The Effect of Ethiopian Orthodox Christians '*Abiy Tsom*' (Lent fasting) on Metabolic Syndrome Indices and Serum Electrolytes

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This is to certify that the thesis prepared by ***Chala Kenenisa Edae*** entitled; **The effects of Ethiopian Orthodