}\right)^2 \overline{pq}(r+1)}{r\,(p_1 - p_2)^2}$$

$$n_2 = rn_1$$

Where:

n_1 = Number in control group

n_2 = Number in the intervention group

$Z_{\alpha/2}$ = Standard normal deviate for two-tailed test based on alpha level (relates to the confidence interval level)

Z_{ρ} = Standard normal deviate for one-tailed test based on beta level (relates to the power level)

r = ratio of intervention to control groups

p_1 = proportion of control with blood sugar levels above the good control cut-off point and q_1 = 1-p_1

p_2 = proportion of intervention with blood sugar levels above the good control cut-off point and q_2 = 1-p_2

$$\overline{p} = \frac{p_1 + rp_2}{r+1}$$

And $\bar{q} = 1 - \bar{p}$

Secondary data from Thika hospital Diabetes clinic shows the prevalence of poor blood sugar control was 43%. The effect was 20% and sample size was calculated as follows;

$n_2 = rn_1$ $\quad q_1 = 1 - p_1$ $\quad q_2 = 1 - p_2$ $\quad Z_{1-\beta} = 0.8$

$p_1 = 0.23$ $\quad p_2 = 0.43$ $\quad r = 1$ $\quad Z_{\alpha/2} = 1.96$ Therefore;

$\bar{p} = \frac{p_1 + r\ p_2}{r+1} = 0.33$ $\quad \bar{q} = 1 - \bar{p} = 0.67$

$$n_1 = \frac{\left[1.96\ \sqrt{(1+1)\ 0.33\ x\ 0.67} \quad + \quad 0.8\sqrt{1\ x\ 0.23\ x\ 0.77\ + 0.43\ \ x\ 0.57}\right]^2}{1(0.23\ -\ 0.43)^2}$$

$$= \frac{\left(1.96\ \sqrt{0.866712} \quad + \quad 0.8\ \sqrt{0.4222}\right)^2}{0.04}$$ **= 84 participants per study group.**

The sample size was subjected to correction factor to cater for attrition using the following formula by Fleiss, (1981).

$$n_{1cc} = \frac{n_1}{4}\left(1 + \sqrt{1 + \frac{2(r+1)}{n_1|P_2 - P_2|}}\right)^2$$

$$= \frac{84}{4} + \left(1 + \sqrt{1 + \frac{2(2+1)}{84\times 2\ |0.43 - 0.25|}}\right)^2$$

$= 21(2.08562)^2$=86.99 that were rounded off to 90 participants per study group making a total sample of 180 participants.

3.6 Sampling technique

Purposive sampling was used to select Thika level five hospital since it is the referral hospital for most parts of Kiambu, Thika town and Muranga County. The Diabetes clinic has observed rising numbers of diabetics and have 5 clinic days a week (Monday-Friday).

The study participants were recruited at the health facility. The intervention and biochemical tests were also conducted at the health facility. The hospital is well equipped with facilities and qualified medical personnel. All the diabetic patients in the facility were screened and those who qualified and willing to participate in the study were randomly assigned to either intervention or control study groups.

3.7 Randomization

The numbers in the sample size (1-180) was randomized by a biostatistician into two study groups using a formula generated in Microsoft office Excel 2007. This was based on a 1:1 ratio. Group 1 was the intervention while group 2 was the control. The diabetic patients who met the inclusion criteria and were willing to participate in the study were randomly assigned into intervention or control group (Figure 3.1). Randomization aimed to ensure that subjects on different treatments were comparable with respect to baseline characteristics as well as known and unknown risk factors. This reduced the risk of confounding factors (Katzenellenbogen *et al.,* 2002).

3.8 Recruitment of study participants

The participants in the study were recruited as they visited the health facility by the researcher assisted by the nurse and nutritionist attached to the hospital Diabetes clinic. The participants were first briefed about the study by the researcher assisted by the nurse attached to Diabetes clinic. They were informed that screening would be done and only those who qualify and willing to participate shall be enrolled into the study. Eligibility of participants was established by the nurse using the medical records against the inclusion criteria. The researcher then explained the objectives of the study to the eligible patients without revealing the study hypothesis. The participants were assured of confidentiality

and informed that they were free to discontinue their participation at any time. Those who accepted to participate then signed the consent form by signature or thumb print. The participants were then referred to the study team where they picked a numbered paper from a box of numbered papers (1-180) and depending on the number picked, were placed in either the intervention or the control group. The researcher and the study team reported at the health facility from Monday- Friday 8a.m to 1p.m to carry out recruitment until the desired number was realized in 3 months (December 2012-February 2013).

3.9 Blinding

This was a double blind study to control for bias. The biostatistician who conducted randomization of the study sample population was blinded of the study treatment and hypotheses. The participants, research assistants, nurse and laboratory technician were blinded to the treatment and hypotheses of the study. The physician was made aware of the treatment but not the study groups since he was the medical person that dealt with clinical issues arising during the study. The researcher was aware of the treatment and the groups to enable accuracy during the intervention. The placebo and the chromium picolinate capsules were procured from the same pharmaceutical company (Power Health Products Limited in United Kingdom). They were physically similar in size, shape, colour and packaging. Only the researcher could tell the difference between the two bottles.

3.10 Description of the intervention

The study comprised of 2 study groups; a control group and an intervention group. During the study, the participants continued receiving services at the health facility. The study intervention was administered as indicated in Figure 3.1. The study participants

were screened against the inclusion criteria. Those who met the criteria were randomly assigned to either intervention or control groups. Baseline data was then collected by administering baseline questionnaire to participants in both study groups. The participants were then given an appointment to visit the health facility after an overnight fast for the baseline blood draw. After fasting blood draw, then the intervention administered where the intervention group received routine treatment at the facility and chromium picolinate supplements- 500mcg/day for a period of 4 months. The control group received the routine treatment at the facility and placebo – 1 capsule/day for a period of 4 months. During the study, participants visited the health facility for monthly follow up where a questionnaire was administered on anthropometry, hypoglycaemic drug used and compliance to chromium and placebo intake. Participants were reminded of the next visit date by phone 3 days before. At month 4 in the study, there was blood draw after an overnight fast; after which, the participants were phased off the study (Figure 3.1)

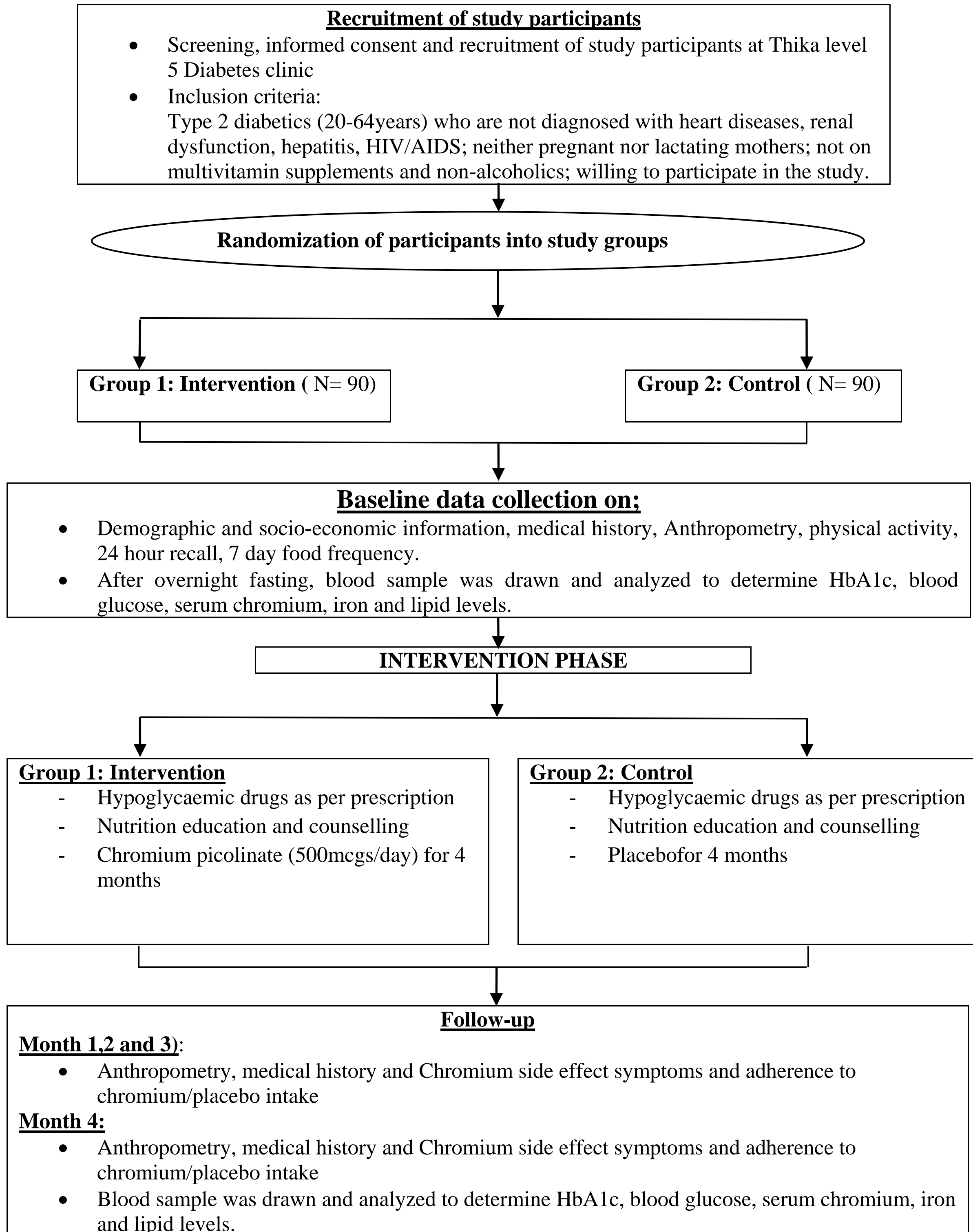

Figure 3.1: A schematic representation of the study design, Intervention and follow-up

3.11 Chromium supplements and placebo

The chromium picolinate supplements and placebo were acquired from Power Health Products Limited in the United Kingdom. The company is registered and licensed to practice and manufactures various food supplements alongside other health products (GMP and specification certificates–Appendices M and N). The acquisition of the supplements was done through a local supplier- Arman Limited located in Nairobi. Chromium specifications were as in table 3.2.

Table 3.2 Specifications of chromium picolinate and placebo

Chromium	Placebo
Composition: Stone ground brown rice flour (249.5mg) Chromium picolinate (0.5mg) **Colour**- Beige free flowing powder filled into Two-Piece clear hard gelatine capsule **Average weight**- 250mg ±7.5% (268mg-232mg range) **Shell composition-** Bovine gelatine size 3(average 47mg)	**Composition**- Stone ground brown rice flour (249.5mg) **Colour**- Beige free flowing powder filled into Two-Piece clear hard gelatine capsule **Average weight**- 250mg ±7.5% (268mg-232mg range) **Shell composition-** Bovine gelatine size 3(average 47mg)

3.12 Data collection tools

3.12.1 Questionnaire;

3.12.1.1 Baseline questionnaire (Appendix B)

This was administered to both study groups upon recruitment. The questionnaire consisted of closed and open ended questions that were used to solicit information on; demographics, socio-economic characteristics and medical history and7day food

frequency. Similarly, information was sought on physical activity using global physical activity questionnaire (GPAQ).Physical body measurements (anthropometry) were also measured which included height, weight, waist circumference and hip circumference.

3.12.1.2 24 hour recall questionnaire (Appendix-B)

The questionnaire was administered to 37% of the participants in both study groups who agreed to be followed up at home on equal basis. Measurements on the foods that were consumed in 24 hours preceding the interview were recorded. The participants reported the foods they consumed in terms of quantities in household measures, the ingredients, method of preparation and quantities consumed in household measures. A weighing scale was used to determine the actual weights while a measuring cylinder was used to approximate the volume of liquids consumed in millilitres and later converted to grams. Food albums were used to help the participants correctly identify and quantify the foods consumed.

3.12.1.3 Monthly questionnaire (Appendix C)

This was administered to all participants at the end of months 1, 2, 3 and 4 in the study. The questionnaire consisted of closed and open ended questions that solicited information on medical history, adherence, side effects/ hyperglycaemia symptoms and physical body measurements (height, weight, waist and hip circumference).

3.12.1.4 Laboratory forms for blood draw (Appendix F)

These consisted of tables with sub titles that were used to record biochemical results on fasting blood glucose, HbAlc, chromium levels, lipid profile and iron levels. The forms

were used at baseline and month 4 laboratory blood draw hence existed in yellow colour for the former and blue colours for the latter.

3.12.1.5 Appointment card (Appendix D)

This was used to record information on recruitment date and the dates for follow up visits. The card was given to the participant upon enrolment into the study and indicated the name, serial number and the return date to the clinic which was after every 4 weeks. The cards were retained by the participants for four months and surrendered to the researcher upon completion of the study.

3.12.1.6 Monitoring sheet (Appendix E)

This was a form used to record information on the participants that were attended to on a daily basis with details of the kind of service offered to them during the visit.

3.12.1.7 Recruitment Sheet (Appendix-G)

This contained the randomized numbers to the two study groups and the names of participants were recorded against the numbers. The follow up dates were also recorded for each visit by the participants.

3.12.1.8 Diary

This was used to record the dates that participants were recruited and booked them for follow up date when they were to visit the clinic.

3.12.1.9 Equipment for drawing blood samples

The following equipment were used**;** vacutainer needles (size 20G to 22G), tubes, vacutainer holder, tourniquet, disinfection swabs, micropore tape, dental rolls, adhesive

dressing, rubber gloves, pillow or other support, separate stoppers for opened vacuum tubes and non-vacuum tubes and needle disposal box.

3.12.1.10 Equipment for handling, transfer and storage of blood samples

The following equipment was used; transfer and storage tubes (freezable), disposable pipettes or pipettes with changeable apex, centrifuge, capable of 3000g with swinging bucket rotor, timer, racks for tubes, set of labels with identification codes (freezable), refrigerator and a freezer.

3.12.1.11 Laboratory machines

Auto-analyzer (Dirui, CS-300B model) was used to analyze the blood samples for blood lipids and fasting blood sugar. It was manufactured in 2011 and was calibrated as per manufacturer instructions at the time of study.

CERA-STAT^{TM} 2000 auto analyzer (H113A240100; CERAGEM MEDISYS Inc, Korea) was used to analyze HbAlc. It was manufactured in 2013 and was calibrated as per manufacturer instructions at the time of study.

3.13 Selection and training of the research team

3.13.1 Selection criteria for research assistants

- Diploma or Bsc. (food, nutrition and dietetics)
- Ability to speak English, Kiswahili and Kikuyu languages
- work experience of at least 6 months in nutrition related fields and willing to work in the study for 6 months

3.13.2 The research team

The research team consisted of;

- The principal researcher (MSc- Foods, Nutrition and Dietetics); that trained and oversaw data collection and intervention.
- Four nutritionists; 2 nutritionists assisted the researcher with administration of the baseline and monthly questionnaires, provision of placebo and chromium at the health facility. The other 2 nutritionists assisted the researcher with collecting 24 hour dietary recall information at the homes of the participants.
- A nurse in charge of Diabetes clinic; who assisted the researcher with screening of eligible willing participants. The nurse verified information on participants that met inclusion criteria from their medical records. The nurse also kept track of adherence and any unusual experiences reported (adverse effects) at each visit.
- A phlebotomist; that drew blood from the participants in readiness for biochemical analysis.
- Laboratory technician; that treated and analyzed the blood samples and
- A physician in charge of Diabetes patients; Identified the diabetic patients with diseases and medical conditions in the exclusion criteria and monitored patients on monthly visits. The physician also attended to the referred patients that laboratory results revealed very high fasting blood sugar during the study.

3.13.3 Training of research team

Training was conducted by the principal researcher who has a Msc. in food, nutrition and dietetics and a lecturer in the University in the area of qualification. Training was conducted through lecture, demonstrations and role play methods. The team members

with the exception of the physician and laboratory technicians were trained for three days. The purpose of the training was for the research assistants to:

- Explain the objectives and the research methodology;
- Understand the various components of the questionnaires;
- Acquire skills to conduct an interview and anthropometry;
- Explain the role and responsibilities of the research assistants in the study.

The content of the training was based on; recruitment of study participants (inclusion and exclusion criteria), anthropometry and the baseline and monthly questionnaire administration. This was followed by a practical session when questionnaires were administered to 10 diabetic patients to test skills learned.

Two nutritionists were further trained for 3more days on 24 hour recall data collection that consisted of:

- Explaining the content and use of food album;
- Explaining the household measures and their use in quantification of foods;
- Administration of the 24 hour recall questionnaire;
- Calculation of quantities of food consumed.

During the training, five households were visited and 24 hour questionnaire administered for practice purposes. One questionnaire administered by the researcher in presence of the assistants and after 2 questionnaires administered by each assistant.

The Physician, Nurse and the laboratory technicians were inducted into the study objectives and procedures by the researcher.

3.14 Data collection procedure

3.14.1 Baseline data collection

Upon recruitment into the study, the researcher assisted by the nutritionist then administered baseline questionnaire that sought information on socio-economic characteristics, medical history, anthropometry and 7 day food frequencies. Information on physical activity was sought using global physical activity questionnaire (GPAQ). The participants were then given appointments for a visit after an overnight fast for baseline blood sample collection.

3.14.2 24 hour recall

The following equipment was used;

- Food albums; consisted of pictures of various household measures and their right descriptions, photos of various foods both cooked and non-cooked to help participants understand and correctly confirm the foods consumed during 24 hour recall interview.
- Calibrated jug; this was used when collecting data on 24 hour recall to estimate quantities of fluids consumed.
- Household measures (250ml cups, shallow bowls, deep bowls, teaspoon and tablespoon); these were used to help participants correctly identify the utensils used to quantify the foods consumed.

The participants were asked to list the foods consumed in 24 hours preceding the study and with the help of food albums identified the type of foods and quantities. The albums were also used to quantify the weights of the solids taken like the fruits and pieces of meats consumed.

3.14.3 Phlebotomy at baseline and end of intervention

Participants were asked to come after an overnight fast. A date was agreed upon with the participants based on their convenience within the first seven days after recruitment. Fasting blood samples were drawn from them by the laboratory phlebotomist for analysis. The laboratory technician then prepared the samples accordingly for analysis of fasting blood sugar and HbAlc and the rest of the sample stored for a weekly analysis of other biochemical indicators (HDL, LDL, Triglycerides, Total cholesterol ferritin and Chromium).

The participants were then given chromium or placebo depending on their study groups. Participants were then given appointment dates for monthly follow up. The date and respective phone numbers were recorded in a diary that was retained by the researcher and copied onto appointment card that was given to the participant to keep as a reminder.

3.14.4 Monthly follow ups

A monthly questionnaire for months 1, 2, 3 and 4 were administered by the researcher and a nutritionist to participants as they visited the clinic that collected information on; adherence to the intervention, medical history and any unusual experiences. Those who reported any unusual experience were referred to the physician attached to the Diabetes clinic and who was part of the research team for review. Appointment dates for the next visit were then given and recorded on the diary and the appointment card. This was repeated for each of the months that the participants came for follow up visits. At end of month 3visit, the participants were reminded of blood draw at the next visit (4th month)

that required them to come to the clinic after an overnight fast. The participants were also reminded of their follow up date three days before to reduce on default.

3.14.5 Data collection at end of intervention

At the completion of 4 months in the study, participants visited the clinic after an overnight fast and blood as drawn from them by the laboratory phlebotomist. The samples were then analyzed for fasting blood sugar and HbAlc and the rest of the samples prepared and stored for weekly analysis of other biochemical indicators such as HDL, LDL, Triglycerides, Total cholesterol ferritin and Chromium (Figure 3.2).

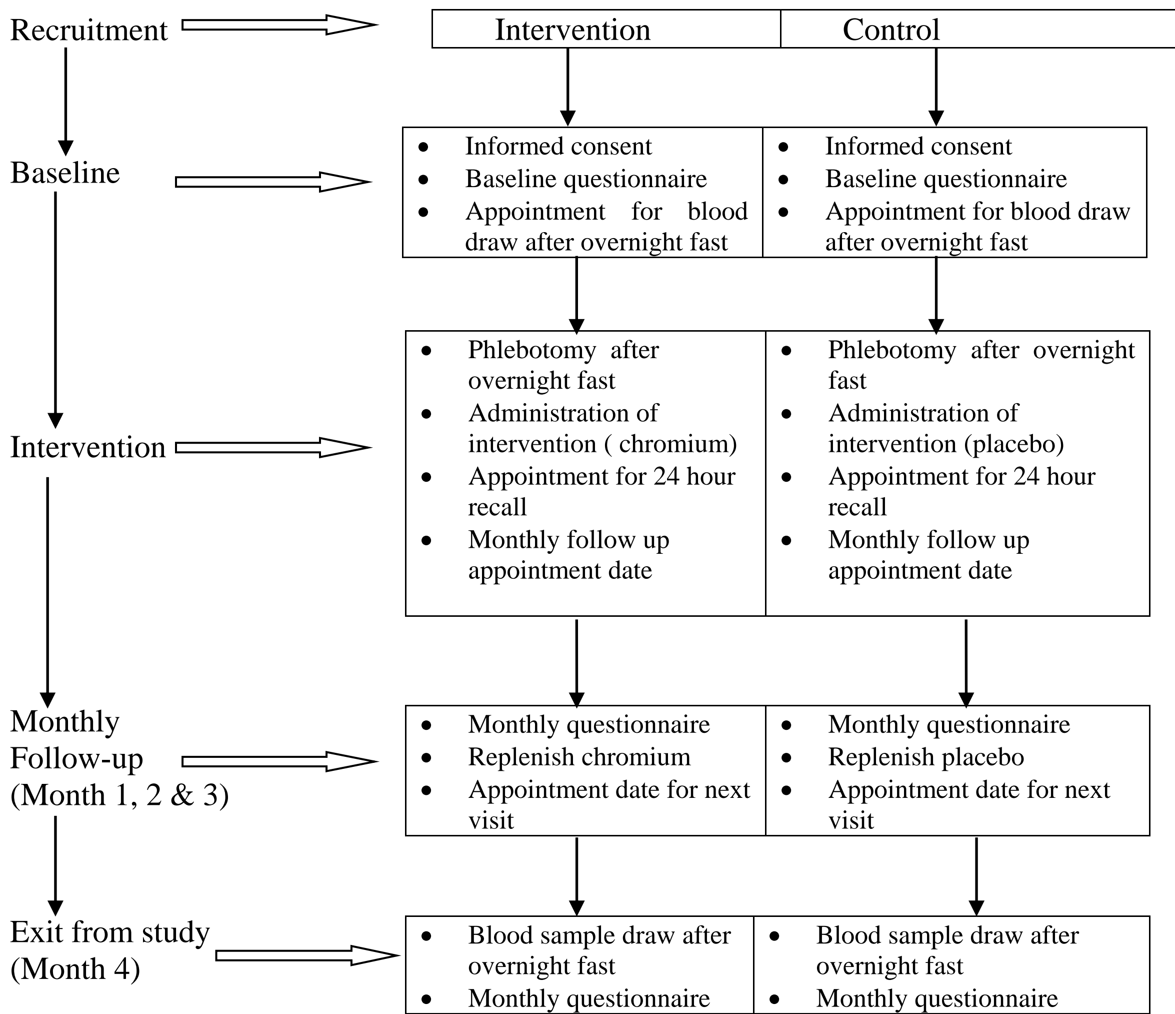

Figure 3.2 Flow chart on data collection process

3.15 Anthropometry

Physical body measurements were taken which included weight, height, waist circumference and hip circumference. The measurements were taken usingthe following equipment;

- Balanced beam scale mounted with a standiometre sliding height measuring rod attached; this was used to measure the weight and height of study participants.
- Flexible, but non-stretchable measuring tape: was used to measure waist and hip circumference of the participants.
- Several calibrated weights (e.g. 2kg, 5kg and 10 kg each); that were used to calibrate the weighing scale on a daily basis.
- Carpenter's level; this was used to verify that the hard floor where the scale was placed was levelled.

3.15.1 Weight measurement

The scale was placed on a hard-floor surface and a carpenter's level was used to verify that the surface on which the scale was placed was horizontal. The scale was then calibrated using known weights for accuracy. Participants were asked to remove their heavy outer garments (jackets, caps, sweaters and scarves) and shoes. They also emptied their trouser, shirt or skirt pockets. The participants were asked to stand in the centre of the platform with weight distributed evenly to both feet. Weights were then read out to the nearest 0.1kg.

3.15.2 Height measurements

Since the sliding height rod was attached to the scale, the participants were asked to stand with their backs to the height rule. The back of the head, back, buttocks, calves should be

upright and touching the rod, feet together and heels on the scale. The top of the external auditory meatus (ear canal) was checked to be at level with the inferior margin of the bony orbit (cheek bone). The participant was then asked to look straight and the sliding part of the measuring rod is lowered so that the hair (if present) is pressed flat. Height was read out and to the nearest 0.1cm and recorded. If the participant was taller than the measurer, the measurer stood on a platform so as to properly read the height rule.

3.15.3 Waist circumference measurement

Participants should have been asked to remove their clothes, except for light underwear but due to cultural reasons, they were asked to remove the heavy outer garments. Tight clothing, including the belts were loosened and the pockets emptied. Participants then stood with their feet fairly close together (about 12-15 cm) with their weight equally distributed to each leg. The measurer then located the top of the hip bone (iliac crest) wound the tape between the iliac crest and the lowest rib. The tape was positioned horizontally parallel to the floor. Participants were asked to breathe normally and the reading taken at the end of gentle exhaling to prevent them from contracting their abdominal muscles or from holding their breath. The circumference was read out to the nearest 0.1cm and recorded.

3.15.4 Hip circumference measurement

The hip girth measurement is taken over minimal clothing, at the level of the greatest protrusion of the gluteal (buttock) muscles. The participants were asked to stand erect with their weight evenly distributed on both feet and legs slightly parted, making sure not to tense the gluteal muscles. The tape was then wound round the fullest part of the gluteal

muscles, not too tight or too loose, lying flat a horizontal and parallel to the ground. The measurements were read out to the nearest 0.1cms and recorded.

3.16 Blood sample collection, preparation and storage

3.16.1 Collection process

Blood samples were drawn by a certified phlebotomist under standard operational procedures after an overnight fast of 10-14 hours. All blood samples were drawn in a sitting position and that the participants remained in sitting position for 15 minutes prior to blood collection. This waiting period was to allow equilibration of the concentrations of blood components. The participant was then asked to remove tight clothing that may constrict the upper arm and rest the left arm on a support prop.

The phlebotomist set the tourniquet around the upper arm of the subject, searched the proper vein by inspecting and palpating in the antecubital fossa and then sterilized the injection site. After that, the needle, bevelled upward was pushed smoothly and quickly into the vein, and the tourniquet immediately released to minimize the effect of hemoconcentration. The vacuum tubes for various assays were then filled with 4mml blood and labelled as indicated in Table 3.3.

Table 3.3 Type of analysis, specimen and type of tubes used for blood draw

Type of analysis	Specimen type	Type of tube
Lipids	serum	10 ml Lithium heparin bottle
Plasma glucose	plasma	4 ml tube filled with glycolytic inhibitors potassium oxalate and sodium fluoride
Glycated haemoglobin	whole blood	3 ml tube with anticoagulant K_2EDTA
Chromium	serum	5ml plain vacutainer with gel
Ferritin	Serum	5ml plain vacutainer with gel

3.16.2 Clotting and Centrifuging

After the identification of the tubes the timer was started and the blood samples are allowed to clot at 15-24 °C (Vacuum gel tubes at temperature s 20-22°C) for at least 30 minutes. Blood specimens were centrifuged at a temperature 15-24°C with stoppers on. Plasma samples for glucose measurements were centrifuged immediately after blood draw for 15minutes at 2000 x g to 3000 x g. Serum samples were centrifuged within one hour after blood collection for10 minutes at 1500 x g and separated immediately after centrifuging. After all serum/plasma is separated to proper transfer/storage tubes, the tubes were carefully marked with stickers with identification code and immediately frozen at -70°c awaiting analysis within 7 days.

3.17 Biochemical analysis procedures

Biochemical analysis was done to determine serum chromium, blood sugar and ferritin levels. Levels of HDL, LDL, triglycerides, total cholesterol and liver functions were also determined in the study participants.

3.17.1 Trivalent chromium test

Quantitative determination of chromium (VI) was done using QuantiChrom^{TM} Chromium assay kit (DCRM-250) given in Appendix M. Total chromium (VI)was determined in two levels; first the amount in the sample, and secondly converting Cr (III) in the sample to Cr (VI) by addition of nitric acids and again determining the levels of chromium (VI) as per the procedure. The amount of chromium (III) was then determined by computing the difference. The reference for normal serum chromium was 0.3-0.9ng/ml (Appendix M).

3.17.2 HbAlc test

The levels of HbAlc were determined using a standard operating procedure given in CERA-STAT^{TM} 2000 HbAlc test kit (Appendix K). An automated analyzer (H113A240100; CERAGEM MEDISYS Inc, Korea) was used to measure absorbance at 415 nm. The reference levels were: normal (<8.0%), good control (8.0-9.0%), fair control (9.0-10.0%) and poor control (>10.0%).

3.17.3 Serum fasting glucose test

Glucose content was analyzed using a colorimetric procedure developed by Centronic GmbH, Wartenburg Germany (Appendix L).Glucose PAP fluid mono-reagent was used as an enzymethat does notlead to de-proteinization. The absorbance of the solution was determined 500 nm using a 1 cm cuvette using an autoanalyzer (Dirui, CS-300B, China). The reference value for normal serum fasting glucose was 4.44-6.38 mmol/l.

3.17.4 Lipid profile

The analysis of lipids, HDL, LDL, total cholesterol and triglycerides was performed using an auto-analyzer (Dirui, CS-300B model-China) as follows.

3.17.4.1 Triglyceride tests

Triglycerides were assessed using the tryglyceridesenzymax assay test kit method developed by VitrioScient by measuring absorbance ranging between 500 – 550 nm using in a 1 cm cuvette (Appendix I). This process targets key enzymes involved in the metabolism of triglycerides: lipoprotein kinase, glycerol kinase, glycerol phosphate oxidase, peroxidase enzymes. The reference standards for serum triglycerides (mg/dl)

were normal (<150mg/dl or 0.45-1.81 mmols/l), borderline high (150-199mg/dl), high (200-499mg/dl) and very high (≥500mg/dl).

3.17.4.2 HDL tests

HDL was analyzed using HDL a cholesterol differential precipitate enzymatic colorimetric test. Absorbance was measured at 500 nm using a 1 cm cuvette(Appendix H).HDL cholesterol levels were classified as low (<40 mg/dl), high (≥60 mg/dl) or normal (>4.2 mmols/litr for males and >1.68 mmols/liter for females).

3.17.4.3 LDL tests

LDL cholesterol concentration was calculated automatically by the synchron system and expressed as part of the test panel using the formula:

LDL= (Total cholesterol- HDL) – (Triglycerides/2.2)

LDL cholesterol levels were then classified as optimal (<100mg/dl), borderline high (30-159mg/dl), near optimal/above optimal (100-129mg/dl), high(160-189mg/dl) and very high (≥190mg/dl). Or, normal (0-4.1 mmols/litre)

3.17.4.4 Total cholesterol

Total cholesterol was analyzed using the cholesterol liquid enzymatic colorimetric test developed by Cypress Diagnostics, Langdorp, Belgium (Appendix J).The levels were determined by measuring absorbance at 505 nm using a 1 cm cuvette. The reference standards for total cholesterol were as follows; normal (<200mg/dl), borderline (200-239mg/dl) and high (>240mg/dl).Or normal (0-5.2mmols/litre)

3.17.5 Liver function test-Alkaline Phosphatase (ALP)-DEA

ALP was analyzed using alkaline phosphatase (ALP) –diethanolamine buffer enzyme assay kit (BioSystems)(Appendix G). Absorbance was measured at 405 nm using a 1 cm cuvette in an auto-analyzer (Dirui, CS-300B, China).The reference for ALP was 270U/L for Men and 240U/L for Women.

3.17.6 Serum ferritin test

Enzyme immunoassay kit for the determination of ferritin in human serum was used to determine serum ferritin in the samples. Serum samples were incubated in Microtiter strip wells for 1 hour before bound/free substrates were separated using a solid-phase material. Tetramethylbenzidine (TMB) substrate was then added, incubated at room temperature for 10 minutes in the dark and sulphuric acid added to stop the reaction before measuring absorbance at 450 nm in an ELISA microwell plate reader. The normal ferritin serum values were categorized as pre-menopausal female (6-180ng/ml), post-menopausal female (8-350ng/ml) and male (20-400ng/ml).

3.18 Pilot study

A pilot study was carried out on 20 Type 2 diabetic patients from Thika level 5 Diabetes Clinic with the same characteristics as the study sample. The participants in the pilot study did not participate in the main study. Piloting was done particularly to validate and standardize the study procedures and research instruments. Questions in the instruments were tested for clarity, consistency, coherence and sensitivity. The research assistants were able to master the flow of the procedure from recruitment to baseline data

collection, to laboratory phlebotomy, intervention, follow up and exit from the study. They also practiced how to keep records in various instruments of the study.

The laboratory assistant had a practical experience with the various procedures that would be used for biochemical test in the study. The researcher and two nutritionists visited the homes of the participants for 24 hour recall data collection after consulting for the convenient date and time.

During the pilot study, various translations into local language (Kikuyu) were sought to give the exact meaning of questions in the questionnaire for accuracy. There were also local vegetables and fruits that were added to the list on the 7 day food frequencies. The laboratory technician realized challenges with collecting adequate samples for the auto analyzer per day hence readjusted to weekly analysis of the specimen other than fasting blood sugar and HbAlc. The study was piloted for a period of 1 month (November-December 2012) and data collected analyzed (Appendix W).

3.18.1 Validity

To ensure validity, the questionnaires were evaluated by a panel of supervisors who were experts in the subject area. The supervisors evaluated the questionnaires individually and gave feedback to the researcher who later shared the final document with all of them.

3.18.2 Reliability

The study was piloted and reliability of the instruments tested using Cronbach alpha coefficient. The coefficient was computed in terms of the average interconnections among the items measuring the concept. Reliabilities of 0.7 range is considered acceptable and those over 0.8 range considered good (Orodho 2008). A Cronbach

coefficient of 0.83was obtained when data was analyzed for reliability.The weighing scale was calibrated using known weights every morning before it was used to weigh participants of the day. During training, the research assistants were provided with practical sessions on anthropometry where the measurements were standardized to reduce on errors.

3.19 Data analysis

Data was entered into Epi data software and transferred to Excel 2007for cleaning. A spread sheet was developed and used to cross-check each entry against the questionnaire. Data was then imported to Statistical Package for Social Sciences (SPSS) version 20 for analysis. Descriptive summary statistics such as frequencies, means, medians and standard deviations were used to describe the characteristics of the study population on; age, sex, education level, socio-economic status, medical history, serum chromium, triglycerides, HDL, LDL, HbAlc, ferritin levels, BMI, waist to Hip ratio, waist circumference and physical level of activity. Principle component analysis was used to compute a wealth index to determine socio economic categories of the study participants.

Inferential statistics such as t-test, chi square and Wilcoxon test were used to determine similarity in the two study groups in their baseline characteristics (baseline comparison) and to determine relationships and associations between variables. Chi-square tests were used to test relationships between categorical variables while independent t-tests was carried out to determine differences between the study groups for continuous variables like blood glucose levels, serum chromium and lipid levels, BMI, and Waist to hip ratio with normal distributions. Wilcoxon signed rank test was used to test for differences

between the study groups for continuous data with non-normal distributions such as 7 day food frequencies. Significance level was set at $p<0.05$.

Physical activity was analyzed using WHO standards indicated in global physical activity questionnaire (GPAQ) guidelines (Appendix X). Physical activity levels were computed per metabolic equivalent activity (MET) per week depending on the type of activity and categorized into low, medium and high activity levels.

The adequacy of dietary nutrient intake was analyzed using Nutri-survey software and compared with the Recommended Daily Allowance. The nutrients analyzed include; energy, protein, fat, vitamins and minerals. RDA for nutrients were obtained from Kenya Food Composition Table (Sehmi, 1993) and American Food Composition Tables. A cut off point of 80% of RDA was used to indicate adequacy of nutrient intakes.

3.20 Logistical and ethical consideration

Authority to conduct research was granted by Kenyatta university graduate school (Appendix S) Ethical clearance was obtained from Kenyatta University Ethical Review Committee (Appendix U) and permission to conduct research was given by National Council of Science Technology and Innovations (Appendix Q and R). Authority to conduct research was granted from Thika level 5 Hospital (Appendix V)

Informed consent (Appendix A) was sought from prospective study participants that met inclusion criteria and recruited upon their informed consent by signature or thumbprint on the form. Before recruitment, the participants were informed of the objectives of the study. They were also informed of the requirement to provide blood samples at baseline and at fourth month of the study. The usual medical attention at the Thika level 5

Diabetes clinic remained. Participants were informed of possible risks and telephone numbers of the researcher, the physician and Kenyatta university ethics review committee were provided for reports of any unusual experiences or clarifications sought during the study.

Participants were informed that they were free to withdraw at any point in time when they choose to however, they were requested to willingly provide reasons for withdrawal. Participants were assured that withdrawal from the study would not affect services received at the hospital Diabetes clinic. Confidentiality during data collection was assured by substituting names with code numbers and information obtained from subjects kept in strict confidence and used only for the purpose of the study by the researcher.

Adequate medical care was provided to study participants by the medical personnel at Thika level 5 Diabetes Clinic by the physician who was part of the research team. There were no adverse reactions to chromium during the study. Two participants were diagnosed with HIV and 2 others diagnosed with hypertension. They were discontinued from the study upon diagnosis and attended to by the physician.

There were cases of very high fasting and very low blood sugars that were referred to the physician for attention. It was also indicated to the participants that results from the study would be communicated to them, personnel at the health facility and disseminated in refereed journals.

CHAPTER FOUR: RESULTS

4.0 Introduction

The purpose of this study was to investigate the effect of chromium supplementation in the management of blood glucose in Type 2 diabetics on routine care (hypoglycaemic therapy, physical activity and diet counselling) at Thika level 5 Hospital Diabetes clinic.

4.1 Enrolment and trial profile for study participants

The Diabetes clinic at Thika level 5 Hospital had approximately 1800 registered Type 2 diabetics at the time of the study. A total of 698 patients with Type 2 Diabetes were screened of which; 276 were eligible to the study from which 180 participants were recruited. The study participants were randomly assigned to two study groups; intervention group with 90 participants and control group with 90 participants.

During the study, the intervention group lost 18 participants while the control group lost 10 participants due to attrition. Attrition was due to various reasons which included fear of phlebotomy, changing residence, diagnosis with a condition that does not meet inclusion criteria or lack of support from the spouse to participate. A total of 152 participants completed the study and analyzed (Intervention group, 72 participants and control group 80 participants; Figure 4.1).

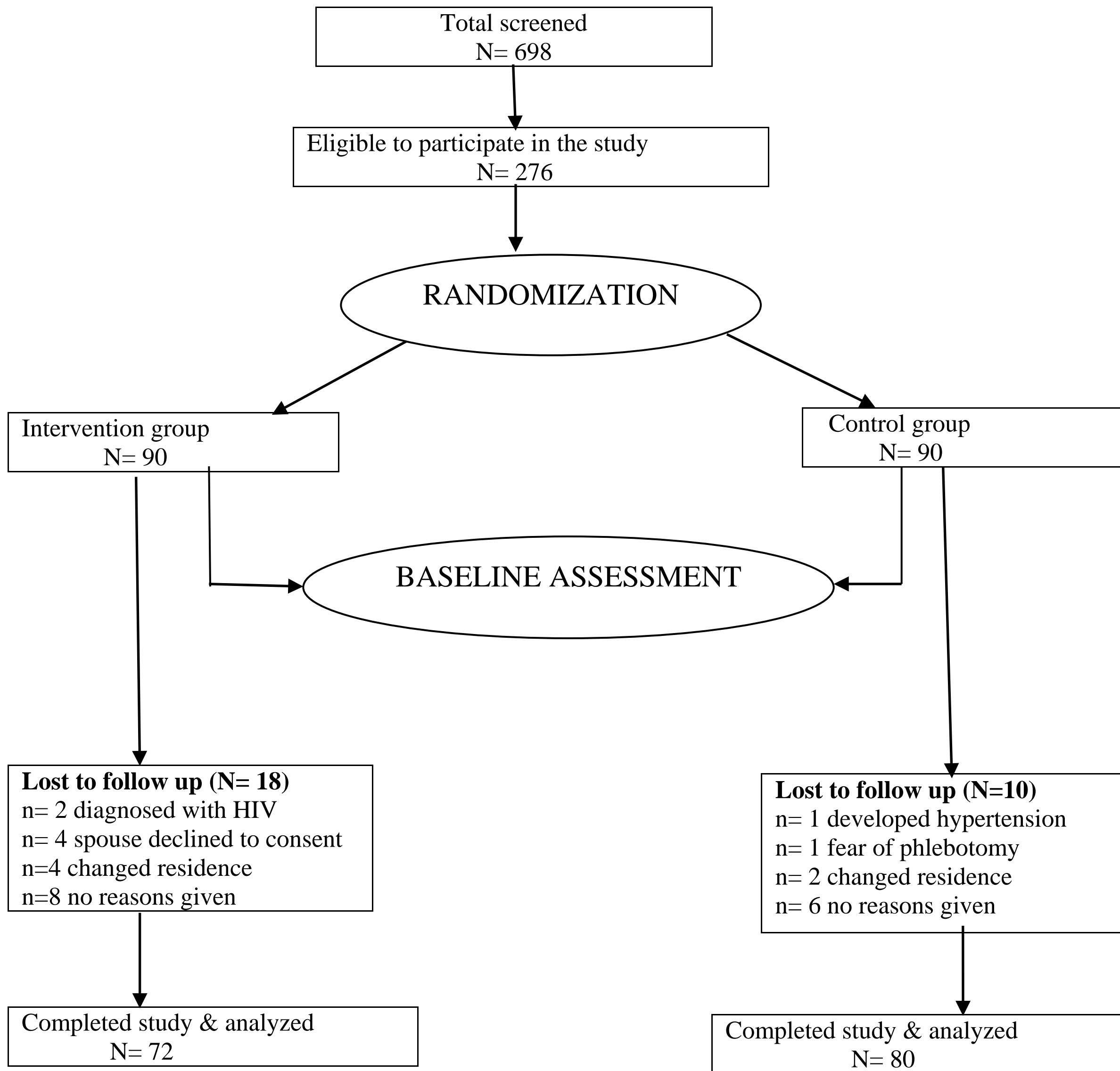

Figure 4.1: Schematic representation of the recruitment process for the participants included in the study

4.2 Participants who were lost to follow up and those who completed the study

A comparison was conducted of baseline characteristics of participants who completed the study and those lost to follow up. Participants who completed primary level of education (90.2%) were more likely complete the study (χ^2 =8.6550, p=0.034). University education had low representation in the study but was more likely to drop out where 50% were lost to follow up. Older participants in the age category of 56-64 years was more likely to drop out of the study while the participants in age category of 46-55 years were more likely to complete the study (χ^2 =11.3130, p=0.010). There was no significant difference in gender and marital status of the participants lost to follow up versus those who completed the study (Table 4.1).

Table 4.1 Demographic characteristics of participants lost to follow up and those who completed the study.

Variable	**Category**	**N=28 Lost to follow up n (%)**	**N=152 Completed n (%)**	**χ^2 value**	**p-value**
Gender	Male	6 (10)	54 (90)	2.1150	0.146
	Female	22 (18.3)	98 (81.7)		
Education Level	Primary level	10 (9.8)	92 (90.2)	8.6550	0.034*
	Secondary level	12 (21.4)	44 (78.6)		
	Tertiary level college	4 (25)	12 (75)		
	University	2 (50)	2 (50)		
Age	20-35	4 (28.6)	10 (71.4)	11.3130	0.010*
	36-45	10 (15.2)	56 (84.8)		
	46-55	4 (6.1)	62 (93.9)		
	56-64	10 (29.4)	24 (70.6)		
marital status	Married	24 (66.7)	128 (84.2)	2.4180	0.120
	Divorced/separated	4 (33.3)	24 (15.8)		

* ***Refers to significance (p<0.05)***

4.3 FINDINGS AT BASELINE AND COMPARISON OF STUDY GROUPS

Findings at baseline have been reported and compared for similarities between the study groups as follows;

4.3.1 Demographic characteristics

There were more females (66.7%) than males (33.3%).There was no significant difference in gender distribution between the study groups (χ^2; p=0.058).The highest proportion of participants was within the age ranges;36-45(36.7%) and 46-55(36.7%).There was no difference in age range between the study groups ((χ^2; p=0.329).Over half of the participants (57.3%) had completed primary education while 31.5% completed secondary level of education. There was no difference in education levels between the study groups (χ^2; p=0.797).Most of the participants were married at the time of the study and living with their spouses (84.4%).Marital status was not different among the participants in both intervention and control groups (χ^2; p=0.487).The highest percentage of participants (51.7%) resided in Kiambu while 47.2% were residents of Muranga County. This observation was similar to both study groups (Table 4.2)

Table 4.2 Demographic characteristics

Characteristics	Study Groups			Chi-square value	p-value (2 sided)
	Intervention N=90 n (%)	**Control N=90 n (%)**	**Total N=180 n (%)**		
Gender					
Male	24 (26.7)	36 (40)	60 (33.3)	3.6000	0.058
Female	66 (73.3)	54 (60)	120 (66.7)		
Age (years)					
20-35	8 (8.9)	6 (6.7)	14 (7.8)	3.4340	0.329
36-45	38 (42.2)	28 (31.1)	66 (36.7)		
46-55	28 (31.1)	38 (42.2)	66 (36.7)		
56-64	16 (17.8)	18 (20)	34 (18.9)		
Education Level					
Primary	52 (59.1)	50 (55.6)	102 (57.3)	1.0170	0.797
Secondary	28 (31.1)	28 (31.1)	56 (31.5)		
Tertiary	7 (6.8)	10 (11.1)	16 (9)		
University	3 (2.3)	2 (2.2)	4 (2.2)		
Marital status					
Single	8 (8.9)	8 (8.9)	16 (8.9)	1.4390	0.487
Married	74(82.2)	78 (86.7)	152 (84.4)		
Divorced/ Widowed	8 (8.9)	4 (4.4)	12 (6.7)		
County of origin					
Kiambu	48 (53.3)	44 (48.9)	92 (51.7)	0.0830	0.773
Muranga	42 (46.7)	42 (46.7)	84 (47.2)		
Machakos	0 (0.0)	4(4.4)	4 (1.1)		

4.3.2 Socio-economic characteristics

Farmers made the highest proportion of the participants (48.7%) followed by business that constituted of 36.8%. Most participants (41.2%) owned houses in their own compounds while 24.4% lived in rented houses. Those who paid rent were more likely to pay less than Kenya shillings 2500 per month. Cement and stone blocks were the main materials used for walling of the participants houses; iron sheets were the main roofing materials while the floor was mainly cemented (Table 4.3). Electricity was the main source of lighting with 58.9% of the participants though the main source of cooking fuel was firewood with 56.7%. In property ownership, the highest percentage (96.6%) owned cell phones followed by radio (95.5%) and sofa sets (85.2%).

Among domesticated animals, most participants (57%) kept chicken while 56.8% owned cows. The wealth index indicated highest proportion (37.2%) of participants to be in low socio-economic category followed by the middle socio-economic category (33.7%). There were no significant differences in the socio economic characteristics and wealth index between the study groups (Table 4.3).

Table 4.3 Socio-economic characteristics

Characteristics	Study Groups			χ^2 value	p-value (2 sided)
	Intervention N=90 (%)	Control N=90 (%)	Total N=180 (%)		
Occupation					
Civil servant	8.1	10.3	9.2	3.3800	0.337
Private sector	8.1	2.6	5.3		
Business	32.4	41.0	36.8		
Farmers	51.4	46.1	48.7		
Housing type					
Rented house	26.7	22.2	24.4	0.4820	0.786
Own house in parental compound	33.3	35.6	34.4		
Own house in own compound	40	42.2	41.1		
Rent/month					
<2500	82.2	88.9	85.6	5.8530	0.054
2500-3500	11.1	2.2	6.7		
>3500	6.7	8.9	7.8		
Walling of house materials					
Iron sheets	13.3	6.7	10	6.3330	0.275
Burnt bricks	6.7	11.1	8.9		
Mud and wood	2.2	6.7	4.4		
Mud and cement	2.2	4.4	3.3		
Cement/stone blocks	60	60	60		
Timber	15.6	11.1	13.3		
Roofing materials					
Iron sheets	97.8	97.7	96.7	0.0010	0.982
Tiles	2.2	4.4	3.3		
Floor of house					
Earthen	17.8	17.8	17.8	1.4510	0.484
Cement	73.3	77.8	75.6		
Other	8.9	4.4	6.7		
Source of lighting					
Kerosene	33.3	33.3	33.3	2.9110	0.233
Electricity	62.2	55.6	58.9		
Solar	4.4	11.1	7.8		

Table 4.3 Continued: Socio-economic characteristics

Characteristics	**Study Groups**			**χ^2 value**	**p-value (2 sided)**
	Intervention N=90 (%)	**Control N=90 (%)**	**Total N=180 (%)**		
Source of cooking fuel					
Firewood	57.8	55.6	56.7	1.3730	0.712
Charcoal	15.6	11.1	13.3		
Kerosene	8.9	11.1	10		
Gas	17.8	22.2	20		
Property ownership					
Phone	95.5	97.7	96.6	0.6900	0.406
Radio	93.2	97.7	95.5	2.0950	0.148
Bicycle	34.1	45.5	39.8	2.3720	0.124
Motorcycle	6.8	6.8	6.8	0.0000	1.000
Television	72.7	68.2	70.5	0.4370	0.509
Vehicle	18.2	18.2	18.2	0.0000	1.000
Sofa set	88.6	81.8	85.2	1.6250	0.202
Video	56.8	56.8	56.8	0.0000	1.000
Land	50	59.1	54.5	1.4670	0.226
Cows	52.3	61.4	56.8	1.4820	0.223
Sheep	9.1	15.9	12.5	1.8700	0.171
Chicken	55.8	58.1	57	0.0950	0.758
SES_Category					
Lower SES	34.9	39.5	37.2	0.9510	0.622
Middle SES	37.2	30.2	33.7		
Higher SES	27.9	30.2	29.1		

SES- Socio economic status category for wealth index

4.3.3 Nutrient intake (7 day food frequency)

A Wilcoxon signed-rank test was used to compare the frequency of consumption for various foodstuffs based on a 7 day food frequency between the intervention and the control study groups. The foods were categorized into six groups namely; cereals, carbohydrates and starches (energy sources); fruits and vegetables; legumes, pulses and nuts (plant proteins); meat, poultry and fish (animal proteins); milk and milk products (Calcium sources) as well as sugar, candy and beverages (sweets).

4.3.3.1 Consumption of carbohydrate and carbohydrate products

The most frequently consumed carbohydrate food was whole meal bread and whole meal *ugali* in a 7 day period, each with a median of 3(1-7) times. There was no significant difference in the frequency of consumption of these food items between the study groups (Wilcoxon test; p=0.242 and p=0.082 respectively).*Githeri*, brown porridge, brown rice, roast maize and potato chips were also frequently consumed with no significant difference in consumption between the study groups (Table4.4). The least frequently consumed foods were white bread, *pasta, chapati, mandazi*, taro roots, yams, sweet potatoes and green bananas. There were no significant differences in the frequency of consumption of these foods between the study groups (Table 4.4).

In contrast, the frequency of consumption of white rice was significantly higher (Wilcoxon test; p=0.035) in the intervention group with a median intake of 2 (1-7)times, compared to that of intervention group that had a median intake of 1 (1-3) times in a 7 day period (Table 4.4).

Table 4.4 Consumption of carbohydrate products

Food item	N=90 Interventiongroup Median(range)	N=90 Controlgroup Median(range)	N= 180 Total Median(range)	Wilcoxon Test p value
Ugali sifted	2.5(1-7)	2.5(1-7)	2.5(1-7)	0.415
White bread	1(1-7)	1(1-3)	1(1-7)	0.127
Whole meal bread	2(1-7)	3(1-7)	3(1-7)	0.242
Brown rice	2(1-2)	1.5(1-3)	2(1-3)	0.794
White rice	2(1-7)	1(1-3)	2(1-7)	0.035*
Pasta	5(1-7)	1(1-1)	1(1-7)	0.407
Brown porridge	2(1-7)	3(1-7)	2(1-7)	0.353
Whole meal *chapati*	1(1-5)	1(1-5)	1(1-5)	0.816
Sifted flour *chapati*	1(1-3)	1(1-2)	1(1-3)	0.914
Githeri	2(1-7)	2(1-7)	2(1-7)	0.117
Mandazi	1(1-7)	1(1-7)	1(1-7)	0.681
Roast maize	1(1-7)	2(1-7)	2(1-7)	0.274
Potato Chips	1(1-7)	2.5(1-7)	2(1-7)	0.297
Taro roots	1(1-3)	1(1-7)	1(1-7)	0.895
Sweet potatoes	1(1-7)	1(1-4)	1(1-7)	0.198
Green bananas	1(1-2)	1(1-3)	1(1-3)	0.118
Whole meal *ugali*	3(1-7)	2(1-7)	3(1-7)	0.082
Mukimo	2(1-2)	2(1-7)	2(1-7)	0.056

Ugali- *stiff porridge**: Chapati-** Thin unleavened wheat flour product**: Githeri-** mixture of maize and beans**: Mukimo-** mashed mixture of maize, beans/peas, green leaves and potato**: Brown porridge-** porridge from either sorghum or millet or a mixture of both*

4.3.3.2 Consumption of milk and milk products

Whole milk was the most frequently consumed food item with median frequency of 4(1-7) times in a 7 day period. There was no significant difference in consumption of the food item between the two study groups (Wilcoxon test; p=0.50). Similarly, the next high frequently consumed food item was low fat milk that was consumed 3(1-7) times. There was no significant difference in consumption of both whole milk and low fat milk between the study groups (Table 4.5).

The least frequently consumed food item was fermented milk and whole fat yoghurt that both registered intake of 1(1-3) time in 7 days period. There was no significant difference in consumption of fermented milk and whole fat yoghurt between the study groups (Wilcoxon test; p=0.272 and p=0.147) respectively.

Table 4.5 Consumption of milk and milk products

Food item	N=90 Intervention group Median(range)	N=90 Controlgroup Median(range)	N= 180 Total Median(range)	Wilcoxon Test P value
Whole milk	4(1-7)	3(1-7)	4(1-7)	0.502
Low fat milk	3(1-7)	2(1-3)	3(1-7)	0.297
Fermented milk	1(1-3)	1(1-1)	1(1-3)	0.272
Yoghurt (whole fat)	1(1-3)	1(1-1)	1(1-3)	0.147
Margarine	1.5(1-7)	3(1-7)	2(1-7)	0.239
Butter	1(1-1)	2(2-2)	1.5(1-2)	0.194

4.3.3.3 Consumption of meat, poultry, fish and their products

Beef was the most frequently consumed source of animal protein with a median of 2(1-7) times. There was no significant difference in the consumption of beef between the two study groups (Wilcoxon test; p=0.811). On the contrary, consumption of pork was significantly higher (Wilcoxon test; p=0.047) with the intervention group frequency 1 (1-3) time in a 7 day period, compared to the control group frequency of 1(1-1) time within the same period.

Similarly, processed meat registered significantly higher consumption (Wilcoxon test; P=0.026) by the control group with median of 2 (1-3) times against the median intake by intervention group that was 1 (1-1) time in a 7 day period.

Table 4.6 Consumption of meat, poultry and fish

Food item	N=90 Intervention group Median(range)	N=90 Control group Median(range)	N= 180 Total Median(range)	Wilcoxon Test P value
Beef	2(1-7)	1.5(1-7)	2(1-7)	0.811
Mutton	1(1-1)	1(1-7)	1(1-7)	0.515
Chicken	1(1-4)	1(1-1)	1(1-4)	0.117
Fish	1(1-2)	1(1-3)	1(1-3)	0.893
Pork	1(1-3)	1(1-1)	1(1-3)	0.047*
Offal	1(1-2)	1(1-2)	1(1-2)	1.003
Processed meat	1(1-1)	2(1-3)	1(1-3)	0.026*
Eggs	1(1-5)	1(1-7)	1(1-7)	0.769

4.3.3.4 Consumption of legumes, pulses and nuts

Among the legumes, dry bean was the most frequently consumed protein with median intake of 3(1-7) times in 7 days period. Dry peas, green beans and black peas were the highest consumed pulses with median intake of 2(1-7) times in a 7 day period. There was no significant difference in consumption of the highly consumed legumes and pulses above between the study groups (Table 4.7).

Lentil consumption was significantly higher (Wilcoxon test; p=0.018) in the control group that had frequency of consumption of 3(3-3) times versus that of the intervention group of 1(1-3) times in 7 days. Similarly, control group registered significantly higher consumption of ground compared to the intervention group (Wilcoxon test; p=0.014).

Table 4.7 Consumption of legumes, pulses and nuts

Food item	**N=90 Intervention group Median(range)**	**N=90 Control group Median(range)**	**N= 180 Total Median(range)**	**Wilcoxon Test P value**
Dry peas	2(1-7)	2(1-4)	2(1-7)	0.941
Dry beans	3(1-7)	3(1-7)	3(1-7)	0.466
Lentils (*kamande*)	1(1-3)	3(3-3)	1(1-3)	0.018*
Green grams	1(1-4)	1(1-7)	1(1-7)	0.352
Green beans (*Mbaazi*)	2(1-2)	2(1-7)	2(1-7)	0.174
Black peas (*Njahi*)	2(1-7)	2(1-7)	2(1-7)	0.567
Ground nuts	1(1-3)	2(1-7)	1.5(1-7)	0.014*

Refers to significance; p <0.05

4.3.3.5 Consumption of fruits and vegetables

Tomato was the most frequently consumed vegetable with a frequency intake of 7(2-7) times. The next frequently consumed food items were carrots and kales were consumed 4(1-7) and 5(1-7) times respectively in a 7 day period. There was no significant difference in consumption of these food items between the study groups (Table 4.8).

The least consumed vegetable was French-bean that had frequency intake of 1.5(1-7) times. Bananas and citrus fruits were the most frequently consumed in the fruit category at 2(1-7) times each in 7 days. There was no significant difference in consumption of French beans, bananas and citrus fruits between the study groups (Table 4.7). The least consumed fruits were passion and avocado that both had frequency intake of 1(1-7) time in a week. There was no significant difference in passion and avocado consumption between the study groups (Wilcoxon test; p=0.685 and p= 0.401 respectively).

Table 4.8 Consumption of fruits and vegetables

Food item	N=90 Intervention group Median(range)	N=90 Control group Median(range)	N= 180 Total Median(range)	Wilcoxon test Pvalue
Kales	4 (1- 7)	4(1-7)	4(1-7)	0.483
Cabbage	2(1- 7)	2(1-7)	2(1-7)	0.685
Tomatoes	7(3- 7)	7(2-7)	7(2-7)	0.806
Carrots	5(1- 7)	5(1-7)	5(1-7)	0.821
Spinach	3(1-7)	2.5(1-7)	3(1-7)	0.588
Cucumber	5(1-7)	4(1-7)	5(1-7)	0.693
Cow pea leaves	2(1-7)	2(1-4)	2(1-7)	0.366
French beans	1.5(1-3)	1.5(1-7)	1.5(1-7)	0.871
African leafy vegetables	2.5(1-7)	4(1-7)	2.5(1-7)	1.004
Apples	1(1-7)	1.5(1-5)	1(1-7)	0.606
Bananas	2(1-7)	2(1-7)	2(1-7)	0.224
Citrus fruits	2(1-7)	2(1-7)	2(1-7)	0.228
Melon	1(1-7)	2(1-7)	1(1-7)	0.139
Passion	1(1-7)	1(1-7)	1(1-7)	0.685
Pawpaw	1(1-7)	2(1-5)	1(1-7)	0.126
Pineapple	1(1-3)	1.5(1-7)	1(1-7)	0.293
Avocado	1(1-7)	1(1-7)	1(1-7)	0.401

4.3.3.6 Consumption of sugars and sugar products

Candy was the most frequently consumed source of sugar with a median of 2(1-7) times. The control group had significantly higher intake of candy compared to the intervention group (Wilcoxon test; p= 0.037). There was no significant difference in the consumption of cakes and soda between the study groups (Table 4.9)

Table 4.9 Consumption of sugar and sugar products

Food item	N=90 Intervention group Median(range)	N=90 Control group Median(range)	N= 180 Total Median(range)	Wilcoxon Test P value
Cakes	1(1-1)	1(1-3)	1(1-3)	0.074
Sweets (Candy)	2(1-7)	3(2-4)	2(1-7)	0.037*
Soda	1(1-1)	1(1-1)	1(1-1)	1.004

****Refers to significancep(p<0.05)***

4.3.4 Nutrient intake from 24 hour recall

The adequacy in nutrient intake was based on WHO (2006) recommendation of ≥80% of the RDA.

4.3.4.1 Adequacy of dietary nutrient intake

All the study participants (100%) consumed adequate vitamin B_6, phosphorus and magnesium. The least consumed nutrients were fats (37%).There was no significant difference between the study groups in consumption of these nutrients(Table 4.10).On the contrary, the control group consumed significantly higher quantities of carbohydrates than the intervention group (t-test; p=0.038).

Table 4.10 Adequacy of dietary nutrient intake

Nutrient	N=30 Intervention		N=34 Control		N =64 Total		t-test P-value
	RDA		RDA		RDA		
	<80% n(%)	≥80% n(%)	<80% n(%)	≥80% n(%)	<80% n(%)	≥80% n(%)	
Energy	12(40)	18(60)	6(17)	28(83)	18(28)	46(72)	0.565
Protein	6(20)	24(80)	2(6)	32(94)	8(13)	56(87)	0.968
Fats	20(67)	10(33)	20(59)	14(41)	40(63)	24(37)	0.109
Carbohydrates	10(33)	20(67)	4(12)	30(88)	14(22)	50(78)	0.038*
Dietary Fiber	2(7)	28(93)	2(17)	32(83)	4(6)	60(94)	0.058
PUFA	8(27)	22(73)	12(35)	22(65)	20(31)	44(69)	0.068
Vit A	0(0)	30(100)	8(24)	26(76)	8(13)	56(87)	0.234
Vit E	2(7)	28(93)	4(12)	30(88)	6(9)	58(91)	0.174
Vit B_1	6(20)	24(80)	4(12)	30(88)	10(16)	54(84)	0.125
Vit B_2	2(7)	28(93)	6(17)	28(83)	8(13)	56(87)	0.343
Vit B_6	0(0)	30(100)	0(0)	34(100)	0(0)	64(100)	0.241
Folate	10(33)	20(67)	10(29)	24(71)	20(31)	44(69)	0.209
Vit C	4(13)	26(87)	8(24)	26(76)	12(19)	52(81)	0.326
Sodium	14(47)	16(53)	24(71)	10(29)	38(59)	26(41)	0.109
Potassium	6(20)	24(80)	8(24)	26(76)	14(22)	50(78)	0.249
Calcium	14(47)	16(53)	22(65)	12(35)	36(56)	28(44)	0.167
Magnesium	0(0)	30(100)	0(0)	34(100)	0(0)	64(100)	0.442
Phosphorus	0(0)	30(100)	0(0)	34(100)	0(0)	64(100)	0.47
Zinc	2(7)	28(93)	0(0)	34(100)	2(3)	62(97)	0.072
Iron	10(33)	20(67)	6(17)	28(83)	16(25)	48(75)	0.929

Refers to significance (p<0.05)
≥80% is the WHO recommendation of daily nutrient adequacy based on the RDA

4.3.4.2 Magnitude of nutrient adequacy based on RDA

There was overconsumption (>100% of RDA) of most nutrients that were considered in the 24 hour recall (Table 4.11). Phosphorus, Magnesium, dietary fibre, vitamins A, B_6 and C were the most over-consumed with intake above 200% of the RDA. There was no significant difference in the intake of these foods between the study groups. On the contrary, sodium intake was low, represented with intake of 70.01±37.4 percent of the RDA. The control group had higher intake of sodium (76.00±42.4) compared to the

intervention group (63.34±29.9).The difference between the study groups in sodium intake was not significant (t-test p=0.178). Similarly fat intake was below the RDA on the whole with 88.74±72.8 percent. There was higher intake of fat in the intervention group (106.68±96.47) compared to control group (74.03±44.66). The difference in intake of fat between the study groups was not significant (t-test p=0.113).

Table 4.11 Magnitude of nutrient adequacy based on RDA

Nutrient type	**Min (%)**	**Max (%)**	**N= 64 Total Mean (%)**	**N=30 Intervention Mean (%)**	**N=34 Control Mean (%)**	**t-test Pvalue**
Energy	55.20	231.00	120.56±54.6	120.75±57.6	119.96±53.7	0.957
Protein	36.90	438.40	141.03±84.6	150.50±109.3	132.27±60.2	0.422
Fats	34.70	322.20	88.74±72.8	106.68±96.4	74.03±44.6	0.113
Carbohydrates	53.20	276.60	130.90±64.6	120.55±49.3	138.87±75.2	0.257
Dietary Fiber	60.30	436.10	206.78±115.0	185.40±85.3	221.71±134.0	0.204
Pufa	35.60	1112.50	181.50±222.4	243.214±323.4	132.31±58.6	0.090
Vit.A	33.30	721.40	244.38±156.1	288.73±192.9	208.51±113.2	0.063
Vit.E	31.00	1000.00	193.35±182.8	226.68±258.2	165.50±86.4	0.247
Vit.B1	52.10	719.20	220.28±177.4	186.50±155.0	246.83±193.8	0.182
Vitb.2	55.70	480.40	165.05±100.2	142.86±84.4	180.35±110.1	0.138
Vit.B6	87.90	523.00	250.42±128.7	232.09±118.7	262.37±137.4	0.360
Folate	56.50	457.10	129.62±84.3	117.84±42.2	137.11±107.0	0.342
Vit.C	21.30	871.70	225.08±191.1	195.81±133.2	244.33±227.7	0.304
Sodium	12.40	162.90	70.01±37.4	63.34±29.9	76.00±42.4	0.178
Potassium	58.00	359.50	140.65±72.0	124.73±51.1	151.22±84.0	0.135
Calcium	28.50	265.40	91.07±49.9	94.63±65.3	85.18±29.4	0.490
Magnesium	104.30	517.50	239.97±125.2	203.50±83.6	266.22±146.4	0.140
Phosphorus	81.90	621.80	259.64±139.88	247.39±149.1	265.12±133.5	0.631
Iron	57.60	349.90	128.40±64.54	136.54±82.2	121.14±47.3	0.392
Zinc	71.00	501.20	224.23±121.44	201.85±96.6	240.31±138.2	0.207

Refers to significance (p<0.05)

4.3.5 Physical activity

4.3.5.1 Types and duration on physical activity

The highest percentage of participants (56.7%) was involved in walking /riding. The control group were significantly more involved in the walking/riding for periods longer than 30 minutes frequently in the week (χ^2test; p=0.001). Participation in vigorous intensity job was the next form of exercise with 55.5% of the participants. There was higher participation in vigorous intensity job for more than 30 minutes by the intervention group (95.8%) compared to the control group (90.5%). The difference in duration of participation in vigorous intensity job between the study groups was not significant (χ^2 test; p=0.309). Vigorous intensity sport was represented by 13.3% of the participants. There was significantly higher participation by the control group in vigorous intensity sport than the intervention group (intervention group= 6.7% versus control group =20%; (χ^2 test; p=0.009).

Table 4.12 Physical activity types and time spent

Variable	Category	N=90 Intervention n (%)	N=90 Controls n (%)	N=180 Total n (%)	χ^2 value	P value
Vigorous intensity job	Yes	48 (53.3)	42 (46.7)	90 (55.5)	0.8000	0.371
Number of days/ week	>=3 days	40 (83.3)	32 (76.2)	72 (40)	0.7140	0.398
Number of minutes	>=30 minutes	46 (95.8)	38 (90.5)	84 (46.7)	1.0330	0.309
Moderate intensity job	Yes	36 (40)	46 (51.1)	82 (45.6)	2.2400	0.134
Number of days/ week	>=3 days	24 (66.7)	20 (43.5)	44 (24.4)	4.3670	0.374
Number of minutes	>=30 minutes	32 (94.1)	40 (87)	72 (40)	1.1140	0.291
Mode of travel Involving walking/riding	Yes	46 (51.1)	56 (62.2)	102 (56.7)	2.2620	0.133
Number of days/ week	>=3 days	36 (78.3)	42 (77.8)	78 (43.3)	0.0030	0.954
Number of minutes	>=30 minutes	20 (43.5)	42 (75)	62 (34.4)	10.5270	0.001*
Vigorous intensity sport	Yes	6 (6.7)	18 (20)	24 (13.3)	6.9230	0.009*
Number of days/ week	>=3 days	4 (66.7)	8 (44.4)	12 (6.7)	0.0690	0.793
Number of minutes	>=30 minutes	6 (100)	12 (66.7)	18 (10)	0.1810	0.671
Moderate intensity sport	Yes	2 (2.2)	6 (6.7)	8 (4.4)	2.0930	0.148
Number of days/ week	>=3 days	0 (0)	6 (100)	6 (3.3)	---	----
Number of minutes/day	>=30 minutes	0 (0)	2 (33.3)	2 (1.1)	---	----
Sedentary behaviour	Yes	6 (6.7)	4 (4.5)	10 (5.6)	0.3780	0.539
Time spent sitting or reclining on a typical day	>=30 minutes	6 (100)	4 (100)	10 (5.6)	3.0130	0.782

Refers to significance (p<0.05)
---- was not compared due to a zero (0) in one of the cells

4.3.5.2 Physical activity levels

The physical activity levels were considered as number of minutes and the metabolic equivalents per type of activity per week. Activity levels have been categorized as per American guidelines for physical activity. Most participants (65.9%) realized high levels of physical activity while 24.2% were of low physical activity levels. There was no significant difference in physical activity levels between the study groups (Table 4.13).

Table 4.13 Physical activity levels

Variable	**N=90 Intervention n (%)**	**N=90 Control n (%)**	**N= 180 Total n (%)**	**χ^2-test p value**
Physical activity levels				
Low: <500 MET-min/wk	20 (22.2)	22 (24.4)	42 (24.2)	0.518
Medium: 500-1000 MET-min/wk	6 (6.7)	12 (13.3	18 (9.9)	
High: >1000 MET-min/week	64 (71.1)	56 (62.2)	120 (65.9)	

wk= week

4.3.6 Type of hypoglycaemic drugs used

Metformin was the most commonly used drug with the highest percentage of 58.89 among the participants. Insulin was the next popular drug used by18.89% of the participants. There was no significant difference in hypoglycaemic drug use between the study groups (χ^2 =4.8340, p=0.184) as indicated in Table 4.14.

Table 4.14Type of hypoglycaemic drugs used

Variable	**N=90 Intervention n (%)**	**N=90 Control n (%)**	**N =180 Total n (%)**	**χ^2value**	**p-value (2 sided)**
Drugtype					
Glucomet	8 (9.5)	12 (14)	20 (11.11)	4.8340	0.184
Glucophage	8 (9.5)	2 (2.3)	10 (5.55)		
Metformin	50 (59.5)	56 (65.1)	106 (58.89)		
Mixtard Insulin	18 (21.4)	16 (18.6)	34(18.89)		

4.3.7 Anthropometry

Body mass index (BMI), waist circumference (WC) and waist to hip ratio (W/H) were the anthropometric measurements considered in this study.

4.3.7.1 Anthropometric measurements

The participants in the study had a mean BMI of 25.88±4.99 with mean W/H ratio of 0.70±0.06 and mean waist circumference of 90.28±12.32 cm. There was no significant difference in BMI, W/H ratio and waist circumference between the study groups (Table 4.15)

Table 4.15 Anthropometric measurements

Variable	N=90 Intervention Mean(sd)	N=90 Control Mean(sd)	N=180 Total Mean(sd)	t value	p-value (2 sided)
BMI	26.00 ±1.04	26.0 ±1.04	25.88±4.99	0.03	0.978
W/H	0.65 ±0.02	0.66 ±0.01	0.70±0.06	-0.60	0.549
Waist Circumference	90.66 ±2.87	90.75 ±2.26	90.28±12.32	-0.05	0.958

4.3.7.2 Anthropometry status

In the BMI category, more than half of the participants were overweight and obese (Table 4.14).There were higher underweight (7.7%) in the intervention group compared to the control group (4.5%). The difference in underweight between the study groups was not significant (χ^2 test; p=0.844). There was no difference in BMI status distribution between the study groups (Table 4.16)

The waist circumference status indicated 49.4% of the participants were more likely to be at severe risk of metabolic complications. The likelihood for metabolic complications was higher in the intervention group (51.3%) compared to the control group (47.7%). There was no significant difference in severe risk of metabolic complications between the

study groups ((χ^2 test; p=0.075). Similarly, W/H ratio status had more participants in the intervention group (7.7%) at risk of mortality compared to the control group (4.5%). The difference between the study groups was not significant (χ^2 test; p=0.507).

Table 4.16 Anthropometry status

Variables	N=78 Intervention n (%)	N=88 Control n (%)	N= 166 Total n (%)	Chi-square	P value
BMI					
Underweight (< 18.50)	6 (7.7)	4 (4.5)	10 (6)	1.399	0.844
Normal (18.51-24.99)	30 (38.5)	40 (45.5)	70 (42.2)		
Overweight (25.00-29.99)	20 (25.6)	28 (31.8)	48 (28.9)		
Obese (>30.0-40.00)	18 (23.1)	14 (15.9)	32 (19.3)		
Morbid obese (>40.00)	2 (2.6)	2 (2.3)	4 (2.4)		
WHR					
Normal (<10)	68 (87.2)	84 (95.5)	152 (91.6)	0.440	0.507
Risk to mortality (≥10)	6 (7.7)	4 (4.5)	10 (6.0)		
Waist circumference (cm)					
Normal(M <94; F<80)	34 (43.6)	30 (34.1)	64 (38.6)	5.183	0.075
Metabolic comp. risk {M-(94-101.9); F-(80-87.9}	2 (2.6)	16 (18.2)	18 (10.8)		
Severe risk(M-≥102; F-≥88)	40 (51.3)	42 (47.7)	82 (49.4)		

4.3.8 Biochemical parameters

Biochemical tests were carried out for selected indicators that included; serum chromium, fasting blood sugar, HbAlc, total cholesterol, HDL, LDL, triglycerides and ferritin.

4.3.8.1 Biochemical status for selected indicators

The study variables were categorized to determine status of the sample population. Most participants (88%) were deficient in serum chromium. The intervention group had higher deficiencies (89.7%) compared to the control group (86.4%). There was no significant difference in chromium status between the study groups (χ^2 test; p=0.401). Most participants had normal ferritin levels (72.3%) with higher proportion in the control

group (81.8%) compared to the intervention group (61.5%). The difference in ferritin levels between the study groups was not significant (χ^2 test; p=0.151).

The highest proportion (78.3%) had high fasting blood sugar of which the control group had a higher proportion (84.15%) compared to the intervention group (71.8%). The difference in fasting blood sugar between the study groups was not significant (Table 4.17). Over half of the participants had elevated HbAlc (>9mmols) and 42.2% had normal HbAlc. There was no significant difference in HbAlc status between the study groups (χ^2 test; p=0.463).

The lipid profile was represented by; 38.6% with high total cholesterol, 13.3% with high triglycerides, 49.4% with low HDL while 12.0% had high LDL. There was no significant difference in lipid profile values between the study groups (Table 4.17).

Table 4.17 Comparison of baseline biochemical status by study groups

Variables	N=78 Intervention n (%)	N=88 Control n (%)	N= 166 Total n (%)	χ^2 value	P value
Serum chromium(ng/ml)					
Normal (0.3-0.9)	6 (7.7)	12 (13.6)	18 (10.8)	1.829	0.401
Deficient (<0.3)	70 (89.7)	76 (86.40)	146 (88.0)		
Excess (>0.9)	2 (2.6)		2 (1.2)		
FBS (mmol/l)					
Normal (4.44-6.38)	20 (25.6)	12 (13.6)	32 (19.3)	1.952	0.377
Low (<4.44)	2 (2.6)	2 (2.3)	4 (2.4)		
High (>6.38)	56 (71.8)	74 (84.1)	130 (78.3)		
HbAlc (%)					
Normal (<8.0)	28 (35.9)	42 (47.7)	70 (42.2)	2.568	0.463
Good control (8.0-9.0)	12 (15.4)	18 (20.5)	30 (18.1)		
Fair control (>9.0-10.0)	10 (12.8)	6 (6.8)	16 (9.6)		
Poor control (>10.0)	28 (35.9)	22 (25.0)	50 (30.1)		
Total cholesterol (mmol/l)					
Normal range (0-5.2)	48 (61.5)	54 (61.4)	102(61.4)	0.001	0.987
High (>5.2)	30 (38.5)	34 (38.6)	64 (38.6)		
Triglycerides					
Normal range (0.45-1.81)	68 (87.2)	76 (86.4)	144 (86.7)	0.012	0.913
High (>1.81)	10 (12.8)	12 (13.6)	22 (13.3)		
HDL:Desirable (>1.42)	48 (61.5)	36 (40.9)	84 (50.6)	3.520	0.061
Low (≤1.42)	30 (38.5)	52 (59.1)	82 (49.4)		
LDL: Normal range (0-4.1)	64 (82.1)	82 (93.2)	146 (88.0)	2.417	0.120
High (>4.1)	14 (17.9)	6 (6.8)	20 (12.0)		
Ferritin(ng/ml)					
Deficient (M-<20; Pr.F-<6;Pt.F-<8)	2 (2.6)	2 (2.3)	4 (2.4)	3.781	0.151
Normal(M-20-400; Pr.F-6-180; Pt.F-8-350)	48 (61.5)	72 (81.8)	120 (72.3)		
High(M->400; Pr.F->180; Pt.F->350)	26 (33.3)	14 (15.9)	40 (24.1)		

Key: *M-males; F-females; Pr.- premenopausal; Pt.-postmenopausal*

Mean serum levels for the biochemical indicators were also considered and compared at baseline between the study groups. The findings were as follows; High fasting blood sugar of 9.84±3.92 was realized on the whole. The intervention group had a higher mean of 10.1±1.39 compared to that of the control group (9.88±1.01).There was no significant difference in fasting blood sugar between the study groups (t-test; p=0.803).On the whole levels of HbAlc were 8.92±2.96. The control group had lower mean of 8.38±0.76 for

HbAlc which was not significantly different from that of intervention group (9.45±1.43; t-test; p=0.109).

For the lipid profile, total cholesterol mean was at 4.87±0.98 on the whole that was within the normal range. There was no significant difference in cholesterol level between the study groups (intervention= 4.85±0.28; control 4.91±0.33; t-test; p=0.789).The mean for HDL was 3.28±2.30. There was no difference in HDL levels between the study groups (t-test; p=0.727). The LDL levels were normal on the whole with a mean of 2.27±1.50.The intervention group had higher LDL levels (2.50±0.51) compared to the control group (2.10±0.44). The difference in LDL levels between the study groups were not significant (t-test; p=0.234). The triglyceride was 1.21±0.81 on the whole and was not significantly different between the study groups (Table 4.18).

Serum chromium was below the desired levels with a mean of 0.23±0.77 that remained low with no difference across the study groups (Table 4.18). Serum ferritin level was adequate (203.88±150.46) on the whole and similarly in each study group. There was no significant difference in ferritin levels between the study groups (Intervention =232.60±55.45; control =185.70±37.95; t-test; p=0.163).

Table 4.18 Baseline biochemical outcomes

Variable	N=78 Intervention Means(sd)	N=88 Control Means(sd)	N=166 Total Mean (sd)	p-value
Fasting blood sugar (mmol/l)	10.1 ±1.39	9.88 ±1.01	9.84±3.92	0.803
HbAlc (%)	9.45 ±1.43	8.38 ±0.76	8.92±2.96	0.109
Total Cholesterol (mmol/l)	4.85 ±0.28	4.91 ±0.33	4.87±0.98	0.789
HDL (mmol/l)	3.18 ±0.82	3.36 ±0.64	3.28±2.30	0.727
LDL (mmol/l)	2.50 ±0.51	2.10 ±0.44	2.27±1.50	0.234
Triglycerides (mmol/l)	1.22 ±0.28	1.21 ±0.23	1.21±0.81	0.981
Serum chromium (ng/ml)	0.23 ±0.03	0.23 ±0.02	0.23±0.77	0.690
Serum ferritin (ng/ml)	232.60 ±55.45	185.70 ±37.95	203.88±150.46	0.163

4.4 MONTHLY FOLLOW UPS

The study participants were followed up on adherence to chromium or placebo and the hypoglycaemic drug intake depending on the study group for the four months.

4.4.1 Adherence to chromium/placebo intake

Both study groups had high adherence to chromium intake above 85% throughout the months 1, 2, 3 and 4 in the study (Figure 4.2). The highest adherence by both groups was in the second month into the study (Intervention =94.7% and control =89.7%). Adherence was least in month 1 for the control group (85.4%) and month 3 for the intervention group (86.1%). There was no significant difference between the study groups in adherence to chromium and placebo intake in the 4 months of the study (Figure 4.2).

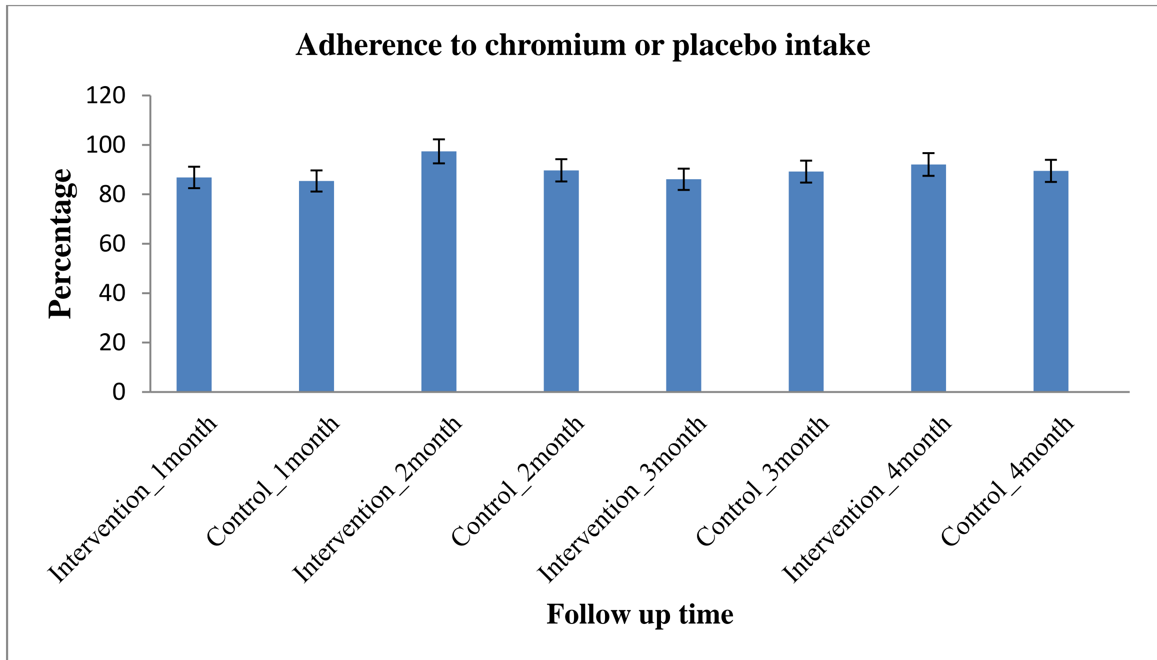

Figure 4.2: Adherence to chromium or placebo intake

4.4.2 Hypoglycaemic drug intake

Different hypoglycaemic drugs were used as part of routine management of blood sugar by Type 2 diabetics. These drugs were monitored on a monthly basis in terms of type of drug and dosage. The types of medication included Glucomet (500/850mg), Glucophage (500mg), Metformin (500/850/1000mg) and insulin. The most frequently used drug was Metformin (500mg), insulin and Glucomet (500mg).There was no significant difference in drug type and dosage between the study groups in months 1,2, 3 and 4 in the study (t-test; Month1; p=0.162; Month2, p=0.772;Month3 p=0.620and Month4 p=0.589).

Table 4.19 Monthly hypoglycaemic drug intake

Drug type	**Month 1**			**Month 2**			**Month 3**			**Month 4**		
	Group 1 (%)	**Group 2 (%)**	**t-test Pvalue**	**Group 1 (%)**	**Group 2 (%)**	**t-test Pvalue**	**Group 1 (%)**	**Group 2 (%)**	**t-test Pvalue**	**Group 1(%)**	**Group 2 (%)**	**t-test Pvalue**
Glucomet												
500mg	21.1	24.4	0.162	15.8	19.4	0.772	14.7	28.6	0.620	18.2	25.7	0.589
850mg	0	0		5.3	5.6		5.9	5.7		9.1	5.7	
Glucophage												
500mg	13.2	2.4		5.3	5.6		8.8	5.7		0	0	
Metformin												
1000mg	10.5	2.4		7.9	8.3		5.9	5.7		15.2	5.7	
850mg	7.9	4.9		2.6	8.3		2.9	8.6		3	8.6	
500mg	15.8	36.6		31.6	36.1		23.5	22.9		24.2	31.4	
Insulin	23.7	17.1		26.3	16.7		32.4	22.9		30.3	22.9	

4.5 FINDINGS AT END OF INTERVENTION

At the completion of the study, participants were assessed on; anthropometry and biochemical characteristics (fasting blood sugar, HbAlc, lipid profile, chromium levels and ferritin levels).

4.5.1 Effects of chromium supplementation on selected outcome indicators

4.5.1.1 BMI, W/H ratio and WC by study groups

The intervention group recorded a decrease in BMI with time compared to the control group that recorded an increase in BMI (Figure 4.3). The decrease in BMI with chromium supplementation in the intervention group was not significantly different to BMI in the control group (Figure 4.3).

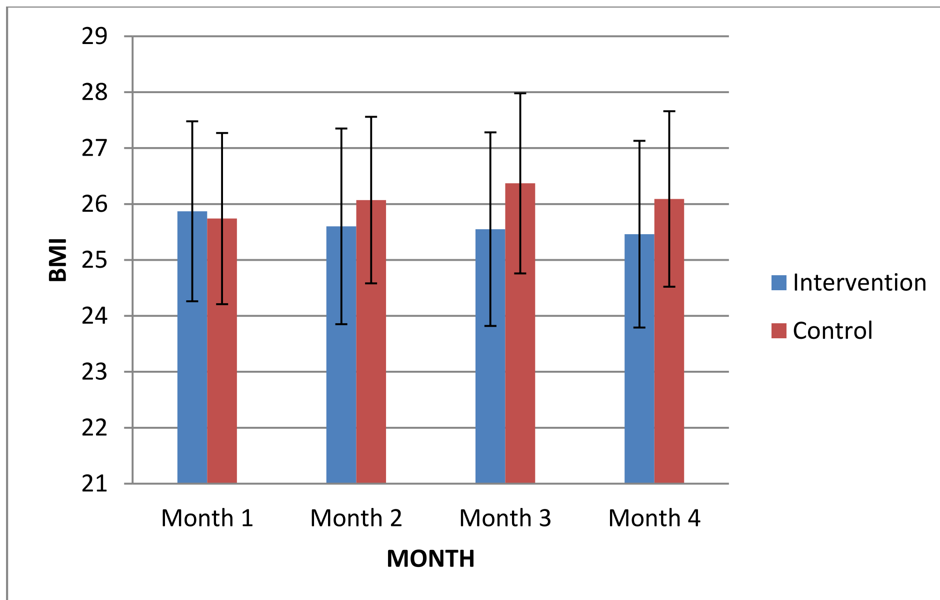

Figure 4.3 Effect of chromium supplementation on BMI

The study findings indicated an increase in W/H ratio in the control group particularly at end of month 3 into the study (Figure 4.4). The increase was not significantly different compared to the intervention group (t-test; p= 0.763).

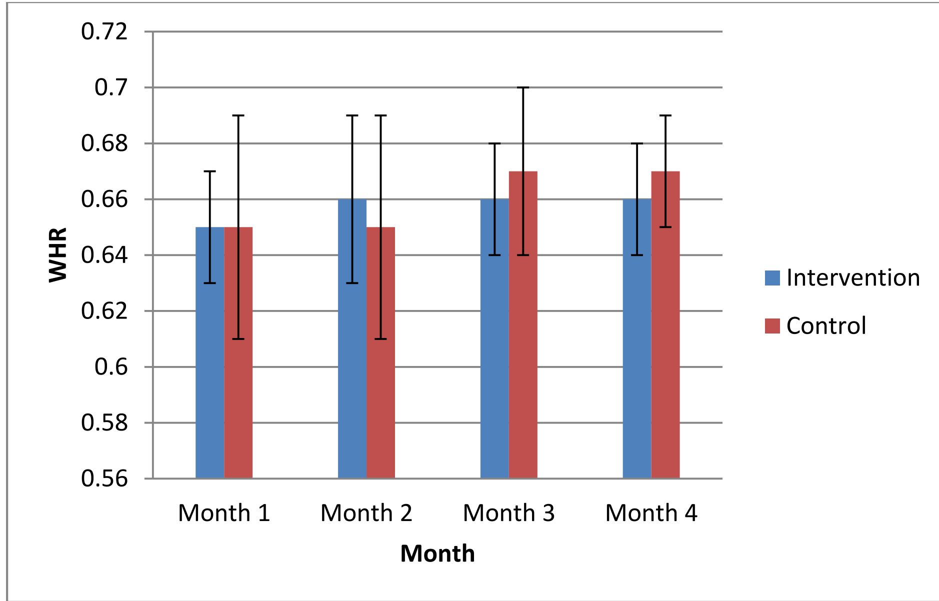

Figure 4.4 Effect of chromium supplementation on waist: hip ratio (W/H)

The intervention group recorded a decrease in waist circumference (WC) with time in the study while the control group recorded an increase in WC with time in the study (Figure 4.5). The difference in WC between the study groups was not significant (Figure 4.5)

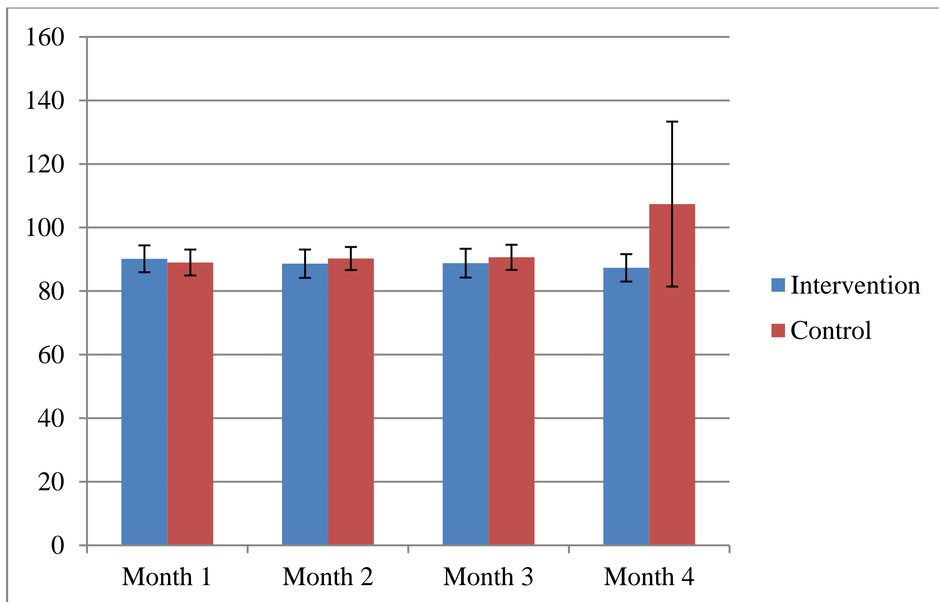

Figure 4.5 Effect of chromium supplementation on waist circumference (WC)

Regression analysis was run to determine associations between serum chromium levels and anthropometric status (BMI, WC and W/H).There was a positive association between BMI and serum chromium levels that was not significant (p=0.774). In contrast, there was negative association between W/H ratio and serum chromium. The association was not significant (p= 0.349). Similarly, a negative association was indicated between WC and serum chromium levels which was not significant (p= 0.807)

Table 4.20 Relationship between serum chromium levels and BMI, W/H and WC

Variable	Regression estimates	Standard error	p-value
BMI	0.02661	0.09266	0.774
Waist to Hip ratio	-0.00131	0.00139	0.349
Waist Circumference	-0.05630	0.22943	0.807

4.5.1.2 Effect of chromium supplementation on Blood sugar

This study investigated effect of chromium supplementation on fasting blood sugar and HbAlc. The control group had higher fasting blood sugar (11.58±1.02) compared to the intervention group (10.30±0.99). There was no difference in fasting blood sugar levels between the study groups (t-test; p= 0.075).

Similarly, the control group had higher HbAlc levels (9.24±0.53) compared to the intervention group (7.90±0.5). There was a significant difference in HbAlc levels between the study groups (t-test; p= 0.003).

Table 4.21 Effect of chromium supplementation on blood sugar

Variable	N=72 Intervention Mean (95% CI)	N=80 Control Mean (95% CI)	t-test P-value
Fasting blood sugar (mmols/l)	10.30±0.99	11.58±1.02	0.075
HbAlc (%)	7.90±0.5	9.24±0.53	0.003*

****Refers to significance (p<0.05)***

4.5.1.3 Effect of chromium supplementation on lipid levels

The total cholesterol levels were not significantly different between the study groups (intervention =4.79±0.25; control =4.76±0.29; t-test; p=0.896).Similarly, the control group had higher levels of LDL (2.86±0.25) compared to that of the intervention group (2.67±0.28). The difference between LDL levels was not significant (t-test; p=0.323). The triglyceride levels were higher in the control group (1.38±0.28) than the intervention group (1.35±0.16). The difference between the study groups was not significant (t-test; p=0.819).On the contrary, the control group was more likely to have significantly higher HDL levels (1.76±0.13) compared to the intervention group (1.53±0.1; t-test; p= 0.005). The liver test (ALP) showed normal functions in both study groups. There was no significant difference in ALP levels between the study groups.

Table 4.22 Effect of chromium supplementation on blood lipids

Variable	**N=72** **Intervention** **Mean (95% CI)**	**N=80** **Control** **Mean (95% CI)**	**t-test** **P-value**
Total Cholesterol(mmol/l)	4.79±0.25	4.76±0.29	0.896
HDL(mmol/l)	1.53±0.1	1.76±0.13	0.005*
LDL(mmol/l)	2.67±0.28	2.86±0.25	0.323
Triglycerides(mmol/l)	1.35±0.16	1.38±0.28	0.819
ALP (U/L)	234±0.18	236±0.14	0.653

Refers to significance (p<0.05)

4.5.1.4 Effect of chromium supplementation on serum levels of chromium and ferritin

The intervention group had higher serum chromium levels (0.31±0.07) compared to the control group (0.27±0.03). The difference in serum chromium levels was not significant (0.241). Similarly, there was no significant difference in ferritin levels between the intervention and the control study groups (Table 4.24).

Table 4.23 Effect of chromium supplementation on serum levels of chromium and ferritin

Variable	**N=72** **Intervention** **Mean (95% CI)**	**N=80** **Control** **Mean (95% CI)**	**t-test** **P-value**
Serum chromium (ng/ml)	0.31±0.07	0.27±0.03	0.241
Serum ferritin (ng/ml)	173.32±37.39	176.18±30.27	0.905

4.5.1.5 Magnitude of change on selected biochemical indicators

The differences in selected biochemical indicators at baseline and month 4 were computed per individual in the study (baseline-month 4) and mean difference computed to determine the magnitude of change between the study groups.

4.5.1.5.1 Magnitude of change in blood sugars

There was a higher increase in fasting blood sugar at end of the study in the control group (-1.92±1.12) compared to the intervention group (-0.52±1.29). There was no significant difference in magnitude of change in fasting blood sugar (t-test; p=0.103). On the contrary, there was a significant decrease in HbAlc in the intervention group compared to an increase in the control group at end of study (Intervention= 1.44±1.03; Control= -0.79±0.84; t-test; p= 0.001).

Table 4.24 Magnitude of change (Baseline versus month 4 mean) on blood sugars

Variable	N=72 Intervention Mean (95% CI)	N=80 Control Mean (95% CI)	t-test P-value
Fast blood sugar (mmol/l)	-0.52±1.29	-1.92±1.12	0.103
HbAlc (%)	1.44±1.03	-0.79±0.84	0.001*

Negative = increase in month 4; Positive = decrease in month 4
Refers to significance (p<0.05)

4.5.1.5.2 Magnitude of change in lipid levels

There was a negligible increase in total cholesterol in the intervention group (-0.00±0.35) while there was a decrease in the control group (0.08±0.43). There was no significant difference in the changes in cholesterol levels between the study groups (t-test; p=0.782). There was a decrease in HDL levels in both study groups. There was no difference in changes of HDL levels between the study groups (t-test; p=0.648; Table 4.26).

There was higher increase in triglyceride levels in intervention (-0.23±0.21) compared to the control group (-0.16±0.2). The change in triglyceride levels between the study groups was not significant (0.648). In contrast, the control group had a significantly higher

increase in LDL levels compared to the intervention group (Intervention= -0.09±0.48; Control= -0.91±0.4; t-test; p=0.010).

Table 4.25 Magnitude of change in lipid levels

Variable	N=72 Intervention Mean (95% CI)	N=80 Control Mean (95% CI)	t-test P-value
Total cholesterol(mmol/l)	-0.00±0.35	0.08±0.43	0.782
HDL (mmol/l)	1.74±0.61	1.57±0.44	0.648
LDL (mmol/l)	-0.09±0.48	-0.91±0.4	0.010*
Triglycerides (mmol/l)	-0.23±0.21	-0.16±0.2	0.648

Negative = increase in month 4; Positive = decrease in month 4
Refers to significance (p<0.05)

4.5.1.5.3 Magnitude of change in chromium and ferritin levels

There was higher increase in serum chromium levels in the intervention group (-0.09±0.07) than the control group (-0.03±0.04). There was no difference in the magnitude of change in chromium levels between the study groups (t-test; p=0.172). Similarly, there was no difference in the magnitude of change between serum ferritin levels between the study groups (Table 4.27)

Table 4.26 Magnitude of change in chromium and ferritin levels

Variable	N=72 Intervention Mean (95% CI)	N=80 Control Mean (95% CI)	t-test P-value
Serum chromium (ng/ml)	-0.09±0.07	-0.03±0.04	0.172
Serum ferritin (ng/ml)	61.07±54.35	52.13±54.91	0.077

Negative = increase in month 4; Positive = decrease in month 4

4.5.2 Association between serum chromium levels and biochemical outcomes

Regression analysis was used to determine associations between serum chromium levels and FBS (fasting blood sugar), HbAlc, blood lipids and ferritin. There was a positive association between chromium levels and FBS where FBS increased with increase in chromium levels. A minimum of 0.25ng/ml of chromium is required to maintain normal FBS (4.44-6.38mmol/l).There was a positive association between serum chromium levels and HbAlc among chromium deficient participants. The HbAlc levels increased with increase in chromium and stabilized (6.5-8.4%) at chromium levels of 0.3ng/ml. At very high HbAlc, chromium levels decreased (Figure 4.6).

Low cholesterol levels were registered at low serum chromium levels thus positive association. Increase in chromium levels did not change the cholesterol levels that remained constant.HDL levels remained low (<4.2) with increase in serum chromium Most of the participants had their LDL levels stabilized (2-2.8 mmols /l) and triglycerides stabilized at 1-2mmols/l when the chromium levels were approximately 0.3ng/ml. There was a negative association between ferritin and chromium levels where, most participants had their ferritin levels less than 200ng/ml at chromium levels 0.2-0.3ng/ml. The chromium levels then maintained an upward trend (Figure 4.6).

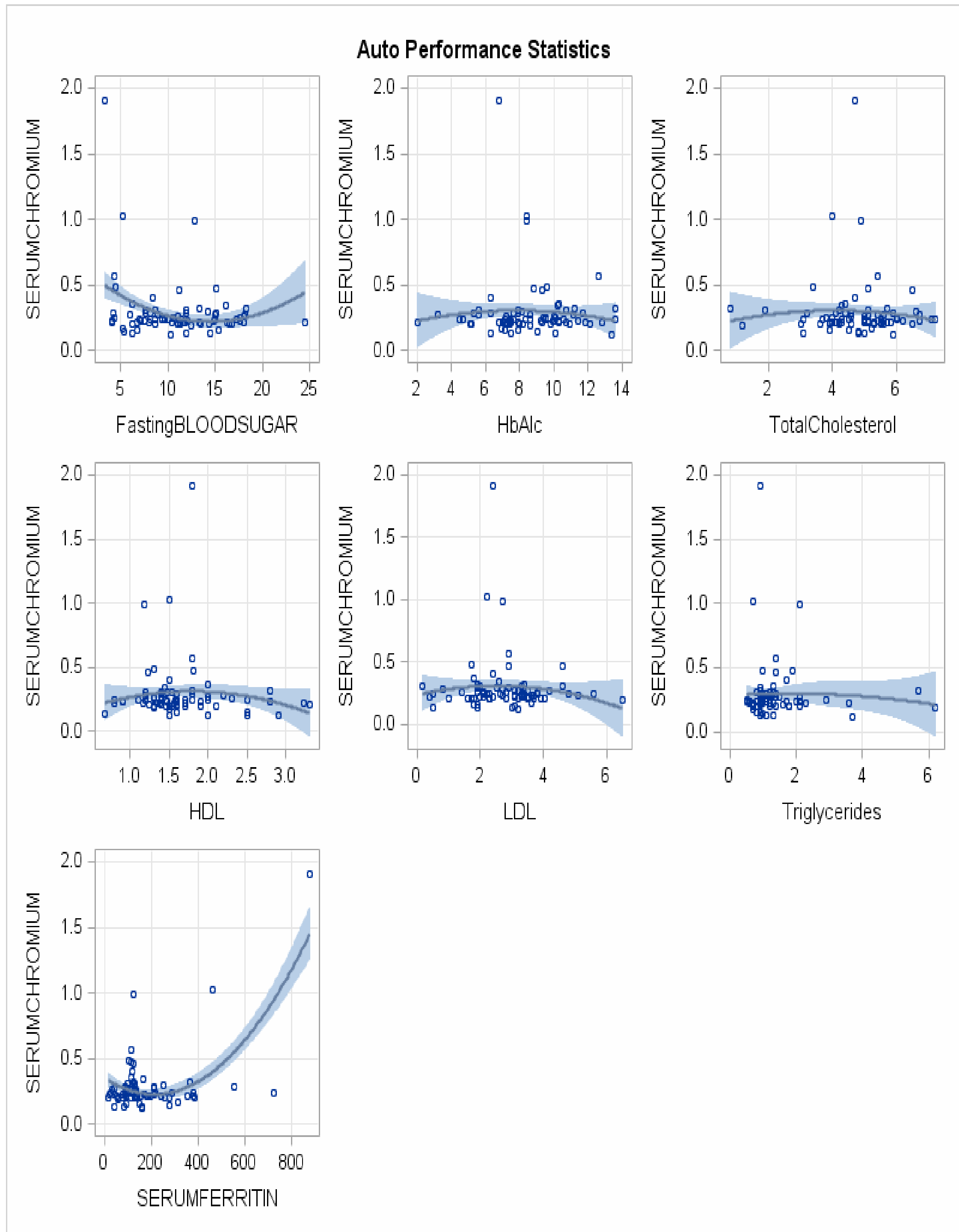

Figure 4.6: Associations between chromium status and FBS, HbAlc, total cholesterol, HDL, LDL, triglycerides and ferritin.

4.5.3 Predictors of elevated HbAlc

Baseline data was used to determine predictors for elevated HbAlc (>9%). This was analyzed using linear regression. There was no significant relationship between elevated HbAlc and the variables - physical activity (TM/week), serum ferritin, serum chromium, total cholesterol, HDL, LDL, Triglycerides, BMI, WHR and Property ownership (Table 4.28). On the contrary, age was a predictor of elevated HbAlc whereas age increases, the participants were more likely to have elevated HbAlc (Linear regression model; p=0.040). Similarly, as fasting blood sugar increases, the higher the likelihood of elevated HbAlc (Linear regression model; p=0.023).

Table 4.27 Predictors of HbAlc

Model		Coefficients[a]			T	Sig.
		Un-standardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta		
1	(Constant)	10.445	5.514		1.894	.062
	Age(completedyears)	.099	.047	.263	2.099	.040*
	Property ownership	-.223	.187	-.140	-1.192	.238
	Fasting blood sugar	.209	.090	.277	2.334	.023*
	Serum chromium	2.469	4.229	.066	.584	.561
	Serum ferritin	.002	.002	.116	.987	.327
	Total cholesterol	-.077	.411	-.027	-.188	.851
	Triglycerides	.458	.466	.128	.982	.330
	HDL	-.076	.144	-.061	-.524	.602
	LDL	.062	.250	.032	.247	.806
	BMI	.065	.078	.112	.826	.411
	WHR	-11.033	5.629	-.262	-1.960	.054
	Physical activity (TM/ week)	1.928E-005	.000	.058	.510	.612

a. Dependent Variable: HbAlc
b. Predictors: Physical activity (TM/week), Fasting blood sugar, serum ferritin, serum chromium, total cholesterol, HDL, LDL, Triglycerides, BMI, WHR, Property ownership and Age in completed years.

CHAPTER FIVE: DISCUSSION

5.1 Introduction

This was a double blind-randomised controlled study to determine the contribution of chromium supplementation in the management of blood sugar and lipids in Type 2 Diabetes. High total cholesterol, LDL and triglycerides are associated with increased oxidative stress that is associated to hyperglycaemia and progression of Type 2 Diabetes. In addition, adiposities measures that indirectly measure body composition such as BMI, WC and W/H ratio have been associated with insulin resistance that in turn increases hyperglycaemia among Type 2 Diabetics. The findings of this study have been discussed on the characteristics of the study participants in relation to socio demographic characteristics, dietary practices and physical activity levels. Effect of chromium supplementation on; blood sugar (FBS and HbAlc), blood lipids (Total cholesterol, triglycerides, HDL and LDL) and adiposity measures (BMI, WC and W/H) have also been discussed. In addition, predictors of elevated HbAlc have also been discussed.

5.2 Socio-demographic characteristics of study participants

Hyperglycaemia increases with age and remains asymptomatic leading to late diagnosis of Type 2 Diabetes (IDF Diabetes Atlas, 2013). The global age range of Type 2 Diabetics has been reported as between 40 and 59 years (IDF, Diabetes Atlas, 2013). In this study, most of the Type 2 diabetics were within 36-55 years age range. This finding does not concur with studies by Brian *et al.,*(2012); Seth *et al.,* (2014) who reported that majority of Type 2 diabetics in their study were aged between 40 and 59 years. Study done in an urban slum in Kenya by Richard *et al.,* (2013) reported increase in diabetes prevalence with age peaking at 45-54 year range. In contrast, this study found that diabetes

prevalence increases with age peaking at 36-55 years. The minimum majority age in the study is 36 years which indicates that Type 2 diabetes does not only affect individuals above 40 years but could also occur earlier as indicated by this study finding.

Reports by IDF Diabetes Atlas (2013) indicate higher but insignificant prevalence of Type 2 diabetes in males than females and same trend projected to 2035. The findings of the current study reported more females than males among the study participants. The finding is in agreement with that reported by Seth *et al.,* (2014) in which women represented 70% of the total participants in the study. Since globally, there is no difference in prevalence of Type 2 Diabetes by gender, the higher proportion of females in this study may be due to the fact that women are more likely to seek health services than men and consequently, more participated in the study.

Due to its chronic nature, Type 2 Diabetes may impose long term expenditure and strain in the health care budget both at national and household levels. The Kenya government's goal is to improve the quality of life and reduce complications associated to hyperglycaemia in Type 2 diabetics. To achieve this, interventions have been put in place by providing hypoglycaemic drugs at subsidized costs in the government hospitals (GOK, 2010). Thika level 5 hospital is a government facility and as expected, most participants in the study were from the low and middle socio economic status. This was further supported by the fact that majority of the participants were of low level of education. These findings prove that Diabetes is no longer a disease of the affluent but is also affecting the low socio-economic class who also share its burden on their finances due to the chronic nature and complications of the disease. An additional blood sugar management strategy such as chromium supplementation would slow down the progress

of hyperglycaemia related complications and the financial burden as well as decreased productivity that come with it.

5.3 Dietary practices of Type 2 diabetics- baseline findings

The long-term and the severity of complications in Type 2 Diabetes are significantly related to the diet quality and nutritional management of the disease (Chrystalleni *et al.,* 2012). People with Diabetes generally have the same nutritional requirements as the general population. The diet of persons with Diabetes should promote health and well-being but should maintain normal blood glucose levels, healthy weight, normal blood lipids levels and normal arterial pressure levels (Mendosa, 2003; Franz *et al.,* 2008). In this study, the findings from the 24 hour recall indicated that at baseline, the participants met the WHO (2006) recommendations of most nutrients except for fat, calcium and sodium. Chromium intake was not analyzed since chromium levels are not included in the Kenya Food Composition Tables.

This study finding indicated over-consumption of some nutrients particularly dietary fibre. High fibre intake could be attributed to the high frequency of consumption of whole grains and cereals as reported from the 7 day food frequency. Studies have reported the benefits of high fibre intake in management of blood sugar among Type 2 diabetics (Aller *et al.,* 2004; Bantle *et al.,* 2008; Qureshi *et al.,* 2002). On the contrary, excess fibre intake and phytates have been reported to negatively affect bioavailability of dietary minerals like calcium, iron and even chromium (Barbara and Donald, 2014).The study participants should be encouraged to consume dietary fibre within the RDAs in

order to maximize micronutrient absorption especially chromium that was found to be deficient among the study participants.

Excess intake of Vitamins (A, B_1, B_6 and C) and minerals (magnesium, phosphorus and zinc) was also reported in this study. The high intake of B vitamins could be attributed to high consumption of whole grains and cereals while vitamin A intake could be due to high frequencies of carrot and tomato intake among the study participants. Excess nutrient intake could lead to toxicities that may increase the health burden for the Type 2 diabetics. It is however important to note that serum levels of these nutrients were not determined in this study.

Dietary carbohydrates from unrefined cereals are recommended to reduce rapid rise in blood sugar and chromium losses resulting from hyperglycaemia due to their low glycaemic index (GI). The whole grains and cereals have also been identified as rich sources of dietary chromium (Anderson, 2007; Wheeler and Pi-Sunyer, 2008). In addition, studies by Buyken *et al.,*(2001); Jetkins *et al.,* (2008); Villegas *et al.,*(2007) that reported that low GI carbohydrates had beneficial effect in reducing HbAlc and HDL among Type 2 diabetics. In this study, the findings from the 7 day food frequency indicated that whole grains and cereals consisting of whole grain bread, *ugali*, beans, *gither*i and sorghum porridge were the most commonly consumed. This is commendable practice which should be encouraged. Nutrient intake adequacy based on 24 hour recall indicated that consumption of dietary carbohydrates for most of the participants was above the RDA. Consumption above the RDA is of concern as this has been associated with blood glucose spikes in Type 2 diabetics (Anderson, 2007). A study done by Hajime *et al.,* (2009) among Japanese and Muller *et al.,* (2011) on German Type 2 diabetics

reported that reduction of dietary carbohydrates to 30% of daily calories coupled with hypoglycaemic drugs significantly reduced HbAlc and serum cholesterol. The study participants should therefore be encouraged to consume carbohydrates within recommended dietary allowances.

Milk intake has been found to be beneficial to Type 2 diabetics as indicated by the findings that milk intake by women reduced the likelihood of developing hyperinsulinemia (Wirfält *et al.,* 2001). In this study, whole milk was the most frequently consumed. This is in agreement with the findings by Karmeen *et al.,* (2004) that reported high preference for whole milk by African Americans because they found it sweet. High intake of whole milk in this study would be based on the fact that majority of the study participants owned livestock. In contrast, whole milk has high content of saturated fats and its high consumption may increase the risk to overweight and obesity that further aggravate insulin resistance. The study population should be encouraged to consume more of low fat milk.

Fat slows down the processing of food in the digestive tract hence lowering the overall glycemic load. This makes steady blood sugar levels over time and long lasting satisfaction after eating (ADA, 2008). On the contrary, study by Barnard *et al.,* (2009), indicated benefits of low fat diet in improving glycaemia and plasma lipids. Additionally, dietary intake of PUFA has been reported to reduce progression of Type 2 Diabetes (Summers *et al.,* 2002; Vessby *et al.,*2001) by improving insulin sensitivity. The low fat diet in these studies were fortified with essential fatty acids (oleic and linoleic fatty acids). The finding of the current study showed low fat intake among the participants despite adequate intake of PUFA. The participants were on regular diet that was neither

supplemented nor fortified with essential fatty acids. Dietary sources of essential fatty acids such as groundnuts and oily fish intake should be encouraged among the study population since their intake was found to be low based on the 7 day food frequency recall.

Beef is an expensive animal source of protein that contains all the essential amino acids. Beef may not be commonly consumed especially in households from low socio-economic status because of its cost. Participants in this study consumed beef twice per week on average. Panagiotakos *et al.,* (2005) reported association between meat and meat products with insulin resistance and hyperinsulinemia and recommended consumption of meat less frequently among Type 2 diabetics. The beef intake among the participants should therefore be commended.

American Diabetes association (2008) guidelines recommend consumption of at least two servings of fish per week to provide sufficient quantities of omega 3 polyunsaturated fatty acids. Fish is a source of unsaturated fatty acids and studies have proved contribution of fish intake to reduced progression of Diabetes (Summers *et al.,* 2002). In this study, fish was consumed once per week on average. The consumption of fish should be encouraged among the participants since it is available in the local markets. The contribution of egg intake to hyperlipidemia is controversial. A study by Karma *et al.,* (2011) on Type 2 diabetics in Australia reported that egg intake of as much as 2 eggs per day was beneficial in metabolic control. On the contrary, a study by Supanee *et al.,* (2013) in Thailand reported negative effect of daily egg intake in addition to low fat Thai diet on total cholesterol and LDL and no effect on HDL, triglycerides and LDL/HDL ratio. This study finding indicated low intake of eggs despite the high ownership of

chickens by the participants. The egg intake should be encouraged as it provides cheap animal proteins that is readily available while maintain normal HDL/LDL ratio.

Dietary guidelines for Americans (2010) recommend groundnut intake as it is a rich source of dietary chromium and healthy fats. In this study, groundnut intake was low among the participants despite is availability in the local markets. Groundnuts are quite expensive and do not culturally form part of the diet of those living in the study area. The type 2 diabetics may be encouraged to increase their intake of groundnuts.

Despite Leafy vegetables and fruits being poor sources of dietary chromium, they are rich in vitamin C that is an enhancer of chromium absorption in the gut. A study in Addis Abbaba-Ethiopia by Amelmal *et al.,* (2012) reported low intake of fruits and leafy vegetables among Type 2 Diabetics that were attributed to unavailability. The current findings indicate Kale (*sukuma wiki*) as the most frequently consumed leafy vegetables despite availability of other leafy vegetables like pumpkin, bean, amaranth and other African leafy vegetables. Kale is a rich source of magnesium that is associated to improved tolerance to carbohydrates by Type 2 diabetics (Franz *et al.,*2002). High frequency in Kale intake could have contributed to 100% realization of RDA for magnesium by the study participants. In addition to that, these study participants consumed at least a fruit per day which is commendable. Variety in the intake of leafy vegetables should be encouraged among the study population to maximize on the available micronutrients.

5.4 Physical activity levels of Type 2 diabetics

Physical activity is core to healthy lifestyle as well as instrumental in management of blood sugar in Type 2 Diabetes. Exercise burns calories which helps in weight loss and maintain a healthy weight and also improves circulation in arms and legs where people with Diabetes have complications (Diabetes Mellitus Information, 2006). Exercise also provides relief from stress, which is the major contributing factor in raising blood sugar levels (www.medicine net.com- accessed in March 2015). In this study, most participants met the WHO requirement of at least 600 minutes of physical activity per week that was mostly work and travel related. This study finding agrees with a study done by Richard *et al.,* (2013) in a rural facility in Kenya that found high physical activity levels that entailed walking and riding.

Studies by Balkau *et al.,*(2008); Ekelund *et al.,*(2009); Esteghamati *et al.,*(2009); Kavouras *et al.,*(2007) reported beneficial effects of participation in physical activity lasting more than 30 minutes daily on increased insulin sensitivity. Further, a meta-analysis by the Boulé *et al.,* (2003) reported that exercise intensity predicted post intervention weighted mean difference in HbAlc to a larger extent than exercise volume. The participants in this study had low participation in both vigorous and medium intensity sports. Participation in intensive exercise lasting at least 30 minutes per day should be encouraged among the study population to reduce hyperglycaemia, overweight and risk to metabolic complications observed in this study.

5.5 Hypoglycaemic drugs profile of the Type 2 diabetics

The medication used to manage hyperglycaemia among the participants in this study included glucomet, glucophage, metformin and insulin which are in line with the reported anti diabetic drugs used by Swindale (2013); Marcheti *et al.,* (2009). In this study, the most commonly used hypoglycaemic drug was metformin which is in agreement with the findings of a study by Papanus and Maltezos, (2009) who found metformin to be a widely used anti-diabetic drug. During the study period, there was no difference in drug intake between the study groups in the 4 months of the study. This observation confirms that randomization was successful and that the blood sugar and lipids findings post-intervention were as a result of chromium supplementation.

5.6 Effect of chromium supplementation on study outcomes

5.6.1 Effect of chromium supplementation on BMI, WC and W/H ratio

Weight reduction is a primary therapeutic aim for people with Type 2 Diabetes and studies have shown association between weight reduction and; reduced insulin resistance, improved glucose measurements, improved lipid profile and decreased arterial blood pressure among Type 2 diabetics (ADA, 2008; Lee and Aronne, 2007; Nield *et al.,*2008).

In this study, the findings indicated that over half of the participants were overweight. After four months of chromium supplementation, there was no significant reduction in BMI. Similarly, there was no significant association between serum chromium levels of the participants and BMI following supplementation with chromium picolinate. These study findings are in contrast to the findings of the study by Se ra Hong et al., (2009) on Korean adult women that reported positive association between hair chromium levels and

BMI. Study by Brownlee *et al.,* (2013) on overweight American adults in North Carolina reported beneficial effects of chromium supplementation in reducing body weight gain and visceral fat accumulation compared with placebo group after supplementing them with 1000mcg chromium per day for 6 months. Similar findings have been reported from meta-analysis by Onakpoya *et al.,* (2013) where chromium supplemented groups had significant weight loss compared to their placebo counterparts. Heterogeneity in measuring and reporting tools for body composition in the reviewed studies was cited. This study used a lower dose of 500mcg/day of chromium for 4 months (16 weeks) hence the difference in duration and dosage may contribute to difference in results. There is growing recognition that waist circumference (WC) rather than BMI may be of higher risk to Type 2 Diabetes and the complications that follow (Depres *et al.,* 2008; Kim *et al.,* 2011; Korsic *et al.,* 2011). Study by Mamtani *et al.,* (2013) on Mexican American families reported that WC was strongly associated with risk of Type 2 Diabetes and insulin resistance. In this study, the participants realized mean WC of 90.7 in both study groups with more than half at risk of metabolic complications. The findings concur with that done by Richard *et al.,* (2013) in Kenya that reported higher WC in type 2 diabetics.

In this study, there was no insignificant reduction in WC with chromium supplementation. This finding agrees with that reported by Yuka et al., (2010) where 80 overweight adults had no significant reduction in central adiposity after supplementing them with chromium Picolinate for 6 months. On the contrary, the current finding does not agree with report from a study by Julie *et al.,* (2006) that demonstrated a significant positive effect of chromium supplementation in reducing WC. This study involved 17 American Type 2 diabetics on Sulfonylurea who were supplemented with 1000mcg/day

of chromium picolinate for 6 months. This sample size was rather small compared to the sample size in the current study. The dosage used was double the dose used in the current study and lasted longer period of time.

5.6.2 Effect of chromium supplementation on serum chromium levels

Healthy human population requires trivalent chromium in trace amounts for sugar and lipid metabolism. Trivalent chromium is naturally available in food though affected by agricultural and processing processes. Dietary chromium is poorly absorbed with an absorption rate between 0.4% -2.5% and the rest (>95%) is egested in faeces. Out of dietary chromium absorbed only 1% is retained (National Institute of Health, 2013; Barbara and Donald, 2014). In this study the quantity of dietary chromium was not investigated since it was not included in Kenya Food Composition Tables.

Anderson, (2007) reported positive association between chromium deficiency and high urinary excretory rates as a result of polyuria in Type 2 diabetics. Similar findings have been reported by Ekmecioglu and Anderson, (2007); Nouramonammadi and Underwood, (2007); Olga *et al.,*(2012) of positive association between chromium deficiency and risk of insulin resistance and hyperglycaemia, predisposing individuals to Type 2 Diabetes. The participants in this study were deficient in chromium at baseline. This study finding concurs with that conducted in Pakistan by Tasneem *et al.,* (2008) reported low serum levels of chromium in the diabetic population. The chromium deficiency observed in this study could be attributed to hyperglycaemia as indicated by the high fasting blood sugars at baseline.

At month 4 in this study, there was an increase in serum chromium levels which reached normal levels of 0.31ng/ml in the intervention group while in control, the levels remained deficient. The supplemented group however, realized the threshold for serum chromium indicating safety of the dosage (500mcg/day) that was used in this study. This finding supports that from a study by Gregg (2011) that reported low absorption of chromium (2%) when administered orally in humans. According to Vincent et al., (2006), toxicity ranged from 1500 - 3300mcg/kg body weight. Supplementation increases serum levels despite insignificant difference between the study groups. Studies on chromium supplementation among Type 2 diabetics have not reported the serum chromium levels and therefore the limitation of comparison of this aspect with other studies.

5.6.3 Effect of chromium supplementation on fasting blood sugar (FBS)

The ultimate goal of Diabetes therapy is to achieve and maintain near normal blood glucose levels in order to prevent Diabetes complications and improve quality of life as well as life expectancy (ADA, 2000; IDF, 2005; Narayan, 2006; GOK, 2010). In this study, the fasting blood sugars were high in both study groups at baseline. Type 2 Diabetes is characterized by hyperglycaemia that predisposes the patients to cardiovascular, neural and renal complications that may result to physical disabilities (IDF, Diabetes atlas, 2013). The findings agree with Kenyan studies by Otieno, (2003); Mortala *et al.,* (2008) that reported challenges in achievement of tight blood sugar levels by Type 2 diabetics that resulted to amputation of their lower limbs. Chromium potentiates the action of insulin by increasing insulin receptor mediated signalling thus reducing insulin resistance in Type 2 diabetics. Studies on chromium picolinate supplementation in addition to hypoglycaemic medication have demonstrated its ability

to increase insulin sensitivity and reduce FBS in Type 2 diabetics (Nair *et al.,* 2008; Shilpi *et al.,* 2011 and Olga *et al.,* 2013). However, this has been controversial since other studies indicate otherwise. In this study, there was no significant difference in FBS between chromium supplemented group and the control. This finding is in agreement with studies conducted by Ali *et al.,* (2011); Bailey, (2014); Mieebi *et al.,* (2014) and Sushi *et al.,* (2012) that reported no effect of chromium supplementation on FBS by doses of 500mcg -1000mcg per day. This study considered fasting blood sugar once at the baseline and at end of study.

5.6.4 Effect of chromium supplementation on glycated haemoglobin (HbAlc)

Glycated haemoglobin (HbAlc) has long been used in the management of established Diabetes care as a biomarker of long-term glycaemic control. Levels of HbAlc correlate well with average ambient blood glucose levels during the previous 3 months (Silvio and Inzucchi, 2012). In this study, the baseline findings indicated an overall elevated HbAlc among participants in both study groups. This could be attributed to the chronic hyperglycaemia in Type 2 diabetics and supported by the high FBS across the study groups. Despite being on medication, there were challenges with maintaining a tight blood glucose control.

In this study, chromium supplementation significantly reduced HbAlc. This finding is in agreement with those of Gosh *et al.,* (2010); Perpetua *et al.,* (2010) that observed marked reduction in HbAlc in the participants supplemented with chromium compared to the control groups. In contrast, the findings of this study do not agree with findings reported by Abdollahi *et al.,* (2013) on randomized, placebo-controlled studies where chromium supplementation had no effect on the levels of HbA1c. Additionally, a study by Sushi *et*

al., (2012) reported no beneficial effects of chromium on HbAlc after supplementing 50 diabetic adults with 400mcg chromium daily for 3 months. The researchers of these studies attributed the findings to short study period. This study lasted 4 months with a dose of 500mcg chromium which significantly reduced HbAlc.

5.6.5 Effect of chromium supplementation on blood lipids (Total cholesterol, HDL LDL and triglycerides)

Lipid and lipoprotein abnormalities in Type 2 diabetes include particularly elevated levels of total and very low-density lipoprotein, triglycerides and reduced levels of high-density lipoprotein (HDL) cholesterol. Total and low-density lipoprotein (LDL) cholesterol levels are usually normal if glycaemic control is adequate (Laakso, 2002). The findings of this study showed that approximately 40% of the participants had high total cholesterol while half of the participants had low HDL levels. This implies a challenge in maintaining normal blood lipids among Type 2 diabetics.

There are contradicting findings on the contribution of chromium supplementation on blood lipids. In this study, there was no significant difference in total cholesterol and triglycerides between the intervention and control group after 4 months of chromium supplementation. This finding contradicts other findings by Vincent *et al.,* (2004); Cefalu *et al.,* (2004); Balk *et al.,* (2007) and Humel *et al.,* (2007) that reported significant reduction in oxidative stress and improved lipid metabolism after supplementing Type 2 diabetics with chromium. The participants in this study had a significant reduction in LDL and this agrees with findings from meta-analysis by Perpetua *et al.,* (2010) that reported significant reduction in LDL after chromium supplementation in type 2 diabetics. There were higher levels of HDL in the control group compared to the

chromium supplemented group post intervention. However, in terms of the magnitude of change, there was no significant difference in HDL levels between the intervention and the control groups. There was a marked change of magnitude of in LDL levels in the intervention group compared to the control group.

Additionally, associations between chromium status and HDL, LDL and triglycerides indicated normal serum levels of the lipids were realized in participants with serum chromium levels of 0.3ng/dl. This indicates that adequate serum chromium is therefore required to maintain normal blood lipid levels.

5.6.6 Effect of chromium supplementation on serum ferritin levels

Ferritin is the major iron storage protein of the body. Ferritin level can be indirectly used to measure iron levels in the body as it is a primary indicator of iron deficiency in the storage tissues. Chromium competes with iron for a binding site on transferring and this may disadvantage absorption of iron from the gut. Clodfelder*et al.,* (2001) and Lukaski*et al.,* (2007) recommended inclusion of chromium interaction with other minerals particularly iron. In a study performed by Campbell *et al.,* (1999), chromium supplementation in dosages of 1,000mcg/day for 13 weeks did not affect serum ferritin levels in moderately obese men. In the current study, there was no significant difference in serum ferritin levels between the intervention and the control groups. This finding concurs with that of other studies conducted on women that reported no effect on serum ferritin with chromium supplementation (Campbel *et al.,* 2002; Volpe *et al.,* 2001). Therefore, chromium supplementation does not affect the ferritin levels.

5.7 Predictors of elevated HbAlc

HbA1c is considered as the ‘gold standard’ of glycaemic control and is regarded as the key factor in reducing the risk of Diabetes-related complications (Hirsch*et al.,* 2005; Hanefeld *et al.,* 2002). It is therefore important to identify the predictors of elevated HbAlc that would be useful in the management of blood sugar among Type 2 diabetics. In this study, age was found to be a predictor of elevated HbAlc, where older adults were more likely to have elevated HbAlc compared to the younger ones. This finding is in agreement with the findings reported by other studies that prevalence of hyperglycaemia increases with age (Brian *et al.,* (2012); Seth *et al.,* 2014) and supported by IDF, Diabetes Atlas, (2013). This shows that advancement in age increases the risk to Type 2 Diabetes.

The other predictor for elevated HbAlc was increased FBS. This finding is in agreement with that of Althias *et al.,* (2002) that reported increase in HbAlc with high FBS. This is an expected finding because chronic hyperglycaemia increases glycation resulting into elevated HbAlc.

CHAPTER SIX: SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.0 Introduction

The purpose of this study was to determine the effect of chromium supplementation in addition to hypoglycaemic drugs on management of blood sugar and lipids among type 2 diabetics. The study tested the relationship between chromium supplementation and HbAlc, HDL, LDL BMI and WC. This chapter gives a summary of the study findings, conclusions and recommendations for policy, practice and further research.

6.1 Summary

The majority of participants in this study were women. Majority of Type 2 diabetics were aged above 36 years and belonged to low socio-economic status. Above half of the participants were married, had completed primary level of education and mainly farmers and business people.

Nutrient intake for carbohydrates, proteins, vitamins and most minerals was adequate as per WHO (2006) RDA standards except for fat and sodium that was below RDA. However, there was overconsumption of energy, dietary fibre, vitamins (A, B_1, B_6 and C) and minerals (magnesium, phosphorous and zinc). Chromium intake was not determined since the Kenyan Food Composition Tables do not have information on chromium levels in different foods.

Whole meal bread and *ugali* were the most frequently consumed whole grains and cereals together with *githeri* and sorghum porridge. Beef was the most frequently consumed animal protein while eggs and processed meat were consumed by a minority of the participants. Dry beans were the most frequently consumed source of plant protein.

Tomatoes and Kales were the highest consumed vegetables while ripe bananas and citrus fruits were the most frequently consumed fruits.

High level of physical activity related to work and travel was reported across the study groups. Most participants were involved in vigorous intensity jobs while walking and cycling were the most popular mode of transport to and from places. There was low participation in strenuous exercises like vigorous intensity sport despite its positive association to insulin sensitivity, weight loss and lean body mass. Low sedentary lifestyle was observed in the study findings as participants' lifestyle was active.

The medication used to manage hyperglycaemia was provided by the hospital from where the participants were recruited. The medication included: glucomet, glucophage, metformin and insulin. Metformin and glucomet were the most commonly used medication. Medication used by the participants in both study groups did not change throughout the 4 months study period. Compliance to drug and chromium/placebo intake was high (>85%) in both study groups during the study period.

There was no significant effect of chromium supplementation on BMI, W/H and WC despite the decrease in BMI and WC in the intervention group. Baseline data indicated chromium deficiency in both study groups which improved with supplementation in the intervention group but not significantly. Chromium supplementation significantly reduced HbAlc. This was further demonstrated by a positive association between chromium status and HbAlc. There was a significant reduction in LDL with chromium supplementation while there was no effect on total cholesterol and triglycerides. There was no effect of chromium supplementation on serum ferritin levels. There was no

significant difference in liver functions (ALP) between the intervention and the control group. The predictors of elevated HbAlc were age and FBS. Older people were more likely to have elevated HbAlc while those with higher levels of FBS were more likely of the same.

6.2 Conclusions

In this study, most participants were females. Majority of the participants were above 36years of age, married and mainly farmers and business people. Majority of the participants were from low and middle socio-economic categories that had completed primary level of education. This finding shows that Type 2 Diabetes not only affects the affluent but is also experienced among the poor individuals in developing countries like Kenya.

Daily nutrient intake was adequate for most of the participants except for fat and sodium whose consumption was below the RDA. In contrast, the consumption of energy, dietary fibre, Vitamins C, $B_{1,}$ $B_{2,}$ and B_6; minerals- potassium, iron and zinc was above the RDA. Excess energy intake not only increases hyperglycaemia but also contributes to overweight and insulin resistance. These excesses would put the diabetics at risk of toxicities especially with the micronutrients while the deficiencies in fat intake would increase risk of deficiencies in fat soluble vitamins. Participants should be encouraged to consume nutrients within the RDAs.

High levels of participation in physical activity were registered among the participants that were work and travel related. However, there was low participation in vigorous and

moderate intensity sport that has been proved to be beneficial in insulin sensitivity and weight loss. Participation in sport activities should be encouraged.

More than half of the participants were overweight placing them at increased risk of metabolic complications. Chromium supplementation had no effect on BMI and WC among the Type 2 diabetics hence, *the null hypothesis that there was no significant relationship between serum chromium levels and anthropometric measurements was not rejected.* The study participants were deficient in serum chromium at baseline implying low serum chromium status in individuals with Type 2 Diabetes attending Thika level 5 Hospital. Despite the increase of serum chromium levels in the intervention group, it was not significantly different from the control group. The increase in the intervention group met the threshold for minimum serum chromium recommended levels.

Baseline findings indicated high FBS and HbAlc that is characteristic of chronic hyperglycaemia. Chromium supplementation had no effect on FBS but significantly reduced HbAlc among Type 2 diabetics. *The hypothesis that there is no significant relationship between serum chromium levels and HbAlc was therefore rejected.*

Chromium supplementation reduced LDL significantly in this study. *The hypothesis that there is no significant relationship between serum chromium levels and the lipid profile was therefore rejected.* The predictors for elevated HbAlc among the study participants were FBS and age where HbAlc levels increased with age and hyperglycaemia. These study findings are supported by literature that chronic hyperglycaemia sets in with age and increases HbAlc levels.

6.3 Recommendations

The following recommendations were made based on the study findings;

6.3.1 Recommendations for policy

- MoH should consider including chromium supplements in the management of Type 2 Diabetes since it significantly reduced HbAlc and LDL in the intervention group.
- MoH to strengthen interventions on dietary intake of chromium to reduce on the deficiencies among Type 2 diabetics.

Recommendations for practice

- Fibre intake, vitamins C, B_1, B_2, and B_6 and minerals –potassium, iron and zinc should be consumed within the recommended daily allowances. Excess fibre intake interacts negatively with the dietary mineral absorption. At the same time, adequate intake of healthy fats and sodium should also be addressed.
- Consumption of adequate fat , sodium and calcium to be encouraged
- Participation in physical activity outside work and travel to be encouraged as this may address the incidences of overweight and obesity observed in this study.

6.3.2 Recommendations for further research

- Similar studies in other parts of Kenya but with a longer intervention period should be conducted to determine the effect on WC and BMI that indicated insignificant reduction in this study.
- Studies testing the impact of chromium supplementation should also determine tissue and urine chromium levels as well to ascertain degrees of deficiency and retention by the body.

- There is need to conduct studies that determine chromium levels in Kenyan foods. Chromium quantities were missing in the food composition tables that made it difficult to quantify dietary chromium intake by participants in this study.

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APPENDIX A: INFORMED CONSENT LETTER

Introduction

I am Judith Munga from Kenyatta University, conducting a research on the contribution of chromium supplement in management blood glucose.Chromium is a naturally occurring micro-nutrient in foods. This study will administer chromium in form of capsules to be taken once a day for a period of four months. The chromium supplement will be taken to monitor its contribution to management of blood sugar and lipids.

Chromium supplements will be provided in capsule form free of charge, to last one month and this will be collected at Thika level 5 Diabetes Clinic on a monthly basis for a period of four months.

Expectations from a study subject

Type 2 diabetics attending Diabetes Care Clinic will voluntarily choose to participate in this study. Participants in the study will be required to visit the clinic once in a month. Transport costs during these visits will be reimbursed by the researcher. During the study, you will continue consultation with your doctor for regular treatment and attention.

Participants will be required to provide blood sample at Thika level 5 laboratory at the beginning of the study and end of four months. The laboratory costs will be met by the researcher. Extra time lasting 30 minutes will be required from the usual time you spend during your clinic visits to fill in questionnaires and take body measurements. You will be free to discontinue your participation at any point within the four months if necessary. Kindly alert the research team on your decision to withdraw.

Risks and safety issues

A qualified phlebotomist will draw blood from the participants in hygienic recommended conditions under standard operating procedures (SOP) for laboratories. Alcohol wipes (70% isopropyl alcohol) will be used to clean venipucture site and vacuum collection tubes used to draw blood. Gauze sponges will be applied on the site where needle was withdrawn with adhesive tapes to protect venepuncture site. The phlebotomist will ware latex gloves to minimize cross infection. Disposable needles and syringes will be used and disposed of in designated containers after every sample collection.

Chromium picolinate may bring about allergic reactions like rashes, hives, loose stool or diarrhea and itchy skin on some people. When you experience any of these symptoms, contact the research team immediately through the contact lines provided below.

Confidentiality

Data collected during this study will be highly confidential and only used for the purposes of the study. The names will be substituted with code numbers for confidentiality purposes. The study findings will be communicated back to participants three months after end of intervention and a copy of the report will be made available at the clinic library for reference.

Communication lines

Any form of communication by participants on clarifications about the study or complaints can be directed to any of the following contacts;

- Investigator: Judith Munga- **0722 974 465** or email munga.judith@yahoo.com
- Physician: Dr. Mbogo-**0722613432** or email **mbogomd@yahoo.com**

- Kenyatta university Ethics Review committee- **020 8710901/12** or email **kuer.chairman@ku.ac.ke**

Acceptance

If you accept to participate in the study, please sign below.

Name ………………………………. Phone Number………………………

Signature……………………   Date…………………………

APPENDIX B: BASELINE QUESTIONNAIRE

Date…………….   Recruitment number…………

1.0 Demographic information(Circle the responses appropriately)

1.1 Sex Male (1) Female (2)

1.2 Age in completed years…………

1.3 What is your current marital status?

a. Single
b. Married
c. Separated
d. Divorced
e. Widowed

If the response to question 1.3 is married, answer question 1.4. If not, go to question 1.6

1.4 What is the occupation of your spouse?

a. Civil servant (Specify)……………………………
b. Private sector specify)……………………………
c. Business (Specify)…………………………………
d. Education sector (Specify)…………………………
e. Other (specify)……………………

1.5 What is your occupation?

a. Civil servant (Specify)………………………………
b. Private sector (specify)……………………………
c. Business (Specify)…………………………………
d. Education sector (Specify)…………………………
e. Other (specify)………………………………………

1.6 What is your completed level of education? Tick appropriately in the box.

a. Primary level (KCPE) or equivalent

b. Secondary level (KCSE) or equivalent

c. Middle level college

d. University

1.7 Which area of Kenya do you mostly live? (Specify in the spaces provided below)

a. County……………………………………………

b. Town……………………………………………..

2.0 MEDICAL HISTORY

2.1 When were you first diagnosed with Type 2 Diabetes mellitus?

Year…………………Month……………………

2.3 What is the name of the drug you are using currently to help you manage your blood sugar level? Drug type……………………………Quantity per day…………………

2.4 Do you use other drugs for your condition?

(1) Yes

(2) No **If Yes, Specify**

Drug type………………… Quantity per day………Source……………………….

2.5 Are there any other member(s) of the nuclear or extended family suffering from Type2 Diabetes mellitus?

Yes (1) No (2)

If Yes, specify……………………………………

2.6 Have you experienced any of the symptoms listed below in the last two days? If Yes, circle the response appropriately.

a) Frequent urination
b) Excessive thirst
c) Excessive hunger

2.7 Have you experienced any of the following symptoms in the last two weeks? If Yes, circle the response appropriately.

a) A coma/Fainting
b) Overeating
c) Blurred vision
d) Illness other than Type 2 Diabetes
e) Constipation
f) Restlessness
g) Numbness of the limbs (legs, hands, fingers)
h) Pins and needles in the legs or hands

3.0 <u>Anthropometry</u>

	Height (m)	**Weight (kg)**	**BMI**	**Waist circumference**	**Hip circumference**
1st					
2nd					
Average					

4.0 Dietary Intake

24-Hour dietary recall

TICK THE DAY OF THE WEEK WHICH YOU ARE RECALLING **(IT SHOULD BE THE DAY BEFORE THE INTERVIEW)**

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday

Step 1: Please think back to when you woke up yesterday morning to the time you went to sleep in the evening. Now, I want you to try and remember what you ate or drank yesterday from the moment you got up until you went to sleep again last night. Run through the whole day in your mind and try to remember everything that you ate or drank. (THE INTERVIEWER MUST GIVE THE RESPONDENT A LITTLE TIME TO DO THIS). Now I would like to you tell me what you ate and drank in the morning after you got up. AFTER THE PARTICIPANT MENTIONS AN ITEM, THE INTERVIEWER SHOULD PROMPT THE RESPONDENT BY SAYING "AND THEN?"
ENTER THE INFORMATION IN COLUMN 1)

STEP 1: Food/drink eaten/drank during the day	**STEP 2:** Forgotten foods (PROMPTED)

STEP 2: NOW ASK THE FOLLOWING QUESTIONS ON FORGOTTEN FOODS AND ENTER THEM IN COLUMN 2.

Did you have any cold drinks or soda yesterday?

Did you have any sweets and or chocolate yesterday?

Did you have any cake and or cookies yesterday?

Did you have any snacks like chips, samosa, yesterday

Did you have any (other) fruit yesterday?

Did you have any (other) vegetable yesterday?

Did you have any bread and or rolls yesterday?

Did you have any mandazi yesterday?

Did you have anything else yesterday?

Q. What you ate/ drank yesterday; was it same as, more than or less than usual? (MARK **X** WHERE APPROPRIATE)

	Same as usual		More than usual		Less than usual

If more or less than usual, explain why (circle appropriately)

1. Celebration
2. Religious activity
3. Little food in the household
4. Other (specify) ________________________________

Step 3: ***"Now I am going to ask you more about each food or drink that you ate/drank yesterday"***.

START WITH THE 1ST ITEM REPORTED IN TABLE 1. TRANSFER THIS ITEM TO THE COLUMN 3 IN THE TABLE BELOW.

ASK "At what time was the item 1 eaten?"REPORT THE TIME IN COLUMN 1. DO NOT SPEND MUCH TIME IN GETTING THE EXACT HOUR.

ASK "for what meal was the item 1 eaten? INDICATE FOR WHAT MEAL ITEM 1 WAS EATEN AND REPORT IT IN COLUMN 2.'

Step 4 "Now I want you to tell me more about this food item…."

THIS INCLUDES A DESCRIPTION OF THE FOOD AS WELL AS THE PREPARATION. **ENTER THIS INFORMATION IN COLUMN 4).** *"Now I want you to tell me more about this food item…….*

THIS INCLUDES A DETAILED DESCRIPTION OF THE FOOD (BRAND NAME, IF UNPROCESSED, SEMI-PROCESSED OR FULLY PROCESSED SIZE, ETC), THE AMOUNT PREPARED AND THE METHOD OF PREPARATION. ENTER THIS INFORMATION IN COLUMN 4.

USE STANDARD HOUSEHOLD MEASURES AND WEIGHTS TO DETERMINE AMOUNTS OF INGREDIENTS USED. INDICATE IF FOOD WAS PURCHASED ALREADY COOKED FROM THE STREETS BY INCLUDING THE FOLLOWING TEXT “STREET FOOD”NEXT TO THE ITEM

S*tep 5. “Now we are going to find out how much of this item was eaten/drank”*

INTERVIEWER AND RESPONDENT USE HOUSEHOLD MEASURES AND WEIGHING EQUIPMENT TO DETERMINE HOUSEHOLD PORTION SIZES.

A DESCRIPTION OF HOUSEHOLD PORTION SIZES IN TERMS OF CUPS, SPOONS, BOWLS, GLASSES, MATCHBOXES, MANUAL PICTURES SIZE OR CENTIMETERS (USING RULER) IS THEN ENTERED IN COLUMN 5.

INFORMATION SHOULD BE OBTAINED FOR TOTAL AMOUNT OF COOKED FOOD AND AMOUNTS CONSUMED SHOULD BE ENTERED

IF THE FOOD CODE AND THE PORTION SIZE IN GRAMS OF THIS PARTICULAR ITEM IS EASY TO FIND, IT CAN BE **ENTERED COLUMN 6).** IF IT IS NOT CLEAR OR EASY, THE CODE AND GRAM WEIGHT CAN BE LEFT OUT TO BE COMPLETED AFTER THE INTERVIEW. THIS PROCESS IS REPEATED FOR EACH FOOD ITEM THAT WAS ENTERED ON FORM 1).

STEP 4: RECORDING SHEET FOR INFORMATION COLLECTED IN STEP 3 OF THE 24-HOUR RECALL

1	2	3	4				5			
Item carried from step 1 and 2	Time	Meal 1=breakfast2=lunch3=dinner4=snack5=other	Detailed description of the item before consumption (ingredients, quantities and cooking method)				Amount of food eaten			
			description of ingredients (*Include* •*brand name,* • *if unprocessed, semi-processed and fully processed,* •*large, medium, small*	Quantity of ingredients		Cooking method	Total amount cooked		Total amount eaten	
				description of ingredients in household measures	*Weight (grams)*		*In HH measures*	*Weight (grams)*	*In HH measures*	*Weight (grams*

FOOD FREQUENCY QUESTIONNAIRE

How many times did you consume the following foods in the last 7 days?

	Frequency of consumption in the last one week (Tick where appropriate)								
	Once	**2 times**	**3 times**	**4 times**	**5 times**	**6 times**	**Daily**	**Never**	**Type**
Cereals, carbohydrates, starch									
Ugali (*specify if sifted or whole meal)*									
White bread									
Whole meal bread									
Brown rice									
White rice									
Pasta									
Porridge									
Whole meal chapatti									
Sifted wheat flour chapatti									
Githeri									
Mandazi									
Roasted maize									
Commercial breakfast cereals									
Chips									
Others (Specify)									
Roots and tubers									
Arrow roots									

Yams									
Sweet potatoes									
Green bananas									
Others (Specify)									
Dairy foods									
Whole milk									
Low fat milk (<2% fat)									
Skimmed milk									
Fermented milk (mala)									
Yogurt(*specify if whole fat)*									
Ice cream									
Butter									
Ghee									
Margarine									

Others (Specify)									
Meats, meat products, eggs									
Beef									
Mutton									
Chicken									
Fish									
Pork									
Matumbo									
Processed meat (sausages, bacon, ham, smokies etc)									
Eggs									
Others (Specify)									
Legumes / pulses and nuts									
Dry peas									
Dry beans									
Lentils *(kamande)*									
Green grams									
Mbaazi									
Black eye peas *(Njahi)*									
Groundnuts									
Simsim									
Coconut									

Others (Specify)									
Vegetables									
Kales									
Cabbage									
Tomatoes									
Carrots									
Spinach									
Cucumber									
Kunde (cow pea leaves)									
French beans									
Other African leafy vegetables (specify)									
Fruits									
Apples									
Bananas									
Citrus fruits									
Melon									
Passion									
Pawpaw									
Pineapple									
Mangoes									
Avocado									
Others (Specify)									

Sugar Alternatives, Sweets									
Sugar									
Honey									
Cakes									
Biscuits									
Sweets									
Others (Specify)									
Beverages and Spreads									
Soda									
Juice									
Alcohol									
Peanut Butter									
Margarine									
Jam									
Others (Specify)									

5.0 Physical Activity

Next I am going to ask you about the time you spend doing different types of physical activity in a typical week. Please answer these questions even if you do not consider yourself to be a physically active person.

Think first about the time you spend doing work. Think of work as the things that you have to do such as paid or unpaid work, study/training, household chores, harvesting food/crops, herding, fishing, hunting for food or seeking employment.

*In answering the following questions **'vigorous-intensity activities'** are activities that require hard physical effort and cause large increases in breathing or heart rate, **'moderate-intensity activities'** are activities that require moderate physical effort and cause small increases in breathing or heart rate.*

Activity at work

5.1. Does your work involve vigorous-intensity activity that causes large increases in breathing or heart rate like [carrying or lifting heavy loads, digging or construction work] for at least 10 minutes continuously?

Yes (1) No (2) **If No go to Question 5. 4**

5.2. In a typical week, on how many days do you do vigorous intensity activities as part of your work? Number of days…………..

5.3. How much time do you spend doing vigorous-intensity activities at work on a typical day? Hours…….. Minutes……….

5.4. Does your work involve moderate-intensity activity that causes small increases in breathing or heart rate such as brisk walking or carrying light loads for at least 10 minutes continuously?
Yes (1) No(2) **If No go to Question 5. 7**

5.5. In a typical week, on how many days do you do moderate intensity activities as part of your work? Number of days………………

5.6. How much time do you spend doing moderate-intensity activities at work on a typical day? Hours………… minutes…………….

Travel to and from places

The next questions exclude the physical activities at work that you have already mentioned.

Now I would like to ask you about the usual way you travel to and from places. For example, to work, for shopping, to market or to place of worship.

5.7. Do you walk or use a bicycle (pedal cycle) for at least 10 minutes continuously to get to and from places? Yes (1) No (2) **If No go to Question 5.10**

5.8. In a typical week, on how many days do you walk or bicycle for at least 10 minutes continuously to get to and from places? Number of days……………….

5.9 How much time do you spend walking or bicycling for travel on a typical day?

Hours………… minutes……………..

Recreational activities

The next questions exclude the work and transport activities that you have already mentioned.

Now I would like to ask you about sports, fitness and recreational activities (leisure).

5.10. Do you do any vigorous-intensity sports, fitness or recreational (leisure) activities that cause large increases in breathing or heart rate like [running or football, hockey, tennis, rugby, badminton, squash, netball, handball, rowing] for at least 10 minutes continuously? Yes (1) No (2) **If No go to Question 5.13**

5.11. In a typical week, on how many days do you do vigorous intensity sports, fitness or recreational (leisure) activities? Number of days…………….

5.12. How much time do you spend doing vigorous-intensity sports, fitness or recreational activities on a typical day? Hours………. Minutes……………

5.12.1 If physical activity lasts more than 30 minutes, do you have a feeding pattern for it?

a. Yes

b. No

5.12.2 If Yes Explain…………………………………………………………………

5.13. Do you do any moderate-intensity sports, fitness or recreational (leisure) activities that cause a small increase in breathing or heart rate such as brisk walking, cycling, swimming or volleyball for at least 10 minutes continuously?

Yes (1)   No(2)   **If No go to Question 5.16**

5.14. In a typical week, on how many days do you do moderate-intensity sports, fitness or recreational /leisure activities?   Number of days………………..

5.15. How much time do you spend doing moderate-intensity sports, fitness or recreational (leisure) activities on a typical day?   Hours………. Minutes…………….

Sedentary behavior

The following question is about sitting or reclining at work, at home, getting to and from places, or with friends including time spent [sitting at a desk, sitting with friends, travelling in car, bus, train, reading, playing cards or watching television], but do not include time spent sleeping.

5.16. How much time do you usually spend sitting or reclining on a typical day?

Hours…………. minutes……………..

5.17. Does the physical activity involved in last more than 30 minutes?

Yes (1)   No (2)

5.18. If Yes, How do you balance your feeding with physical activity?

a) Before commencement of physical activity………………………………….

b) 30 minutes into the physical activity……………………………………....

c) End of physical activity…………………………………………………….

APPENDIX C: MONTHLY QUESTIONNAIRE

Date……………. Recruitment number……….. Completed Month :- **(Circle appropriately**

1 2 3 4

Take the following measurements and record readings to the nearest 0.1 units. Each measurement is to be taken twice and the average determined.

1.0 Anthropometry

	Height (m)	**Weight (kg)**	**BMI**	**Waist circumference(cm)**	**Hip circumference(cm)**	**WHR**
Measurement 1						
Measurement 2						
Average						

3.0 MEDICAL HISTORY

3.2 What is the name of the prescribed drug you are using TODAY to help you manage your blood sugar level and what quantity do you take per day? **(Confirm this with file records)** Drug type…………………… Quantity per day…………………

3.3 Do you use any other drugs towards management of your blood sugar besides those prescribed byThika Hospital Doctor? **(Circle the responses appropriately)**

(1) Yes

(2) No

If Yes, Specify; Drug type…………………… Quantity per day……… Source……………………….

3.5 Have you experienced any of the symptoms listed below in the last two days? **(Circle the response appropriately.)**

d) Frequent urination 1. Yes 2. No

e) Excessive thirst 1. Yes 2. No

f) Excessive hunger 1. Yes 2. No

3.6 Have you experienced any of the following symptoms in the last two weeks? **(Circle the response appropriately.)**

i) A coma/Fainting 1. Yes 2. No

j) Overeating 1. Yes 2. No

k) Blurred vision 1. Yes 2. No

l) Illness other than Type 2 Diabetes 1. Yes 2. No **If Yes,**
Specify………………………………………………………………………………………..

m) Constipation 1. Yes 2. No

n) Restlessness 1. Yes 2. No

o) Numbness of the limbs (legs, hands, fingers) 1. Yes 2. No

p) Pins and needles in the legs or hands 1. Yes 2. No

3.7 If you have experienced any of the symptoms in 3.6 above, how did you manage it?

3.8 What influenced your choice of management in3.7 above?

3.9. Are there any challenges you experience with management of Type 2 Diabetes? **(Circle the response)**

1. Yes 2. No

3.10 If Yes, What are the challenges?

<u>Drug adherence</u>

3.13 Were you able to take chromium supplements every day in the past one month?

1. Yes 2. No

3.14 If No, How many times did you miss……………………….

Give
Reason………………………………………………………………………………………………

3.15 Did you get any unusual experiences in your health in the past one month?

1. Yes 2. No

3.16 If YES,
Explain……………………………………………………………………………………………………

3.17 How did you address the unusual occurrence………………………………………………………………………….

APPENDIX D: APPOINTMENT CARD

NAME………………………………

Serial number………………………

NEXT VISIT DATE	REMARKS

APPENDIX E: DAILY MONITORING SHEET

Date	S/No	Name	Nature of visit				
			B.L	M.1	M.2	M.3	M.4

SUMMARY

Total number attended………………………………….

Total number sent to the laboratory…………………………………….

Done by…………………Sign……………………………….Date……………

APPENDIX F: LABORATORY REQUEST FORM

Name…………………………………………….. Date

Serial N^{o}--------------File N^{O}--------------------

fasting blood sugar	Liver test	serum ferritin	HbAlc	fasting lipid profile	Serum Chromium
	ALP--------			Total Cholesterol------ Triglycerides---------- HDL----------------- LDL----------	

Phlebotomy:

Done by (Name) ____

Date ____

Sign ____

Analysis

Done by (Name) ____

Date ____

Sign ____

APPENDIX G: LIVER TEST (ALP)

COD 11590 50 mL	COD 11591 200 mL	COD 11597 500 mL
STORE AT 2-8ºC		
Reagents for measurement of ALP concentration Only for *in vitro* use in the clinical laboratory		

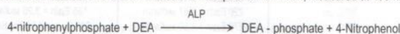
ALKALINE PHOSPHATASE (ALP) - DEA

BioSystems REAGENTS & INSTRUMENTS

ALKALINE PHOSPHATASE (ALP) - DEA
DIETHANOLAMINE BUFFER

PRINCIPLE OF THE METHOD

Alkaline phosphatase (ALP) catalyzes in alkaline medium the transfer of the phosphate group from 4-nitrophenylphosphate to diethanolamine (DEA), liberating 4-nitrophenol. The catalytic concentration is determined from the rate of 4-nitrophenol formation, measured at 405 nm[1,2].



$$\text{4-nitrophenylphosphate + DEA} \xrightarrow{ALP} \text{DEA - phosphate + 4-Nitrophenol}$$

CONTENTS

	COD 11590	COD 11591	COD 11597
A. Reagent	1 x 40 mL	1 x 160 mL	4 x 100 mL
B. Reagent	1 x 10 mL	1 x 40 mL	2 x 50 mL

COMPOSITION

A. Reagent: Diethanolamine 1.2 mol/L, magnesium chloride 0.6 mmol/L, pH 9.8.

Harmful (Xn): R22: Harmful if swallowed. S28.1: After contact with skin, wash immediately with plenty of water. S45: In case of accident or if you feel unwell, seek medical advice immediately.

B. Reagent: 4-Nitrophenylphosphate 60 mmol/L.

STORAGE

Store at 2-8ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagents: Presence of particulate material, turbidity, absorbance of the blank over 1.200 at 405 nm (1 cm cuvette).

REAGENT PREPARATION

Working Reagent:

- Cod. 11590 and 11591: Transfer the contents of one Reagent B vial into a Reagent A bottle. Mix gently. Other volumes can be prepared in the proportion: 4 mL Reagent A + 1 mL Reagent B. Stable for 2 months at 2-8ºC.
- Cod. 11597 : Transfer 25 mL of one Reagent B vial into a Reagent A bottle. Mix gently. Other volumes can be prepared in the proportion: 4 mL Reagent A + 1 mL Reagent B. Stable for 2 months at 2-8ºC.

ADDITIONAL EQUIPMENT

– Analyzer, spectrophotometer or photometer with cell holder thermostatable at 25, 30 or 37ºC and able to read at 405 nm.

– Cuvettes with 1 cm light path.

SAMPLES

Serum and plasma collected by standard procedures.

Alkaline phosphatase in serum or plasma is stable for 7 days at 2-8ºC. Heparin may be used as anticoagulant.

PROCEDURE

1. Bring the Working Reagent and the instrument to reaction temperature.
2. Pipette into a cuvette: (Note 1)

Working Reagent	1.0 mL
Sample/Calibrator	20 µL

3. Mix and insert the cuvette into the photometer. Start the stopwatch.
4. Record initial absorbance and at 1 minute intervals thereafter for 3 minutes.
5. Calculate the difference between consecutive absorbances, and the average absorbance difference per minute (ΔA/min).

CALCULATIONS

The ALP concentration in the sample is calculated using the following general formula:

$$\Delta A/min \times \frac{Vt \times 10^6}{\varepsilon \times l \times Vs} = U/L$$

The molar absorbance (ε) of 4-nitrophenol at 405 nm is 18450, the lightpath (l) is 1 cm, the total reaction volume (Vt) is 1.02, the sample volume (Vs) is 0.02, and 1 U/L are 0.0166 µkat/L. The following formulas are deduced for the calculation of the catalytic concentration:

ΔA/min	x 2764 = U/L x 46.08 = µkat/L

REFERENCE VALUES

Reaction temperature	men[3]	women[3]
25ºC, up to	180 U/L = 3.00 µKat/L	160 U/L = 2.67 µKat/L
30ºC, up to	220 U/L = 3.67 µKat/L	195 U/L = 3.25 µKat/L
37ºC, up to	270 U/L = 4.50 µKat/L	240 U/L = 4.00 µKat/L

Concentrations in growing children are higher and highly variable.These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

– Detection limit: 1.6 U/L = 0.027 µkat/L.

– Linearity limit: 900 U/L = 15.0 µkat/L. For higher values dilute sample 1/2 with distilled water and repeat measurement.

– Repeatibility (within run):

Mean Concentration	CV	n
117 U/L = 1.95 µkat/L	1.1 %	20
431 U/L = 7.18 µkat/L	0.7 %	20

– Reproducibility (run to run):

Mean Concentration	CV	n
117 U/L = 1.95 µkat/L	4.5 %	25
431 U/L = 7.18 µkat/L	2.2 %	25

– Sensitivity: 0.362 ΔmA·L/U·min = 0.022 ΔmA·L/µkat·min

– Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

– Interferences: Lipemia (triglycerides < 10 g/L) and bilirubin (< 20 mg/dL) do not interfere. Hemoglobin (> 5 g/L) interfere. Other drugs and substances may interfere[4].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Alkaline phosphatase catalyzes the hydrolysis of organic phosphate monoesters at alkaline pH. The enzyme is present in practically all tissues of the body, especially at or in the cell membranes, and it occurs at particularly high concentrations in placenta, intestinal epithelium, kidney tubules, osteoblasts and liver.

The form present in the sera of normal adults originates mainly in the liver and bone.

Elevated serum ALP is found in patients with bone disease associated with increased osteoblastic activity (Paget's disease, primary and secondary hyperparathyroidism, bone tumors, rickets, osteomalacia, bone fractures) and also in patients with hepatobiliary disease (obstructive jaundice, hepatitis, hepatotoxicity caused by drugs, liver cancer). Physiological changes, such as bone growth and pregnancy, may cause increases in ALP levels[5,6].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.

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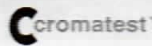
07/2012

APPENDIX H: ASSAY KIT FOR HDL



LINEAR Chemicals, S.L.

HDL-CHOLESTEROL CE

REF 1133010
2 x 40 mL
CONTENTS
R1. Reagent 2 x 40 mL
CAL. Standard 1 x 3 mL
For *in vitro* diagnostic use only

HDL-CHOLESTEROL
DIFFERENTIAL PRECIPITATION
Enzymatic colorimetric test
ENDPOINT

PRINCIPLE

This technique[1] uses a separation method based on the selective precipitation of apoliprotein B-containing lipoproteins (VLDL, LDL and (a)Lpa) by phosphotungstic acid/$MgCl_2$, sedimentation of the precipitant by centrifugation, and subsequent enzymatic analysis of high density lipoproteins (HDL) as residual cholesterol remaining in the clear supernatant.

REAGENT COMPOSITION

R1 **Precipitating reagent**. Phosphotungstic acid 0.63 mmol/L, magnesium chloride 25 mmol/L. Stabilizers.

CAL **Cholesterol standard**. Cholesterol 50 mg/dL 1.3 mmol/L). Organic matrix based primary standard. Concentration value is traceable to Standard Reference Material 1951a.

R2 **Cholesterol MR.** Optative. Ref: 1118005, 1118010, 1118015.

STORAGE AND STABILITY

Store at 2-8ºC.
All the kit compounds are stable until the expiry date stated on the label.
Discard If appear signs of deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 500 nm > 0.100 in 1cm cuvette.

REAGENT PREPARATION

The Reagents and Standard are ready-to-use. Store the vials tightly closed, protected from light and prevented contaminations during the use.

SAMPLES

Serum .EDTA or heparinized plasma, free of hemolysis. Obtained from the patient after an overnight fast. Remove from cells within 3 hours of venipuncture. Samples may be kept at 4-8ºC for 2 weeks, and at –20ºC for 3 months with no alteration of HDL cholesterol.
The supernate containing the HDL fraction is conveniently prepared on the day of sample collection and may be analysed after 2 weeks at 4-8ºC or 3 months at –20ºC in a non-selfdefrosting freezer.[2]

INTERFERENCES

- Lipemia (triglycerides 10 g/L) does not interfere.
- Bilirubin (10 mg/dL), hemoglobin (5 g/L), may affect the results.
- Other drugs and substances may interfere[3].

MATERIALS REQUIRED

I. *Precipitation*

- Dilutor and pipettes.
- Centrifuge tubes (13 x 100 m/m).
- Vortex mixer.
- Desktop centrifuge.

II . *Colorimetry*

- Kit for measuring Total Cholesterol.
- Constant temperature incubator set at 37ºC.
- Photometer or colorimeter capable of measuring absorbance at
- 500 ± 10 nm.

PROCEDURE

I. *Precipitation*

1. Bring reagents and samples to room temperature.
2. Pipette into labelled centrifuge tubes:

Sample or Standard	0.2 mL	Ratio $\frac{\text{Sample}}{\text{Reagent}} = \frac{1}{2}$
Precipitating reagent	0.4 mL	Dil. factor = 3

3. Vortex and allow to stand for 10 minutes at room temperature.
4. Centrifuge for 10 minutes at 4000 r.p.m., or 2 minutes at 12000 r.p.m.
5. Separate off the clear supernatant within 2 hours.

In case of turbid supernatants caused by elevated triglycerides (>350 g/dL) the sample should be diluted 1:2 with saline and steps 2,3,4 and 5 repeated. Multiply the result of the colorimetry by 2.

QUALITY SYSTEM CERTIFIED
ISO 9001 ISO 13485

LINEAR CHEMICALS S.L. Joaquim Costa 18 2ª planta. 08390 Montgat, Barcelona, SPAIN Telf. (+34) 934 694 990 Fax. (+34) 934 693 435. website www.linear.es

II. *Colorimetry*

1. Bring the Cholesterol MR Monoreagent and the cholesterol standard (50 mg/dL) of the kit to room temperature
2. Pipette into labelled tubes:

TUBES	Blank	Sample Supernat	Standard Supernat
Monoreagent	1.0 mL	1.0 mL	1.0 mL
Supernate	–	50 µL	–
Standard	–	–	50 µL

3. Mix and let the tubes stand for 10 minutes at room temperature or 5 minutes at 37ºC.
4. Read the absorbance (A) of the supernatant and the standard at 500 nm against the reagent blank.

The color is stable for at least 30 minutes protected from light.

CALCULATIONS

$$\frac{A_{Supernatant}}{A_{Standard}} \times C_{Standard} = \text{mg/dL HDL-Cholesterol}$$

If results are to be expressed as SI units apply:
mg/dL x 0.0259 = mmol/L

REFERENCE VALUES[4]

Clinical values of HDL-Cholesterol used to classify risk groups.

Cholesterol from lipoproteins of high density		RISK
Men	> 55 mg/dL (> 1.42 mmol/L)	Low
	35-55 mg/dL (0.90-1.42 mmol/L)	Moderate
	< 40 mg/dL (< 1.04 mmol/L)	High
Women	> 65 mg/dL (> 1.68 mmol/L)	Low
	45-65 mg/dL (1.16-1.68 mmol/L)	Moderate
	< 45 mg/dL (< 1.16 mmol/L)	High

QUALITY CONTROL

The use of a standard to calculate results allows to obtain an accuracy independent of the system or instrument used.
To ensure adequate quality control (QC) each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.
If the values are found outside of the defined range, check the instrument, reagents and procedure.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

CLINICAL SIGNIFICANCE

Low HDL-cholesterol is a strong independent predictor of coronary heart disease. In ATP III[4], low HDL cholesterol is defined categorically as a level < 40 mg/dL (1.04 mmol/L), a change from the level of < 35 mg/dL in ATPII (1993).

Low HDL cholesterol is used as a risk factor to estimate 10-year risk for coronary heart disease, having several causes: elevated triglycerides, overweigh and obesity, physical inactivity, and type 2 diabetes. Other causes are, cigarrete smoking, very high carbohydrate intakes (> 60% of calories), and certain drugs as anabolic steroids and progestional agents.

ANALYTICAL PERFORMANCE

- ***Detection limit:*** 1.2 mg/dL
- ***Linearity:*** Up to 200 mg/dL
- ***Precision:***

mg/dL	Within-run			Between-run		
Mean	42.1	45.8	54.6	42.1	45.8	54.6
SD	0.23	0.23	0.2	0.27	0.28	0.31
CV%	0.54	0.5	0.34	0.64	0.61	0.52
N	10	10	10	10	10	10

- ***Sensitivity:*** 2,5 mA/mg/dL HDL
- ***Correlation:*** This assay (y) was compared with a similar commercia method (x). The results were:

N = 25   r = 0.995   y = 0.985 + 2.6

The analytical performances have been generated using on automatic instrument. Results may vary depending on the instrument.

NOTES

1. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meets the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact to the distributor for any question on the application method.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

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B1133-2/0901
R1.ing

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APPENDIX I: ASSAY KIT FOR TRIGLYCERIDES

TRIGLYCERIDES
(Enzymax)

VITRO SCIENT.

INTENDED USE

Vitro triglycerides reagent is intended for the in vitro quantitative determination of triglycerides in serum and plasma on both automated and manual systems.

METHOD

Enzymatic colorimetric method (GPO/PAP) with glycerol phosphate oxidase and 4-aminoantipyrine.

Liquid stable single reagent.

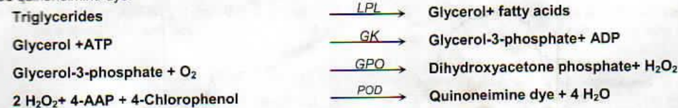
BACKGROUND

Triglycerides are esters of the trihydric alcohol glycerol with 3 long chain fatty acids. They are the main lipids present in human plasma; the others are cholesterol, phospholipids, and non-esterified fatty acids. Triglycerides are synthesized in the intestinal mucosa by the esterification of glycerol and free fatty acids. They are then released into the mesenteric lymphatics and distributed to most tissues for storage. Triglycerides are the main storage lipids in humans, where they constitute about 95% of adipose tissue lipids. Elevated levels of triglycerides have been associated with high risk in severe atherosclerosis. High triglycerides levels and hyperlipidemia in general can be an inherited trait or can be secondary to disorders including diabetes mellitus, nephrosis, biliary obstruction, and metabolic disorders associated with endocrine disturbances[1-3].

ASSAY PRINCIPLE

Triglycerides are generally determined by a combination of hydrolysis to glycerol and free fatty acids and measurement of the amount of glycerol released. The most commonly used methods involve alkaline hydrolysis and either chemical or enzymatic measurement of glycerol. Chemical means of analysis generally rely on measurement of the product of periodate oxidation of glycerol. Eggstein and Kreutz developed an enzymatic method for measuring glycerol released from triglycerides by alkaline hydrolysis[4]. This method was based on the coupled reaction sequence catalyzed by glycerol kinase, pyruvate kinase, and lactate dehydrogenase. A method for complete enzymatic hydrolysis to triglycerides avoiding the need for serum pretreatment was described by Bucolo and David, using a combination of lipase and at least one proteolytic enzyme[5]. Wahlefeld reported that certain esterases could be combined with a lipase to achieve complete triglycerides hydrolysis[6]. Both methods employed a coupled enzymatic reaction sequence[7] to measure glycerol. Vitro triglycerides reagent combines the use of lipoproteinlipase, glycerol kinase, and glycerol phosphate oxidase with the peroxidase/4-chlorophenol/4-aminoantipyrine system of Trinder[8] for the measurement of triglycerides in human serum. The series of reactions involved in the assay system are as follows:

1. Triglycerides are hydrolyzed by lipoprotein lipase (LPL) to glycerol and fatty acids.
2. Glycerol is then phosphorylated to glycerol-3-phosphate by ATP in a reaction catalyzed by glycerol kinase (GK).
3. The oxidation of glycerol-3-phosphate is catalyzed by glycerol phosphate oxidase (GPO) to form dihydroxyacetone phosphate and hydrogen peroxide (H_2O_2).
4. In presence of peroxidase (POD), the hydrogen peroxide (H_2O_2) formed effects the oxidative coupling of 4 - chlorophenol and 4-aminoantipyrine (4-AAP) to form a red-colored quinoneimine dye.



Triglycerides $\xrightarrow{LPL}$ **Glycerol+ fatty acids**

Glycerol +ATP $\xrightarrow{GK}$ **Glycerol-3-phosphate+ ADP**

Glycerol-3-phosphate + O_2 $\xrightarrow{GPO}$ **Dihydroxyacetone phosphate+ H_2O_2**

2 H_2O_2+ 4-AAP + 4-Chlorophenol $\xrightarrow{POD}$ **Quinoneimine dye + 4 H_2O**

The intensity of the color produced is directly proportional to triglycerides concentration. It is determined by measuring the increase in absorbance at 500 – 550 nm.

EXPECTED VALUES

Males:	40 – 160 mg/dl (0.45 – 1.82 mmol/l)
Females:	35 – 135 mg/dl (0.4 – 1.54 mmol/l)

For the recognition of the risk factor hyper-triglyceridemia. The following limits are recommended:

Suspicious	>150 mg/dl (1.71 mmol/l)
Elevated	>200 mg/dl (2.28 mmol/l)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes, the triglycerides results should always be assessed in conjunction with the patient's medical history, clinical examination, and other findings.

REAGENTS

R_1	Triglycerides standard	200 mg/dl
R_2	Pipes buffer, pH 7.8	50 mmol/l
	p-Chlorophenol	2.0 mmol/l
	Lipoprotein lipase	1500 U/l
	Glycerolkinase	800 U/l
	Glycerol phosphate oxidase	4000 U/l
	Peroxidase	1500 U/l
	4-Aminoantipyrine	0.4 mmol/l
	ATP	0.3 mmol/l
	Mg2+	40 mmol/l
	Sodium cholate	0.2 mmol/l

- **Reagent Preparation & Stability**

All reagents are ready for use and stable up to the expiry date given on label when stored at 2–8°C.

SPECIMEN

- Serum or plasma* from fasting patients.
- The only accepted anticoagulants are heparin and EDTA.

Specimen Preparation & Stability For serum specimen

Patients should refrain from eating for 10 to 14 hours before blood is drawn. Samples must be drawn in a soap and glycerol free collection device. Blood should be collected by venipuncture, after the patient has been in a seated position for at least 5 minutes. Tourniquet usage should be kept to a minimum and the specimen should be allowed to clot for 30 minutes at room temperature[8]. The best specimen is unhemolysed serum, and should be analyzed on the day of collection. Specimens are stable for 7 days when stored at 4°C; several months at –20°C and for years at –70°C[1].

PROCEDURE

- **Manual Procedure**

Wavelength	500 - 550 nm
Cuvette	1 cm light path
Temperature	20-25 or 37 °C
Zero adjustment	against reagent blank
Specimen	Serum or plasma

	Blank	Standard	Specimen
R_2	1.0 ml	1.0 ml	1.0 ml
Standard	......	10 µl	......
Specimen	......	......	10 µl

Mix, incubate for 5 minutes at 37°C or 10 minutes at 20-25°C. Measure the absorbance of specimen ($A_{specimen}$) and standard ($A_{standard}$) against reagent blank.

The color is stable for 60 minutes.

- **Automated Procedure**

User defined parameters for different auto analyzers are available upon request

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Article # 131-EN
Date of Revision : 10/2012

APPENDIX J: ASSAY KIT FOR TOTAL CHOLESTEROL

Cholesterol Liq

Enzymatic-colorimetric test.
CHOD-POD.Liquid.
Code HBL010 2 x 125 ml

CE

Store at 2-8°C. Standard Included.

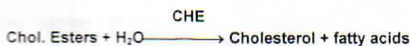
Clinical significance

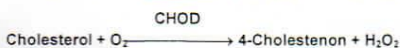
Cholesterol is fatty substance found in blood, bile and brain tissue; it serves as a precursor to bile acids, steroids and vitamin D. The determination of serum cholesterol is a major aid in the diagnosis and classification of lipemia. High blood cholesterol is one of the major risk factors for heart disease. Other conditions such as hepatic thyroid diseases influence cholesterol levels[4,5].

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

Principle

Cholesterol and its esters are released from lipoproteins by detergents. Cholesterol esterase hydrolyses the esters and H_2O_2 is formed in the subsequent enzymatic oxidation of cholesterol by cholesterol-oxidase according to the following equation. In the last reaction a red dye quinonimine dye is formed of which the intensity is proportional to the cholesterol concentration.


$$\text{Chol. Esters} + H_2O \xrightarrow{CHE} \text{Cholesterol} + \text{fatty acids}$$


$$\text{Cholesterol} + O_2 \xrightarrow{CHOD} \text{4-Cholestenon} + H_2O_2$$

$$2H_2O_2 + \text{4-AP} + \text{Phenol} \xrightarrow{POD} \text{Quinonimine} + 4H_2O$$

Reagents

Reagent	Pipes pH 7,0 ....70 mmol/l Phenol ....6 mmol/l Cholesterol esterase (CHE)...........400 U/l Cholesterol oxidase (CHOD).........400 U/l Peroxidase.............................1000 U/l 4-Aminophenazone (4-AP)......0,5 mmol/l
Standard	Cholesterol aqueous..............200 mg/dl

For *in vitro* diagnostic use only.

Preparation

All the reagents are ready to use.

Storage and stability

All the components of the kit are stable at 2-8°C up to the date of expiration as specified, when stored tightly closed, protected from light and contaminations prevented during their use. Handle standard very carefully to prevent contamination.

The reagent should be a clear solution. If turbidity or precipitation has occurred or if blank absorbance at 505 nm ≥ 0,26, the reagent should be discarded.

Additional equipment

- Spectrophotometer or colorimeter measuring at 505 nm
- Matched cuvettes 1,0 cm light path
- General laboratory equipment

Samples

Serum or plasma[1,2]: Stability of the sample for 7 days at 2-8°C or freezing at –20°C will keep samples stable for 3 months.

Procedure

1. Wavelength 505 nm (500-550); Temperature 37°C/15-25°C; Cuvette 1 cm light path.
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

	Blank	Standard	Sample
Standard[note1]	---	10 µl	---
Sample	---	---	10 µl
Working reagent	1 ml	1 ml	1 ml
Mix, incubate 5 min at 37°C or 10 min at 15–25°C. Measure the absorbance (Abs) of standard and sample against blank. The colour is stable for at least 60 min.			

Calculation

Cholesterol conc. (mg/dl)
= (Abs Sample / Abs Stand.) x 200 (stand. conc.)
Conversion factor: mg/dl x 0,0258 = mmol/l.

Quality control

Control sera are recommended to monitor the performance of assay procedures. If control values are found outside the defined range, check the instrument, reagents and calibrator for problems. Each laboratory should establish its own QC scheme and corrective actions if controls do not meet the acceptable tolerances.

Normal and pathological human (HBC01, HBC02) or bovine (HBC03, HBC04) sera are available.

Reference values

Less than 200 mg/dl	Normal
200-239 mg/dl	Borderline
240 mg/dl and above	High

These values are for orientation purpose. Each laboratory should establish its own reference range.

Performance characteristics

Measuring range: from 0,113 mg/dl (detection limit) to 750 mg/dl (linearity limit). If the obtained results are greater than 750 mg/dl, dilute the sample 1:2 with saline solution, repeat the determination, and multiply the result by factor 2.

Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dl)	98,59	186,02	96,55	184,09
SD	3,43	3,64	2,67	6,92
CV (%)	3,48	1,95	2,77	3,76

Sensitivity: 1 mg/dl = 0,00143 A

Accuracy: Results obtained using CYPRESS DIAGNOSTICS reagents did not show systematic differences when compared with other commercial reagents.

Interferences

Hemoglobin up to 4,5 g/l, ascorbic acid up to 10 mg/dl and bilirubin up to 40 mg/dl do not interfere. A list of drugs and other interfering substances with cholesterol determination has been reported by Young et al.

Notes

1. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In this case, it is recommended to use a serum calibrator (HBC03).

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4. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999
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Langdorp, 06.2011

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APPENDIX K: ASSAY KIT FOR HBALC

CERA-STATTM 2000 HbA1c Test Kit

Read this entire insert thoroughly before using the CERA-STAT™ 2000 HbA1c Test Kit. Only use the CERA-STAT™2000 HbA1c Test Kit with the CERA-STAT™ 2000 Analyzer. Keep this insert for future reference. If you have any inquiry or question, please contact your local distributor.

Product description

- Intended use

The CERA-STAT™ 2000 HbA1c Test Kit is intended for the quantitative determination of glycated hemoglobin in human blood. It also provide the Estimated average glucose(eAG) value, in addition to the glycated hemoglobin result, allowing for easier diabetes care.

- Test principle

The CERA-STAT™ 2000 is a boronate affinity assay. The CERA-STAT ™ 2000 HbA1c Test Kit consists of the cartridges, the R1/Reagent and the R2/Reagent. The R1/Reagent contains the agents that lyse erythrocytes and precipitate hemoglobin specifically, as well as a blue boronic acid conjugate that binds cis-diol of glycated hemoglobin. When blood is added to the R1/Reagent, the erythrocytes are lysed and all hemoglobin precipitates, as well as the boronic acid conjugate binds to the cis-diol configuration of glycated hemoglobin. An aliquot of the reaction mixture is added to the cartridge and all the precipitated hemoglobin, conjugate-bound and unbound, remains on top of the filter. Any unbound boronate is removed with the R2/Reagent. The precipitate is evaluated by measuring the blue (glycated hemoglobin) and the red (total hemoglobin) color intensity respectively with the CERA-STAT™ 2000 Analyzer, the ratio between them being proportional to the percentage of glycated hemoglobin in the sample.

- Test Kit contents
  - Cartridge(with the membrane filter) .... 30 units
  - R1/Reagent .... 30 X 0.2 mL
  - R2/Reagent .... 2.0 mL
  - Insert Paper .... 1 Sheet

- Reagent composition

R1/Reagent
- Boronate derivative .... 0.04 mg
- Organic solvent .... 6.2 %
- Lysing agent .... 0.15 %

R2/Reagent
- Detergent .... 0.5 %

Catridge
- Filter (Glass Fiber)
- Membrane(Nylon)
- Absorption pad (Glass Fiber)

- Material needed (not supplied with the kit)
  - Capillary tubes
  - Capillary tube holder
  - Volume fixed pipette and pipette tips
  - CERA-STAT™ 2000 Analyzer

- Warnings and precautions
  - For in vitro diagnostic use only.
  - Do not transfer components from or to any different kit lots.
  - Do not use the kit after the expiration date.
  - The R1/Reagent and R2/Reagent contain a toxic agent(0.05%). Avoid direct contacts to the skin.
  - Do not drink the R1/Reagent and R2/Reagent.
  - The R1/Reagent and cartridge are single use only.
  - Dispose of used reagents and cartridges according to the local guidelines.
  - Exercise the normal precautions required for handling all laboratory reagents.
  - Blood specimens, used reagents, pipette tips and tubes should be considered potentially infectious.
  - This CERA-STAT™ 2000 HbA1c Test Kit shall be used with the CERA-STAT™ 2000 Analyzer only. Do not use it with other brands' analyzers.
  - Change the pipette tip between each pipetting step.
  - The test will be applied on a routine basis and not in emergency situation.

Test characteristics

- Measuring range

HbA1c : 3.0 ~ 15.0 % or 9 ~ 140 mmol/mol
eAG : 39 ~ 384 mg/dL or 2.2 ~ 21.3 mmol/L

- Measuring interval

HbA1c : 0.1 % or 1 mmol/mol
eAG : 1 mg/dL or 0.1 mmol/L

- Reference range[1]

	NGSP	IFCC
Prediabetes	5.7~6.4 %	39~46 mmol/mol
Presence of diabetes	≥6.5 %	≥48 mmol/mol
Target in diabetes	<7.0 %	<53 mmol/mol

- Accuracy

The Accuracy of the CERA-STAT™ 2000 system was evaluated at three clinical sites from 120 Patients with replicate measurement. The correlation obtained between CERA-STAT™ 2000 system results and the reference method was : N=120, y=1.0134x-0.0863; R^2=0.9851

- Precision

The precision of the CERA-STAT™ 2000 system was estimated with venous blood samples and control solution in the laboratory. Readings obtained with the CERA-STAT™ 2000 system were compared to those obtained using Tosoh HLC-723 GHb G7(Tosoh Bioscience).

Within Run Precision(venous blood)

HbA1c concentration(%)	5.5	8.6	11.3
Mean	5.5	8.7	11.4
STD	0.15	0.21	0.27
CV(%)	2.7	2.4	2.3

Day to Day Precision(control solution)

HbA1c concentration(%)	5.3	8.7	11.4
Mean	5.3	8.6	11.4
STD	0.17	0.23	0.32
CV(%)	3.1	2.7	2.8

- Limitations of the test
  - Operation temperature and humidity
    Temperature Range: 15~35℃(59~95°F), the recommended is 20~25℃(68~77°F)
    Humidity Range: 15~75 % RH
  - The reagent must be stored in the designated temperature range (2~8℃). If the reagents are stored in the temperature out of the designated temperature range(2~8℃), the test result can be inaccurate.
  - Do not keep the reagents for more than 3 hours in room temperature.
  - Use only fresh capillary whole blood or venous blood. Do not use serum or plasma.
  - Interference substances

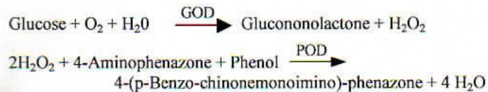
APPENDIX L: ASSAY KIT FOR FASTING BLOOD SUGAR

GLUCOSE PAP FLUID MONOREAGENT

Enzymatic Colorimetric Test Method (GOD-PAP method) without Deproteinization

MEASUREMENT PRINCIPLE

The glucose oxidized by glucose oxidase in the presence of oxygen to gluconolactone. The formed hydrogen peroxide reacts under catalysis of peroxidase with phenol and 4-Aminophenazone to a red-violet quinoneimine dye as indica-tor. The color intensity of the red dye is directly proportional to the glucose concentration.



$$\text{Glucose} + O_2 + H_2O \xrightarrow{GOD} \text{Gluconolactone} + H_2O_2$$

$$2H_2O_2 + \text{4-Aminophenazone} + \text{Phenol} \xrightarrow{POD} \text{4-(p-Benzo-chinonemonoimino)-phenazone} + 4\ H_2O$$

CONCENTRATION OF WORKING SOLUTION

Monoreagent	
Phosphate buffer pH 7.5	0.1 mol/l
4-Aminophenazone	0.3 mmol/l
Phenol	1 mmol/l
Glucose oxidase	> 20.0 KU/l
Peroxidase	> 1.5 KU/l
Stabilizers	
(Standard	100 mg/dl)

STORAGE AND STABILITY

The sealed reagents are stable up to the indicated expiry date if stored at +2° - +8°C.

INTENDED USE

In vitro test for the quantitative determination of Glucose in human serum and plasma.

PRECAUTIONS AND WARNINGS

For in vitro diagnostic use only. Attend to the normal precautions required for handling all laboratory reagents. The reagent contain sodium azide as preservative. Do not swallow. Avoid contact with skin and mucous membranes

EXPECTED VALUES

Serum, Plasma (fasting)

		mg/dl	mmol/l
Newborn		40 – 80	2.22 – 4.44
Children:		60 – 110	3.33 – 6.11
Adults:		70 – 105	3.89 – 5.83
	> 60 y	80 – 115	4.44 – 6.38
	> 70 y	83 – 110	4.61 – 6.11

SAMPLE MATERIAL

Serum, Plasma. The glucose is stable for 24 hours at +2° - +8°C, if serum or plasma is prepared within 30 min. after collection.

QUALITY CONTROL

Centronorm, Centropath or all other control sera with values determinated by this method may be employed.

LINEARITY

The test is linear up to glucose concentration of 400 mg/dl or 22.2 mmol/l. If the glucose concentration of the sample is over this limit dilute the sample 1+2 with physiollogical saline (0.9%) and repeat the determination. Multiply the result by 3.

CONVERSION FACTOR:

mg/dl ⇨ mmol/l Factor: 0.0555

mmol/l ⇨ mg/dl Factor: 18.02

APPLICATIONS

Applications are available for many analysers

TEST SPECIFICATIONS

Reproducibility in Intraassay and Interassay on Hitachi 717:

	Within run (n= 30)			Between run (n= 30)		
Sample	Mean mg/dl	SD mg/dl	%CV	Mean mg/dl	SD mg/dl	%CV
Level 1	77.6	1.40	1.80	81.6	1.96	2.40
Level 2	178.1	2.66	1.49	187.2	3.31	1.77
Level 3	244.8	2.90	1.18	265.4	4.36	1.64

TEST COMPARISION

Comparision with another commercial available test (Hexokinase method) on the Hitachi 717:

$n = 20;\ r = 0.9998;\ y = 1.016x - 1.899$

ASSAY PROCEDURE

Wavelength : **500 nm, 546 nm**
Temperature : **25° or 37° C**
Measurement: **against reagent blank**
Only one reagent blank per series is required.

Pipette into cuvettes:

Reagent	1000 µl
Standard/Sample	10 µl

Mix, incubate for 15 min. at 25°C or 10 min. at 37°C. Measure the absorbance of the standard and the sample against the reagent blank within 60 min. (δA)

CALCULATION

with standard

$$c = 100 \times \frac{\delta A\ (\text{sample})}{\delta A\ (\text{standard})}\ (\text{mg/dl})$$

$$c = 5.55 \times \frac{\delta A\ (\text{sample})}{\delta A\ (\text{standard})}\ (\text{mmol/l})$$

LITERATURE

Barham, D., Trinder, P.: Analyst 97, (1972), 142-145
Teuscher, A., and Richterrich, P.,: Schweiz med. Wschr. 101 (1971), 345 and 390

PACKAGE SIZES

KIT SIZE		ORDER NO.
4 x 100 ml	manual	GF03000100
1 x 400 ml	manual	GF03R05100
2 x 500 ml	manual	GF03000500
1 x 1000 ml	manual	GF03001000
10 x 50 ml	for Hitachi 704	GF03704050
5 x 100 ml	for Hitachi 717	GF03717100
10 x 50 ml	for Hitachi 911	GF03911050
5 x 100 ml	for Hitachi 911	GF03911100

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Edition 04/2008, TV-040-CE-002

APPENDIX M: ASSAY KIT FOR CHROMIUM

QuantiChrom™ Chromium Assay Kit (DCRM-250)

Quantitative Colorimetric Determination of Chromium (VI)

DESCRIPTION

CHROMIUM is widely used in various industries such as electroplating, leather tanning, chrome paint, dying, hardened steel, ceramic and glass industry. Chromium exists in two stable oxidation states, hexavalent Cr(VI) and trivalent Cr(III). Cr(VI) is produced solely by industrial processes, whereas in nature, chromium exists in its trivalent form. Cr(III) is generally regarded as nontoxic due to poor absorption. Cr(VI) is considered a pulmonary carcinogen and has tested positive in genotoxicity tests. It is one of the most serious pollutants in many water streams due to its carcinogenic potential. Most countries apply a legal limit of 50-100 µg/L Cr in drinking water.

BioAssay Systems' Chromium Assay Kit provides a simple one-step colorimetric means to directly measure Cr(VI) in a sample. In the assay, Cr(VI) forms a stable complex with a specific chromogenic dye. The optical density at 480nm is directly proportionate to the Cr(VI) concentration in the sample. Cr(III) can be converted to Cr(VI) with nitric acid/hydrochloric acid, thus allowing the determination of Cr(III) or total Cr [Cr(III) + Cr(VI)] in the sample. The assay is sensitive with a detection limit of 20 µg/L Cr.

KEY FEATURES

Sensitive and accurate. Linear detection range of 20 - 2000 µg/L Chromium.

Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. No wash and reagent transfer steps are involved. Can be readily automated for processing thousands of samples per day.

APPLICATIONS

Determination of chromium in biological (serum, plasma etc), environmental (water, soil etc), food and beverage samples.

KIT CONTENTS (sufficient for 250 tests in 96-well format)

Reagent A: 300 µL **Reagent B:** 20 mL
Cr(VI) Standard: 300 µL 40 mg/L

Storage conditions: store Reagent A at -20°C and other reagents at 2-8°C. Shelf life of 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

96-WELL ASSAY PROCEDURE

Use clear flat-bottom plates. Prior to assay, bring all reagents to room temperature. Unused Reagent A should be stored at -20°C.

Samples should be clear, colorless and free from particles or precipitates. Substances that may potentially interfere with the assay include: azide, Ba^{2+}, Pb^{2+}, Fe^{3+}, Gold(III), Sn(II), Ti (IV).

If necessary, water samples can be concentrated by evaporation. If determination of Cr(III) or total Cr [Cr(III) + Cr(VI)] is desired, please refer to the General Sample Treatment Procedure.

1. *Standards.* Prepare 600 µL 2000 µg/L Cr(VI) Standard Premix by mixing 30 µL Standard and 570 µL deionized water dH_2O (>18 megaohm). Dilute standard as follows.

No	Premix + dH_2O	Standard (µg/L)
1	300 µL + 0 µL	2000
2	150 µL + 150 µL	1000
3	75 µL + 225 µL	500
4	0 µL + 300 µL	0

Transfer 250 µL standards into separate wells of the plate.

Samples: transfer 250 µL of each sample into separate wells of the plate.

2. *Assay.* Prepare enough Working Reagent, for each well, by mixing 1 µL Reagent A and 55 µL Reagent B. Add 50 µL Working Reagent to each well. Tap plate to mix. Incubate for 20 min at room temperature.

Read optical density at 480 nm (430-505nm).

CUVETTE ASSAY PROCEDURE

The cuvette assay procedure is essentially the same as the 96-well plate assay. The Working Reagent is prepared by mixing 4 µL Reagent A and 220 µL Reagent B. Assay by mixing 1000 µL Standard or Sample with 200 µL Working Reagent.

CALCULATION

Subtract the blank control OD (#4) from the OD values of the standards. Plot the Standard Curve and determine its Slope. Cr(VI) concentration of a Sample is calculated as,

$$[Cr(VI)] = \frac{OD_{SAMPLE} - OD_{BLANK}}{Slope} \times n \quad (\mu g/L)$$

where OD_{SAMPLE} and OD_{BLANK} are the OD_{480nm} values of the Sample and Blank Control (#4), respectively. Slope is the slope of the standard curve.

Note: if the Sample Cr(VI) concentration is higher than 2000 µg/L, dilute sample in deionized water and repeat the assay. Multiply result by the dilution factor *n*.

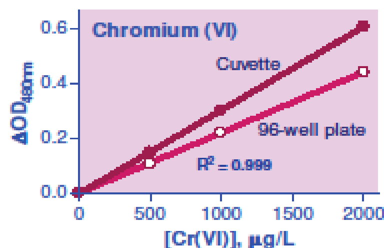
Conversion factor: 1000 µg/L chromium is equivalent to 19.2 µM or 1 ppb.

GENERAL SAMPLE TREATMENT PRECEDURE

The following procedure converts Cr(III) in a sample to Cr(VI) by oxidation with nitric acid. This experiment should be performed with special care in a chemical fume hood. Weigh 0.5 g solid sample (e.g. alloy, food, hair), or transfer 1-2 mL blood or serum samples, into a 50 mL beaker. Add 10 mL concentrated HNO_3 and 1 mL concentrated HCl. Cover with a watch glass until the initial brisk reaction is subsided. Add another 5 mL concentrated HNO_3 and heat the solution gently until all carbides are decomposed. After cooling down to room temperature, neutralize the solution with 3% ammonia. Filter the solution with Whatman No. 42 and use the filtrate for assay.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat bottom 96-well plates, plate reader, cuvette, spectrophotometer, concentrated HNO_3, concentrated HCl, ammonia and chemical hume hood.



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APPENDIX N: PLACEBO INFORMATION

powerhealth

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www.powerhealth.co.uk

SPECIFICATION

Product: **PLACEBO HARD GEL CAPSULES**

Product Code: **ARMA01P**

Spec No: **HG: 0292**

Fill Weight: **250mg**
47mg

Shell Weight: **Average**

Ingredients in Weight Descending Order	Function	Provides Active
Stoneground Brown Rice Flour	Filler	N/A

Shell Composition: Bovine Gelatin Size 3 (Average 47mg)
No Preservatives

Characteristics

Description:	Beige Powder Filled Into Two-Piece Clear Gelatin Capsules
Odour:	Characteristic
Taste:	Characteristic
Fill Wt. Uniformity:	250mg (+/- 7·5%) Range NLT 232mg - NMT 268mg
Disintegration:	NMT 30 Minutes
Shelf Life:	3 Years in Specified Storage Conditions

Spec Issue No: 001
Date Effective: August 2012

Company Directors: M D McIver A C McIver C Brookshaw V C McIver H E Whilesmith G Morgan
Registered No. 1041196 England

APPENDIX O: CHROMIUM SUPPLEMENTS INFORMATION

POWER HEALTH PRODUCTS LTD
Airfield Estate, Pocklington, York, YO42 1NR. UK
Tel: 01759 302595 Fax: 01759 304286
www.powerhealth.co.uk

CERTIFICATE OF CONFORMITY

DATE: 18.10.12

CUSTOMER: ARMAAN LTD - KENYA

SPEC No: HG: 0290

PRODUCT: CHROMIUM PICOLINATE 500iu/g HARD GEL CAPSULES

Manufactured by: Power Health Products Ltd
Airfield Industrial Estate
POCKLINGTON, York.
YO42 1NR. ENGLAND. U.K.

PRODUCT CODE: ARMA01

BATCH NUMBER: 114396 **QTY:** 378 X 1

MANUFACTURED: AUGUST 2012 **EXPIRY DATE:** AUGUST 2015

COLOUR: Beige Free-Flowing Powder Filled Into Two-Piece Clear, Hard Gelatin Capsule **SIZE:** '3' Average 47mg

PHYSICAL CHARACTERISTICS	TOLERANCE	RESULTS
APPEARANCE	INTERNAL	CONFORMS
ORGANOLEPTIC	INTERNAL	CONFORMS
FILL WEIGHT UNIFORMITY	BP 1988	CONFORMS

CONTENTS	mg/cap	LABEL CLAIM	RESULTS
			INPUT CHECK
Stoneground Brown Rice Flour	249.50	N/A	CONFORMS
Chromium Picolinate	0.50	Chromium Picolinate 500iu/g	CONFORMS

FILL WEIGHTS	-	Average:	250mg (+/- 7.5%)
	-	Range:	NLT 268mg – NMT 232mg
SHELL WEIGHT:	-	Average	47mg

Shell Composition: Bovine Gelatin
No Preservatives

REMARKS:

SIGNED:

Quality Assurance

Shelf Life and Stability: Verified to maintain label potency and physical characteristics for three years under specified storage conditions

Storage Conditions: Store in a cool dry place below

Company Directors: M D McIver A C McIver C Brookshaw V C McIver H E Whilesmith G Morgan
Registered No. 1041196 England

APPENDIX P: GMP CERTIFICATE

The Health Food Manufacturers' Association
1 Wolsey Road
East Molesey
Surrey KT8 9EL

t 020 8481 7100
f 020 8481 7101
e hfma@hfma.co.uk

CERTIFICATE OF GOOD MANUFACTURING PRACTICE

To whom it may concern

This is to certify that Power Health Products Limited have registered with us as manufacturers following Good Manufacturing Practice (GMP) for manufacturing products derived from natural source.

GMP requires that quality checks on the various stages of manufacture and packaging are carried out as appropriate and these tests and checks are carried out in accordance with various Pharmacopoeial and other guidelines to ensure that the principles of Good Manufacturing Practice are in place.

Signed:

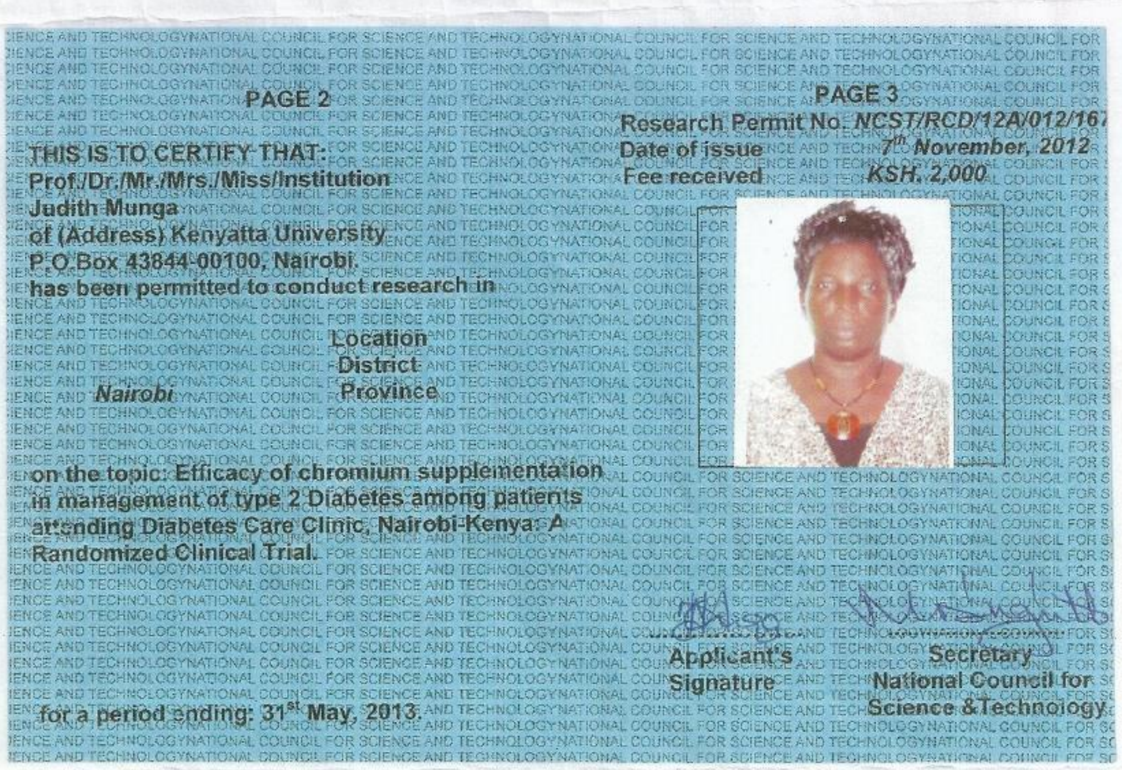
Date: 29th July 2010

Graham Keen
Executive Director

The Health Food Manufacturers' Association is registered in England
as a company limited by guarantee Company No. 5873676 VAT No. 233 6384 65
Registered Office: 1 Wolsey Road, East Molesey, Hampton Court, Surrey KT8 9EL

www.hfma.co.uk

APPENDIX Q: NACOSTI PERMIT



PAGE 2

THIS IS TO CERTIFY THAT:
Prof./Dr./Mr./Mrs./Miss/Institution
Judith Munga
of (Address) Kenyatta University
P.O.Box 43844-00100, Nairobi.
has been permitted to conduct research in

Location
District
Nairobi Province

on the topic: Efficacy of chromium supplementation in management of type 2 Diabetes among patients attending Diabetes Care Clinic, Nairobi-Kenya: A Randomized Clinical Trial.

for a period ending: 31st May, 2013.

PAGE 3

Research Permit No. *NCST/RCD/12A/012/167*
Date of issue *7th November, 2012*
Fee received *KSH. 2,000*

Applicant's Signature

Secretary
National Council for Science &Technology

APPENDIX R: NACOSTI APPROVAL

REPUBLIC OF KENYA

NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

Telephone: 254-020-2213471,2241349
254-020-310571,2213123, 2219420
Fax: 254-020-318245,318249
when replying please quote
secretary@ncst.go.ke

P.O. Box 30623-00100
NAIROBI-KENYA
Website: www.ncst.go.ke

Our Ref:

NCST/RCD/12A/012/167

Date:

7th November 2012

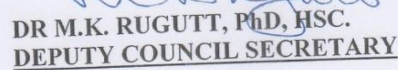
Judith Munga
Kenyatta University
P.O.Box 43844-00100
Nairobi.

RE: RESEARCH AUTHORIZATION

Following your application for authority dated ***29th October, 2012*** to carry out research on "***Efficacy of chromium supplementation in management of type 2 Diabetes among patients attending Diabetes Care Clinic, Nairobi-Kenya: A Randomized Clinical Trial,"*** I am pleased to inform you that you have been authorized to undertake research in **Nairobi Province** for a period ending **31st May, *2013.***

You are advised to report to **the Provincial Commissioner, the Provincial Director of Education and the Provincial Director of Medical Services, Nairobi Province** before embarking on the research project.

On completion of the research, you are expected to submit **two hard copies and one soft copy in pdf** of the research report/thesis to our office.



DR M.K. RUGUTT, PhD, HSC.
DEPUTY COUNCIL SECRETARY

Copy to:

The Provincial Commissioner
The Provincial Director of Education
The Provincial Director of Medical Services
Nairobi Province.

"The National Council for Science and Technology is Committed to the Promotion of Science and Technology for National Development".

APPENDIX S: APPROVAL FROM GRADUATE SCHOOL

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: kubps@yahoo.com
dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 8710901 Ext. 57530

Internal Memo

FROM: Dean, Graduate School **DATE:** 2nd July, 2013

TO: **Ms. Judith Munga** **REF:** H87/13219/09
C/o Foods, Nutrition & Dietetics Dept.

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that the Graduate School Board, at its meeting of **9th July, 2012**, approved your research proposal for the Ph.D. degree entitled **"Efficacy of Chromium Supplementation in Management of Type 2 Diabetes among Patients Attending Diabetes Care Clinic, Nairobi-Kenya: A Randomized Clinical Trial".**

You may now proceed with your data collection.

Thank you.

JOHN M. ODONGI
FOR: DEAN, GRADUATE SCHOOL

c.c. Chairman, Foods, Nutrition & Dietetics Department

Supervisors:

1. Prof. Judith Kimiywe
   Foods, Nutrition & Dietetics Department
2. Dr. Sophie Ochola
   Foods, Nutrition & Dietetics Department
3. Prof. Hudson Nyambaka
   Chemistry Department
4. Dr. George M. Moturi
   Medicine, Therapeutics & Psychiatry Department

JMO/*cww*

APPENDIX T: CHANGE OF STUDY LOCATION APPROVAL

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
admissions-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 8710901 Ext. 57530

Internal Memo

FROM: Dean, Graduate School **DATE:** 22nd January, 2013

TO: **Judith Munga** **REF:** H87/13219/09
C/o Foods, Nutrition & Dietetics Dept.

SUBJECT: CHANGE OF STUDY LOCATION

==

Your request to change study location from Diabetes Care Clinic in Nairobi to Thika Level 5 Hospital in Thika Town was approved in the Graduate School Board meeting held on 16th January, 2013.

Thank you.

JOSEPHINE K. NJAGI
FOR: DEAN, GRADUATE SCHOOL

CC. Prof. Judith Kimiywe
Foods, Nutrition & Dietetics Department

Dr. Sophie Ochola
Foods, Nutrition & Dietetics Department

Prof. Hudson Nyambaka
Chemistry Department

JKN/cww

Kenyatta University ...ISO 9001: 2008 Certified

APPENDIX U: KENYATTA UNIVERSITY ERC APPROVAL

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Fax: 8711242/8711575
Email: kuerc.chairman@ku.ac.ke
kuerc.secretary@ku.ac.ke
Website: www.ku.ac.ke

P. O. Box 43844
Nairobi, 00100
Tel: 8710901/12
Tel: 8710901/12

Our Ref: KU/R/COMM/51/94

Date: October 22nd 2012

Judith Munga
School of Applied Human Sciences
Kenyatta University
P.O. Box 43844, Nairobi.

Dear Ms. Munga,

APPLICATION NUMBER **PKU/060/I53** OF 2012 - **'EFFICACY OF CHROMIUM SUPPLEMENTATION IN MANAGEMENT OF TYPE 2 DIABETES AMONG PATIENTS ATTENDING DIABETES CARE CLINIC, NAIROBI – KENYA: A RANDOMIZED CLINICAL TRIAL – *VERSION 2'*.**

1. IDENTIFICATION OF PROTOCOL

The application before the committee is with a research topic - **Efficacy of Chromium Supplementation in Management of type 2 Diabetes Among Patients Attending Diabetes Care Clinic Nairobi, Kenya: a Randomized Clinical Trial** – ***version 2*** dated 10th October 2012.

2. APPLICANT

Judith Munga
School of Applied Human Sciences
Kenyatta University
P. O. Box 43844, Nairobi.

3. SITE

Nairobi, Kenya

4. DECISION

The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee Guidelines, and is of the view that against the following elements of review,

(i) Scientific design and conduct of study,
(ii) Recruitment of research participant,
(iii) Care and protection of research participants,
(iv) Protection of research participant's confidentiality,
(v) Informed consent process,
(vi) Community considerations.

AND APPROVED that the research may proceed for a period of ONE year from 22nd October, 2012.

5. **ADVICE/CONDITIONS**

i. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.

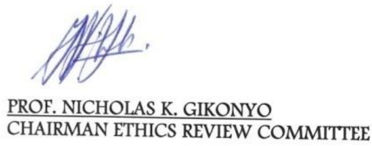
ii. Serious and unexpected adverse events related to the conduct of the study are reported to this board immediately they occur.

iii. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.

iv. Submit an electronic copy of the revised proposal to KU-ERC.

When replying, kindly quote the application number above.

If you accept the decision reached and advice and conditions given please sign in the space provided below and return to KU-ERC a copy of the letter.



PROF. NICHOLAS K. GIKONYO
CHAIRMAN ETHICS REVIEW COMMITTEE

I ...Judith Mungo............ accept the advice given and will fulfill the conditions therein.

Signature.................................. Dated this day ...23rd... of ...OCTOBER... 2012.

cc. Vice-Chancellor
Director: Institute for Research Science and Technology

APPENDIX V: THIKA LEVEL 5 HOSPITAL ERC APPROVAL

MINISTRY OF HEALTH

Tel.Thika 067 21621/2 fax 21778
All correspondence should be addressed to
MED.SUPT.
When replying please quote

Ref; NO. MOH/TKA/

THIKA LEVEL 5 HOSPITAL
P.O. BOX 227
THIKA

Date: 11th January, 2013

TO: JUDITH MUNGA

REF: RESEARCH APPROVAL

Title: **CHROMIUM SUPPLEMENTATION IN MANAGEMENT OF BLOOD SUGAR AMONG TYPE 2 DIABETICS ATTENDING THIKA LEVEL 5 HOSPITAL . A RANDOMIZED CONTROLLED STUDY**

Having discussed your research proposal, the Thika Level 5 Hospital research and ethics committee hereby gives you the green light to conduct above research after you clear the requisite fees.

You are adviced to strictly adhere to the data collection period as you outlined in the proposal. Request for extra data collection time must be made to the committee in writing. You are further advised to strictly stick to research ethics and staff and patients/clients confidentiality must not be breached.
Any data or information you may come across which does not form part of your research must not be used/ broadcast/divulged to other people without express authority of the hospital Medical Superintendent.

As you conduct your research, you will be attached to **Dr. Mbogo M. D** during your data collection.

On completion of the research you are expected and required to inform the hospital of your findings. This gives you an opportunity to help improving the provision of quality heath care at Thika Level 5 hospital.

In case you are found to contravene or violate the code of ethics the hospital reserves the right to terminate your research without prior warning.

We look forward to the findings of the research and we wish you the best.

Thank you.

Dr. Mbogo
Chair
Research & Ethics committee
Thika level 5 hospital

I...Judith Munga...Agree and will adhere to the above terms.
Signed...........
Date..14/1/2013..

APPENDIX W: PILOT STUDY FINDINGS

Pilot study findings on Socio-demographic characteristics

Characteristics	Study Groups			p-value (2 sided)
	Intervention N=11 (%)	**Control N=9 (%)**	**Total N=20 (%)**	
Gender				
Male	29.3	44	38	0.062
Female	70.7	56	62	
Age (years)				
20-35	5.48	6.3	7.1	0.473
36-45	48.6	42.1	45.3	
46-55	39.7	44.2	35.9	
56-65	6.2	8.4	11.7	
Education Level				
Primary	58.1	54.8	56.5	0.865
Secondary	33.1	36.4	32.5	
Tertiary	7.0	7.5	8.9	
University	1.8	2.3	2.1	
Marital status				
Single	10.3	9.8	9.7	0.510
Married	86.2	84.9	88.1	
Divorced/ Widowed	3.5	5.3	3.2	
Socio-economic status (SES)				
Lower SES	37.4	41.2	39.3	0.697
Middle SES	40.7	36.9	36.5	
Higher SES	21.9	21.9	24.2	

APPENDIX X: GPAQ ANALYSIS GUIDELINES

Global Physical Activity Questionnaire
(GPAQ)

Analysis Guide

Surveillance and Population-Based Prevention
Prevention of Noncommunicable Diseases Department
World Health Organization
20 Avenue Appia, 1211 Geneva 27, Switzerland
For further information: www.who.int/chp/steps

Global Physical Activity Questionnaire (GPAQ) Analysis Guide

Table of contents

1 Overview 2
2 The questionnaire 4
3 GPAQ Question by Question Guide 6
4 Cleaning GPAQ data 9
5 Cleaning GPAQ data with EpiInfo 11
6 Analysis Guidelines and Calculations 14

1 Overview

Introduction	The Global Physical Activity Questionnaire was developed by WHO for physical activity surveillance in countries. It collects information on physical activity participation in three settings (or domains) as well as sedentary behaviour, comprising 16 questions (P1-P16). The domains are: • Activity at work • Travel to and from places • Recreational activities
Using GPAQ	Prior to using GPAQ, you should review the question by question section. This section, which follows the actual questions, will guide the interviewer in asking the questions and recording responses. When using GPAQ, all the questions must be asked. Skips of questions do ONLY apply to the corresponding day and time variables if P1, P4, P7, P10, or P13 have been answered negatively. Skipping any other questions or removing any of the domains will restrict the results that you will be able to calculate.
Administration of the GPAQ	The GPAQ has been developed for face-to-face interviews conducted by trained interviewers. It had been tested in large scale population-based surveys with the general adult population.
Show cards	It is advised that show cards be used when the GPAQ is administered. Show cards should be developed for each of the activity types covered by the GPAQ: vigorous and moderate activity at work, transport activity, vigorous and moderate activity during leisure time, as well as sitting. Show cards will help the respondents to know what activities are meant by each question. They should be showing typical physical activities for the setting that the GPAQ is used in. Examples of generic show cards that will need to be adapted to the local context can be found on the GPAQ website: http://www.who.int/chp/steps/GPAQ/en/index.html
GPAQ version 1 and 2	This document provides information on version 2 of GPAQ. It is advised that you use version 2 of GPAQ. If you have already used GPAQ version 1 and need advise on analysing this information, please contact the STEPS team at steps@who.int.
Calculating and cleaning physical activity data	This document includes information on how to clean and analyse GPAQ data in general as well as specifically with the statistical package EpiInfo. The coding column of GPAQ is used as a reference for all the calculations. If you insert this questionnaire into another questionnaire, you should not change the coding column.

Continued on next page

1 Overview, Continued

Metabolic Equivalent (MET)

METs (Metabolic Equivalents) are commonly used to express the intensity of physical activities, and are also used for the analysis of GPAQ data.

MET is the ratio of a person's working metabolic rate relative to the resting metabolic rate. One MET is defined as the energy cost of sitting quietly, and is equivalent to a caloric consumption of 1 kcal/kg/hour. For the analysis of GPAQ data, existing guidelines have been adopted: It is estimated that, compared to sitting quietly, a person's caloric consumption is four times as high when being moderately active, and eight times as high when being vigorously active.

Therefore, when calculating a person's overall energy expenditure using GPAQ data, 4 METs get assigned to the time spent in moderate activities, and 8 METs to the time spent in vigorous activities.

2 The questionnaire

Physical Activity		
Next I am going to ask you about the time you spend doing different types of physical activity in a typical week. Please answer these questions even if you do not consider yourself to be a physically active person. Think first about the time you spend doing work. Think of work as the things that you have to do such as paid or unpaid work, study/training, household chores, harvesting food/crops, fishing or hunting for food, seeking employment. *[Insert other examples if needed]*. In answering the following questions 'vigorous-intensity activities' are activities that require hard physical effort and cause large increases in breathing or heart rate, 'moderate-intensity activities' are activities that require moderate physical effort and cause small increases in breathing or heart rate.		
Question	**Response**	**Code**
Work		
Does your work involve vigorous-intensity activity that causes large increases in breathing or heart rate like *[carrying or lifting heavy loads, digging or construction work]* for at least 10 minutes continuously? *[INSERT EXAMPLES] (USE SHOWCARD)*	Yes 1 No 2 *If No, go to P 4*	P1
In a typical week, on how many days do you do vigorous-intensity activities as part of your work?	Number of days └─┘	P2
How much time do you spend doing vigorous-intensity activities at work on a typical day?	Hours : minutes └─┴─┘ : └─┴─┘ hrs mins	P3 (a-b)
Does your work involve moderate-intensity activity, that causes small increases in breathing or heart rate such as brisk walking *[or carrying light loads]* for at least 10 minutes continuously? *[INSERT EXAMPLES] (USE SHOWCARD)*	Yes 1 No 2 *If No, go to P 7*	P4
In a typical week, on how many days do you do moderate-intensity activities as part of your work?	Number of days └─┘	P5
How much time do you spend doing moderate-intensity activities at work on a typical day?	Hours : minutes └─┴─┘ : └─┴─┘ hrs mins	P6 (a-b)
Travel to and from places		
The next questions exclude the physical activities at work that you have already mentioned. Now I would like to ask you about the usual way you travel to and from places. For example to work, for shopping, to market, to place of worship. *[Insert other examples if needed]*		
Do you walk or use a bicycle *(pedal cycle)* for at least 10 minutes continuously to get to and from places?	Yes 1 No 2 *If No, go to P 10*	P7
In a typical week, on how many days do you walk or bicycle for at least 10 minutes continuously to get to and from places?	Number of days └─┘	P8
How much time do you spend walking or bicycling for travel on a typical day?	Hours : minutes └─┴─┘ : └─┴─┘ hrs mins	P9 (a-b)

Continued on next page

2 The questionnaire, Continued

Physical Activity, Continued		
Question	**Response**	**Code**
Recreational activities		
The next questions exclude the work and transport activities that you have already mentioned. Now I would like to ask you about sports, fitness and recreational activities (leisure), *[Insert relevant terms]*.		
Do you do any vigorous-intensity sports, fitness or recreational *(leisure)* activities that cause large increases in breathing or heart rate like *[running or football]* for at least 10 minutes continuously? *[INSERT EXAMPLES] (USE SHOWCARD)*	Yes 1 No 2 *If No, go to P 13*	P10
In a typical week, on how many days do you do vigorous-intensity sports, fitness or recreational *(leisure)* activities?	Number of days └─┘	P11
How much time do you spend doing vigorous-intensity sports, fitness or recreational activities on a typical day?	Hours : minutes └─┴─┘ : └─┴─┘ hrs mins	P12 (a-b)
Do you do any moderate-intensity sports, fitness or recreational *(leisure)* activities that cause a small increase in breathing or heart rate such as brisk walking, *[cycling, swimming, volleyball]* for at least 10 minutes continuously? *[INSERT EXAMPLES] (USE SHOWCARD)*	Yes 1 No 2 *If No, go to P16*	P13
In a typical week, on how many days do you do moderate-intensity sports, fitness or recreational *(leisure)* activities?	Number of days └─┘	P14
How much time do you spend doing moderate-intensity sports, fitness or recreational *(leisure)* activities on a typical day?	Hours : minutes └─┴─┘ : └─┴─┘ hrs mins	P15 (a-b)
Sedentary behaviour		
The following question is about sitting or reclining at work, at home, getting to and from places, or with friends including time spent sitting at a desk, sitting with friends, traveling in car, bus, train, reading, playing cards or watching television, but do not include time spent sleeping. *[INSERT EXAMPLES] (USE SHOWCARD)*		
How much time do you usually spend sitting or reclining on a typical day?	Hours : minutes └─┴─┘ : └─┴─┘ hrs mins	P16 (a-b)

3 GPAQ Question by Question Guide

Physical Activity		
Next I am going to ask you about the time you spend doing different types of physical activity in a typical week. Please answer these questions even if you do not consider yourself to be a physically active person. Think first about the time you spend doing work. Think of work as the things that you have to do such as paid or unpaid work, study/training, household chores, harvesting food/crops, fishing or hunting for food, seeking employment. *[Insert other examples if needed]*. In answering the following questions 'vigorous-intensity activities' are activities that require hard physical effort and cause large increases in breathing or heart rate, 'moderate-intensity activities' are activities that require moderate physical effort and cause small increases in breathing or heart rate. *Read this opening statement out loud. It should not be omitted. The respondent will have to think first about the time he/she spends doing work (paid or unpaid work, household chores, harvesting food, fishing or hunting for food, seeking employment [Insert other examples if needed]), then about the time he/she travels from place to place, and finally about the time spent in vigorous as well as moderate physical activity during leisure time.* *Remind the respondent when he/she answers the following questions that 'vigorous-intensity activities' are activities that require hard physical effort and cause large increases in breathing or heart rate, 'moderate-intensity activities' are activities that require moderate physical effort and cause small increases in breathing or heart rate. Don't forget to use the showcard which will help the respondent when answering to the questions.*		
Question	**Response**	**Code**
Work		
Does your work involve vigorous-intensity activity that causes large increases in breathing or heart rate like *[carrying or lifting heavy loads, digging or construction work]* for at least 10 minutes continuously? *[INSERT EXAMPLES] (USE SHOWCARD)* *Ask the participant to think about vigorous-intensity activities at work only. Activities are regarded as vigorous intensity if they cause large increases in breathing and/or heart rate.*	Yes 1 No 2 *If No, go to P 4*	P1
In a typical week, on how many days do you do vigorous-intensity activities as part of your work? *"Typical week" means a week when the participant is engaged in his/her usual activities. Valid responses range from 1-7.*	Number of days └─┘	P2
How much time do you spend doing vigorous-intensity activities at work on a typical day? *Ask the participant to think of a typical day he/she can recall easily in which he/she engaged in vigorous-intensity activities at work. The participant should only consider those activities undertaken continuously for 10 minutes or more. Probe very high responses (over 4 hrs) to verify.*	Hours : minutes └─┴─┘ : └─┴─┘ hrs mins	P3 (a-b)
Does your work involve moderate-intensity activity, that causes small increases in breathing or heart rate such as brisk walking *[or carrying light loads]* for at least 10 minutes continuously? *[INSERT EXAMPLES] (USE SHOWCARD)* *Ask the participant to think about moderate-intensity activities at work only. Activities are regarded as moderate intensity if they cause small increases in breathing and/or heart rate.*	Yes 1 No 2 *If No, go to P 7*	P4
In a typical week, on how many days do you do moderate-intensity activities as part of your work? *"Typical week" means a week when the participant is engaged in his/her usual activities. Valid responses range from 1-7.*	Number of days └─┘	P5
How much time do you spend doing moderate-intensity activities at work on a typical day? *Ask the participant to think of a typical day he/she can recall easily in which he/she engaged in moderate-intensity activities at work. The participant should only consider those activities undertaken continuously for 10 minutes or more. Probe very high responses (over 4 hrs) to verify.*	Hours : minutes └─┴─┘ : └─┴─┘ hrs mins	P6 (a-b)

Continued on next page

3 GPAQ Question by Question Guide, Continued

Physical Activity, Continued		
Question	**Response**	**Code**
Travel to and from places		
The next questions exclude the physical activities at work that you have already mentioned. Now I would like to ask you about the usual way you travel to and from places. For example to work, for shopping, to market, to place of worship. *[Insert other examples if needed]* *The introductory statement to the following questions on transport-related physical activity is very important. It asks and helps the participant to now think about how they travel around getting from place-to-place. This statement should not be omitted.*		
Do you walk or use a bicycle *(pedal cycle)* for at least 10 minutes continuously to get to and from places? *Select the appropriate response.*	Yes 1 No 2 *If No, go to P 10*	P7
How much time do you spend walking or bicycling for travel on a typical day? *Ask the participant to think of a typical day he/she can recall easily in which he/she engaged in transport-related activities. The participant should only consider those activities undertaken continuously for 10 minutes or more. Probe very high responses (over 4 hrs) to verify.*	Hours : minutes ⎵⎵ : ⎵⎵ hrs mins	P9 (a-b)
Recreational activities		
The next questions exclude the work and transport activities that you have already mentioned. Now I would like to ask you about sports, fitness and recreational activities (leisure) *[Insert relevant terms]*. *This introductory statement directs the participant to think about recreational activities. This can also be called discretionary or leisure time. It includes sports and exercise but is not limited to participation in competitions. Activities reported should be done regularly and not just occasionally. It is important to focus on only recreational activities and not to include any activities already mentioned. This statement should not be omitted.*		
Do you do any vigorous-intensity sports, fitness or recreational *(leisure)* activities that cause large increases in breathing or heart rate like *[running or football]* for at least 10 minutes continuously? *[INSERT EXAMPLES] (USE SHOWCARD)* *Ask the participant to think about recreational vigorous-intensity activities only. Activities are regarded as vigorous intensity if they cause large increases in breathing and/or heart rate.*	Yes 1 No 2 *If No, go to P 13*	P10
In a typical week, on how many days do you do vigorous-intensity sports, fitness or recreational *(leisure)* activities? *"Typical week" means a week when the participant is engaged in his/her usual activities. Valid responses range from 1-7.*	Number of days ⎵	P11
How much time do you spend doing vigorous-intensity sports, fitness or recreational activities on a typical day? *Ask the participant to think of a typical day he/she can recall easily in which he/she engaged in recreational vigorous-intensity activities. The participant should only consider those activities undertaken continuously for 10 minutes or more. Probe very high responses (over 4 hrs) to verify.*	Hours : minutes ⎵⎵ : ⎵⎵ hrs mins	P12 (a-b)
Do you do any moderate-intensity sports, fitness or recreational *(leisure)* activities that cause a small increase in breathing or heart rate such as brisk walking, *[cycling, swimming, volleyball]* for at least 10 minutes continuously? *[INSERT EXAMPLES] (USE SHOWCARD)* *Ask the participant to think about recreational moderate-intensity activities only. Activities are regarded as moderate intensity if they cause small increases in breathing and/or heart rate.*	Yes 1 No 2 *If No, go to P16*	P13
In a typical week, on how many days do you do moderate-intensity sports, fitness or recreational *(leisure)* activities? *"Typical week" means a week when the participant is engaged in his/her usual activities. Valid responses range from 1-7.*	Number of days ⎵	P14

Continued on next page

3 GPAQ Question by Question Guide, Continued

Physical Activity, Continued		
Question	**Response**	**Code**
How much time do you spend doing moderate-intensity sports, fitness or recreational *(leisure)* activities on a typical day? *Ask the participant to think of a typical day he/she can recall easily in which he/she engaged in recreational moderate-intensity activities. The participant should only consider those activities undertaken continuously for 10 minutes or more. Probe very high responses (over 4 hrs) to verify.*	Hours : minutes ⎿⏊⏌ : ⎿⏊⏌ hrs mins	P15 (a-b)
Sedentary behaviour		
The following question is about sitting or reclining at work, at home, getting to and from places, or with friends including time spent sitting at a desk, sitting with friends, traveling in car, bus, train, reading, playing cards or watching television, but do not include time spent sleeping. *[INSERT EXAMPLES] (USE SHOWCARD)*		
How much time do you usually spend sitting or reclining on a typical day? *Ask the participant to consider total time spent sitting at work, in an office, reading, watching television, using a computer, doing hand craft like knitting, resting etc. The participant should not include time spent sleeping.*	Hours : minutes ⎿⏊⏌ : ⎿⏊⏌ hrs mins	P16 (a-b)

4 Cleaning GPAQ data

Introduction

It is important to standardize the way in which the data collected are cleaned and analysed. Please use the guidelines below when cleaning and analysing your data.
The cleaning and analysis guidelines use the coding column in the questionnaire as an identifier.

Cleaning

You should clean all domains as a combined set. While some of the calculations of results use all the domains and others use only one of the domains, it is necessary that each respondent has an overall "clean" response to all physical activity questions. To be included in the analyses, each participant must have a valid response for at least one domain and have no invalid responses for any domains.

Check for the following for all the domains.

If…	Then…
Values in the hours column are 15, 30, 45, or 60	move them into the corresponding minutes variable, if the corresponding minutes variable is empty or zero (most likely a data recording error).
Maximum values: If for at least one "sub-domain" (vigorous work, moderate work, transport, vigorous recreation, or moderate recreation activity) the value of hours+minutes >16 hours	remove the case from all analyses.
If a respondent reports implausible values (eg., >7 days in any days column)	remove the case from all analyses.
If a respondent has inconsistent answers (eg., 0 days, but values >0 in the corresponding time variables)	remove the case from all analyses.
If one whole "sub-domain" (vigorous work, moderate work, transport, vigorous recreation, or moderate recreation activity) has missing values, but the other "sub-domains" are valid	include the case in the analysis, assuming no activity (0 days, 0 time) for this "sub-domain". That means that, as long as at least one "sub-domain" has valid answers, and all others are missing, this person will be included in analyses.

Notes

Overall, this cleaning method should result in the same denominator across all domains and all analyses.

For information on how to create P3, P6, P9, P12, and P15 see the Cleaning GPAQ with EpiInfo section at the end of this document (p. 12).

Continued on next page

4 Cleaning GPAQ data, Continued

Detailed cleaning instructions

There are detailed cleaning instructions on how to clean each variable in the Cleaning GPAQ with EpiInfo section of this document (p. 11). This section includes details on how to clean the variables and the associated EpiInfo code.

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5 Cleaning GPAQ data with EpiInfo

Introduction

GPAQ collects information on three domains. These domains are:
- Activity at work
- Travel to and from places
- Recreational activities.

For analysis purposes these domains can be further broken down into six different "sub-domains". These "sub-domains" are:
- Vigorous work (codes P1-P3)
- Moderate work (codes P4-P6)
- Travel (codes P7-P9)
- Vigorous recreation (codes P10-P12)
- Moderate recreation (codes P13-P15)
- Sitting (code P16)

Grouping the GPAQ sections

The GPAQ data are cleaned as a whole. Thus is a participant gave an invalid answer to any domain, then their entire response is not included in any analyses. However, a participant needs only to give a valid response to a minimum of one domain, leaving the remaining domains blank, to be included in the analyses.

Cleaning Programs

A "CleanRecode" program exists for each subset of physical activity questions. These are: **CleanRecode P1-P3**, **CleanRecode P4-P6**, **CleanRecode P7-P9**, **CleanRecode P10-P12**, **CleanRecode P13-P15**, and **CleanRecode P16**. The first 5 of these programs are identical with the only exception being that the question codes are changed.

All programs can be downloaded from http://www.who.int/chp/steps/resources/database/en/index.html by clicking on "EpiInfo Analysis Programs".

CleanRecode P1-P3 is described in the following table. This same description applies to CleanRecode P4-P6, CleanRecode P7-P9, CleanRecode P10-P12, and CleanRecode P13-P15. Since the program CleanRecode P16 differs from the other 5 CleanRecode programs, its description is provided in the second table below.

Continued on next page

5 Cleaning GPAQ data with EpiInfo, Continued

CleanRecode P1-P3	
Questions Used	P1, P2, P3a, P3b
General Information	Before checking for valid responses to P1 through P3a&b, P3a and P3b are checked for possible data entry errors (i.e. minutes entered where hours are expected). To have a "clean" response, respondents must have answered all 3 questions correctly and consistently (P1t3CLN=1).
Modified Variables	Before any new variables are created, P3a and P3b are modified using the following logical tests. To summarize, these tests try to correct obvious data entry errors where minute values of 15, 30, 45, or 60 were entered as hour values in P3a. These changes are only saved to the temporary dataset used for analysis, the actual dataset is left unchanged.

Condition	**New P3a Value**	**New P3b Value**
P3a=15 AND (P3b=(.) OR P3b=0 OR P3b=15 OR P3b=77 OR P3b=88 OR P3b=99)	0	15
P3a=30 AND (P3b=(.) OR P3b=0 OR P3b=30 OR P3b=77 OR P3b=88 OR P3b=99)	0	30
P3a=45 AND (P3b=(.) OR P3b=0 OR P3b=45 OR P3b=77 OR P3b=88 OR P3b=99)	0	45
P3a=60 AND (P3b=(.) OR P3b=0 OR P3b=60 OR P3b=77 OR P3b=88 OR P3b=99)	1	0
(P3a=7 AND P3b=77) OR (P3a=8 AND P3b=88) OR (P3a=9 AND P3b=99)	0	0
P3a=77 OR P3a=88 OR P3a=99	0	(leave as is)
P3b=77 OR P3b=88 OR P3b=99	(leave as is)	0

Created Variables

Name	**Purpose**	**Value**	**Condition**
P3amin	Computes min value for P3a.	0	P3a=(.)
		P3a*60	ELSE
P3bmin	Set equal to P3b, with 0's replacing missing values.	0	P3b=(.)
		P3b	ELSE
P3	Total time in mins.	P3amin+P3bmin	
P2CLN	Checks for a valid response to P2	1	P1=1 AND P2>0 AND P2<8 **OR** P1=2 AND (P2=0 OR P2=(.) OR P2=99)
		2	ELSE
P3CLN	Checks for a valid response to P3: P2 must have a valid response with nr. of days = 1 through 7, and P3 must be at least 10 mins. and at most 960 mins. (max. of 16 hrs. per day)	1	P2CLN=1 AND P2>0 AND P2<8 AND P3>9 AND P3<961 **OR** P2CLN=1 AND (P2=0 OR P2=(.) OR P2=99) AND P3=0
		2	ELSE
P1t3CLN	Checks for valid response to P1 through P3a&b. Allows for respondents to skip entire section but a check in the physical activity programs that use these cleaning programs ensures that at least one section of all physical activity sections has a response.	1	P3CLN=1 AND Valid=1 **OR** P1=(.) AND (P2=0 OR P2=(.) OR P2=99) AND P3=0 AND Valid=1
		2	ELSE

Continued on next page

5 Cleaning GPAQ data with EpiInfo, Continued

<table>
<tr><th colspan="6">CleanRecode P16</th></tr>
<tr><td>Questions Used</td><td colspan="5">P16a, P16b</td></tr>
<tr><td>General Information</td><td colspan="5">Responses are first checked for possible data entry errors (i.e. minutes entered where hours are expected). To have a "clean" response, respondents must have given a valid response to P16 (P16CLN=1).</td></tr>
<tr><td rowspan="9">Modified Variables</td><td colspan="5">Before any new variables are created, P16a and P16b are modified using the following logical tests. To summarize, these tests try to correct obvious data entry errors where minute values of 15, 30, 45, or 60 were entered as hour values in P16a. These changes are only saved to the temporary dataset used for analysis, the actual dataset is left unchanged.</td></tr>
<tr><td colspan="3">Condition</td><td>New P16a Value</td><td>New P16b Value</td></tr>
<tr><td colspan="3">P16a=15 AND (P16b=(.) OR P16b=0 OR P16b=15 OR P16b=77 OR P16b=88 OR P16b=99)</td><td>0</td><td>15</td></tr>
<tr><td colspan="3">P16a=30 AND (P16b=(.) OR P16b=0 OR P16b=30 OR P16b=77 OR P16b=88 OR P16b=99)</td><td>0</td><td>30</td></tr>
<tr><td colspan="3">P16a=45 AND (P16b=(.) OR P16b=0 OR P16b=45 OR P16b=77 OR P16b=88 OR P16b=99)</td><td>0</td><td>45</td></tr>
<tr><td colspan="3">P16a=60 AND (P16b=(.) OR P16b=0 OR P16b=60 OR P16b=77 OR P16b=88 OR P16b=99)</td><td>1</td><td>0</td></tr>
<tr><td colspan="3">(P16a=7 AND P16b=77) OR (P16a=8 AND P16b=88) OR (P16a=9 AND P16b=99)</td><td>0</td><td>0</td></tr>
<tr><td colspan="3">P16a=77 OR P16a=88 OR P16a=99</td><td>0</td><td>(leave as is)</td></tr>
<tr><td colspan="3">P16b=77 OR P16b=88 OR P16b=99</td><td>(leave as is)</td><td>0</td></tr>
<tr><td rowspan="9">Created Variables</td><td>Name</td><td>Purpose</td><td>Value</td><td colspan="2">Condition</td></tr>
<tr><td rowspan="2">P16amin</td><td rowspan="2">Computes min value for P16a</td><td>0</td><td colspan="2">P16a=(.)</td></tr>
<tr><td>P16a*60</td><td colspan="2">ELSE</td></tr>
<tr><td rowspan="2">P16bmin</td><td rowspan="2">Set equal to P16b, with 0's replacing missing values</td><td>0</td><td colspan="2">P16b=(.)</td></tr>
<tr><td>P16b</td><td colspan="2">ELSE</td></tr>
<tr><td>P16</td><td>Total time in mins</td><td>P16amin+P16bmin</td><td colspan="2"></td></tr>
<tr><td rowspan="2">P16CLN</td><td rowspan="2">Checks for a valid response to P16 (can be from 0 mins. to 1440 mins. (24 hrs.))</td><td>1</td><td colspan="2">P16<1441 AND Valid=1</td></tr>
<tr><td>2</td><td colspan="2">ELSE</td></tr>
</table>

6 Analysis Guidelines and Calculations

Introduction

A population's physical activity (or inactivity) can be described in different ways. The two most common ways are

(1) to estimate a population's mean or median physical activity using a continuous indicator such as MET-minutes per week or time spent in physical activity, and
(2) to classify a certain percentage of a population as 'inactive' or 'insufficiently active' by setting up a cut-point for a specific amount of physical activity.

The following guidelines describe both how to derive at continuous as well as categorical indicators when analysing GPAQ data.

Continuous indicator

As described in the overview (p. 3), MET values are applied to the time variables according to the intensity (moderate or vigorous) of the activity. Applying MET values to activity levels allows us to calculate total physical activity.

For the calculation of a person's overall energy expenditure using GPAQ data, the following MET values are used:

Domain	MET value
Work	• Moderate MET value = 4.0 • Vigorous MET value = 8.0
Transport	Cycling and walking MET value = 4.0
Recreation	• Moderate MET value = 4.0 • Vigorous MET value = 8.0

WHO recommend-dations on physical activity for health

For the calculation of a categorical indicator, the total time spent in physical activity during a typical week and the intensity of the physical activity are taken into account.

Throughout a week, including activity for work, during transport and leisure time, adults should do at least

- 150 minutes of moderate-intensity physical activity OR
- 75 minutes of vigorous-intensity physical activity OR
- An equivalent combination of moderate- and vigorous-intensity physical activity achieving at least 600 MET-minutes.

6 Analysis Guidelines and Calculations, Continued

Not meeting WHO recommendations on physical activity for health

Description: Percentage of respondents not meeting WHO recommendations on physical activity for health (respondents doing less than 150 minutes of moderate-intensity physical activity per week, or equivalent).

Instrument questions:

- **P1-P6a&b:** activity at work
- **P7-P9a&b:** travel to and from places
- **P10-P15a&b:** recreational activities

Not meeting WHO recommendations on physical activity for health									
Age Group (years)	Men			Women			Both Sexes		
	n	% not meeting recs	95% CI	n	% not meeting recs	95% CI	n	% not meeting recs	95% CI

Questions Used	P1-P15a&b
Program	**Pnotmeetingrecs** (unweighted), **PnotmeetingrecsWT** (weighted)
Equations	Total physical activity MET-minutes/week (= the sum of the total MET minutes of activity computed for each setting) Equation: Total Physical Activity MET-minutes/week = [(P2 * P3 * 8) + (P5 * P6 * 4) + (P8 * P9 * 4) + (P11 * P12 * 8) + (P14 * P15* 4)] **WHO recommendations** — **Physical activity cutoff value** Not meeting recommendations — • IF: Total Physical Activity MET minutes per week is < 600
Program Information	Reports percentage of respondents who do not meet WHO recommendations on physical activity for health. Before any of the below variables are created ALL CleanRecode programs are called. To be included in the output, the respondent must have either left blank or given a valid response to each subset of the physical activity questions AND have given a valid response to at least one subset of the physical activity questions (CLN=1).

Created Variables	Name	Purpose	Values	Condition
	P1t3	MET value of vigorous work activity per week	P2*P3*8	P1t3CLN=1
			(.)	ELSE
	P4t6	MET value of moderate work activity per week	P5*P6*4	P4t6CLN=1
			(.)	ELSE
	P7t9	MET value of transport activity per week	P8*P9*4	P7t9CLN=1
			(.)	ELSE
	P10t12	MET value of vigorous recreational activity per week	P11*P12*8	P10t12CLN=1
			(.)	ELSE
	P13t15	MET value of moderate recreational activity per week	P14*P15*4	P13t15CLN=1
			(.)	ELSE
	Ptotal	Sum of all activity per week	p1t3+p4t6+p7t9+p10t12+p13t15	
	CLN	Checks to see if all physical activity responses, as a combined set, are valid: all subsets of responses must be clean and at least one subset of responses must have a response (not missing)	1	Valid=1 AND P1t3CLN=1 AND P4t6CLN=1 AND P7t9CLN=1 AND P10t12CLN=1 AND P13t15CLN=1 **AND** P1≠(.) OR P4≠(.) OR P7≠(.) OR P10≠(.) OR P13≠(.)
			2	ELSE
	C	Output table values	"Does not meet recommendations"	Ptotal<600
			"Meets recommendations"	Ptotal≥600

Total physical activity

Description: Mean / median time of total physical activity on average per day.

Instrument questions
- **P1-P6a&b:** activity at work
- **P7-P9&b:** travel to and from places
- **P10-P15a&b:** recreational activities

Mean/Median minutes of total physical activity on average per day									
Age Group (years)	Men			Women			Both Sexes		
	n	# minutes	95% CI	n	# minutes	95% CI	n	# minutes	95% CI

Questions Used	P1-P15a&b			
Program	**Ptotal** (unweighted mean & median values), **PtotalWT** (weighted mean values), **PtotalmedianWT** (weighted median values)			
Program Information	Reports the mean or median amount of physical activity per day in minutes. Before any of the below variables are created ALL CleanRecode programs are called. To be included in the output, the respondent must have either left blank or given a valid response to each subset of the physical activity questions AND have given a valid response to <u>at least one subset</u> of the physical activity questions (CLN=1).			
Created Variables	**Name**	**Purpose**	**Values**	**Condition**
	P1t3	Vigorous work activity in minutes per week	P2*P3	P1t3CLN=1
			(.)	ELSE
	P4t6	Moderate work activity in minutes per week	P5*P6	P4t6CLN=1
			(.)	ELSE
	P7t9	Transport activity in minutes per week	P8*P9	P7t9CLN=1
			(.)	ELSE
	P10t12	Vigorous recreational activity in minutes per week	P11*P12	P10t12CLN=1
			(.)	ELSE
	P13t15	Moderate recreational activity in minutes per week	P14*P15	P13t15CLN=1
			(.)	ELSE
	Ptotalday	Sum of all activity per week divided by 7 to get avg. per day	(p1t3+p4t6+p7t9+p10t12+p13t15)/7	
	CLN	Checks to see if all physical activity responses, as a combined set, are valid: all subsets of responses must be clean and at least one subset of responses must have a response (not missing)	1	Valid=1 AND P1t3CLN=1 AND P4t6CLN=1 AND P7t9CLN=1 AND P10t12CLN=1 AND P13t15CLN=1 **AND** P1≠(.) OR P4≠(.) OR P7≠(.) OR P10≠(.) OR P13≠(.)
			2	ELSE

Setting-specific physical activity- mean / median

Description: Mean / median number of minutes spent on average per day, in work-, transport- and recreation-related physical activity.

Instrument questions
- **P1-P6a&b:** activity at work
- **P7-P9&b:** travel to and from places
- **P10-P15a&b:** recreational activities

Mean/Median minutes of [*insert domain*]-related physical activity on average per day									
Age Group (years)	Men			Women			Both Sexes		
	n	# minutes	95% CI	n	# minutes	95% CI	n	# minutes	95% CI

Questions Used	P1-P15a&b			
Program	**Psetspecific** (unweighted mean & median values), **PsetspecificWT** (weighted mean values), **PsetspecificmedianWT** (weighted median values)			
Program Information	Reports the mean or median amount of physical activity in minutes. Before any of the below variables are created ALL CleanRecode programs are called. To be included in the output, the respondent must have either left blank or given a valid response to each subset of the physical activity questions AND have given a valid response to at least one subset of the physical activity questions (CLN=1).			
Created Variables	**Name**	**Purpose**	**Values**	**Condition**
	P1t3	Vigorous work activity in minutes per week	P2*P3	P1t3CLN=1
			(.)	ELSE
	P4t6	Moderate work activity in minutes per week	P5*P6	P4t6CLN=1
			(.)	ELSE
	P7t9	Transport activity in minutes per week	P8*P9	P7t9CLN=1
			(.)	ELSE
	P10t12	Vigorous recreational activity in minutes per week	P11*P12	P10t12CLN=1
			(.)	ELSE
	P13t15	Moderate recreational activity in minutes per week	P14*P15	P13t15CLN=1
			(.)	ELSE
	Pwork-day	Average work-related activity per day	(p1t3+p4t6)/7	
	Ptravel-day	Average transport-related activity per day	p7t9/7	
	Precday	Average recreation-related activity per day	(p10t12+p13t15) /7	
	CLN	Checks to see if all physical activity responses, as a combined set, are valid: all subsets of responses must be clean and at least one subset of responses must have a response (not missing)	1	Valid=1 AND P1t3CLN=1 AND P4t6CLN=1 AND P7t9CLN=1 AND P10t12CLN=1 AND P13t15CLN=1 **AND** P1≠(.) OR P4≠(.) OR P7≠(.) OR P10≠(.) OR P13≠(.)
			2	ELSE

No physical activity by setting

Description: Percentage of respondents classified as doing no work-, transport-, or recreation-related physical activity.

Instrument questions

- **P1-P6a&b:** activity at work
- **P7-P9&b:** travel to and from places
- **P10-P15a&b:** recreational activities

No [*insert domain*]-related physical activity									
Age Group (years)	**Men**			**Women**			**Both Sexes**		
	n	%	95% CI	n	%	95% CI	n	%	95% CI

<table>
<tr><td>Questions Used</td><td colspan="4">P1-P15a&b</td></tr>
<tr><td>Program</td><td colspan="4">Pnoactivitybyset (unweighted), PnoactivitybysetWT (weighted)</td></tr>
<tr><td>Program Information</td><td colspan="4">Reports the percentage of respondents who reported no work-, transport-, or recreation-related physical activity. Before any of the below variables are created ALL CleanRecode programs are called. To be included in the output, the respondent must have either left blank or given a valid response to each subset of the physical activity questions AND have given a valid response to <u>at least one subset</u> of the physical activity questions (CLN=1).</td></tr>
<tr><td rowspan="9">Created Variables</td><td>Name</td><td>Purpose</td><td>Values</td><td>Condition</td></tr>
<tr><td rowspan="2">Work</td><td rowspan="2">Indicates whether or not respondent did any work-related activity</td><td>"did work activity"</td><td>P1=1 OR P4=1</td></tr>
<tr><td>"did no work activity"</td><td>ELSE</td></tr>
<tr><td rowspan="2">Trans</td><td rowspan="2">Indicates whether or not respondent did any transport-related activity</td><td>"did transport activity"</td><td>P7=1</td></tr>
<tr><td>"did no transport activity"</td><td>ELSE</td></tr>
<tr><td rowspan="2">Rec</td><td rowspan="2">Indicates whether or not respondent did any recreation-related activity</td><td>"did recreation activity"</td><td>P10=1 OR P13=1</td></tr>
<tr><td>"did no recreation activity"</td><td>ELSE</td></tr>
<tr><td rowspan="2">CLN</td><td rowspan="2">Checks to see if all physical activity responses, as a combined set, are valid: all subsets of responses must be clean and at least one subset of responses must have a response (not missing)</td><td>1</td><td>Valid=1 AND P1t3CLN=1 AND P4t6CLN=1 AND P7t9CLN=1 AND P10t12CLN=1 AND P13t15CLN=1 AND
P1≠(.) OR P4≠(.) OR P7≠(.) OR P10≠(.) OR P13≠(.)</td></tr>
<tr><td>2</td><td>ELSE</td></tr>
</table>

Composition of total physical activity	Description: Percentage of total physical activity on average per day that comes from each of the 3 types of activity: work-, transport-, or recreation-related. Instrument questions • **P1-P6a&b:** activity at work • **P7-P9&b:** travel to and from places • **P10-P15a&b:** recreational activities

Composition of total physical activity							
Age Group (years)	**Gender**						
	n	% Work	95% CI	% Transport	95% CI	% Recreation	95% CI

Qu. Used	P1-P15a&b			
Program	**Pcomposition** (unweighted), **PcompositionWT** (weighted)			
Program Infor-mation	Reports the percentage of activity that comes from each of the three types of activity (work, transport, or recreation). Before any of the below variables are created ALL CleanRecode programs are called. To be included in the output, the respondent must have either left blank or given a valid response to each subset of the physical activity questions AND have given a valid response to at least one subset of the physical activity questions (CLN=1).			
Created Variables	**Name**	**Purpose**	**Values**	**Condition**
	P1t3	Vigorous work activity in minutes per week	P2*P3	P1t3CLN=1
			(.)	ELSE
	P4t6	Moderate work activity in minutes per week	P5*P6	P4t6CLN=1
			(.)	ELSE
	P7t9	Transport activity in minutes per week	P8*P9	P7t9CLN=1
			(.)	ELSE
	P10t12	Vigorous recreational activity in minutes per week	P11*P12	P10t12CLN=1
			(.)	ELSE
	P13t15	Moderate recreational activity in minutes per week	P14*P15	P13t15CLN=1
			(.)	ELSE
	Ptotal	Sum of all activity per week	p1t3+p4t6+p7t9+ p10t12+p13t15	
	Percent-Work	Percent of all activity from work-related activities	(p1t3+p4t6)/Ptota l*100	
	Percent-Trans	Percent of all activity from transportation-related activities	p7t9/Ptotal*100	
	Percent-Rec	Percent of all activity from recreational activities	(p10t12+p13t15)/ Ptotal*100	
	CLN	Checks to see if all physical activity responses, as a combined set, are valid: all subsets of responses must be clean and at least one subset of responses must have a response (not missing)	1	Valid=1 AND P1t3CLN=1 AND P4t6CLN=1 AND P7t9CLN=1 AND P10t12CLN=1 AND P13t15CLN=1 **AND** P1≠(.) OR P4≠(.) OR P7≠(.) OR P10≠(.) OR P13≠(.)
			2	ELSE

No vigorous physical activity

Description: Percentage of respondents not engaging in vigorous physical activity.

Instrument questions

- **P1-P6a&b:** activity at work
- **P7-P9&b:** travel to and from places
- **P10-P15a&b:** recreational activities

No vigorous physical activity									
Age Group (years)	Men			Women			Both Sexes		
	n	%	95% CI	n	%	95% CI	n	%	95% CI

Qu. Used	P1-P15a&b			
Program	**Pnovigorous** (unweighted), **PnovigorousWT** (weighted values)			
Program Infor-mation	Reports percentage of respondents who did no vigorous physical activity. Before any of the below variables are created ALL CleanRecode programs are called. To be included in the output, the respondent must have either left blank or given a valid response to each subset of the physical activity questions AND have given a valid response to at least one subset of the physical activity questions (CLN=1).			
Created Variables	**Name**	**Purpose**	**Values**	**Condition**
	C	Output table values	"did vigorous physical activity"	P1=1 OR P10=1
			"did no vigorous physical activity"	ELSE
	CLN	Checks to see if all physical activity responses, as a combined set, are valid: all subsets of responses must be clean and at least one subset of responses must have a response (not missing)	1	Valid=1 AND P1t3CLN=1 AND P4t6CLN=1 AND P7t9CLN=1 AND P10t12CLN=1 AND P13t15CLN=1 AND P1≠(.) OR P4≠(.) OR P7≠(.) OR P10≠(.) OR P13≠(.)
			2	ELSE

Sedentary Description: Minutes spent in sedentary activities on average per day.

Instrument questions

- **P16:** sedentary behaviour

Mean/Median minutes spent in sedentary activities on average per day									
Age Group (years)	Men			Women			Both Sexes		
	n	# minutes	95% CI	n	# minutes	95% CI	n	# minutes	95% CI

Questions Used	P16a&b			
Program	**Psedentary** (unweighted mean & median values), **PsedentaryWT** (weighted mean values), **PsedentarymedianWT** (weighted median values)			
Program Information	Reports the mean or median amount of sedentary activity in minutes. Before any of the below variables are created ALL CleanRecode programs are called. To be included in the output, the respondent must have either left blank or given a valid response to each subset of the physical activity questions AND have given a valid response to at least one subset of the physical activity questions (CLN=1). Note: P16 was created in CleanRecodeP16 from P16a and P16b. It contains the total sedentary time in mins.			
Created Variables	**Name**	**Purpose**	**Values**	**Condition**
	CLN	Checks to see if all physical activity responses, as a combined set, are valid: all subsets of responses must be clean and at least one subset of responses must have a response (not missing)	1	Valid=1 AND P16CLN=1
			2	ELSE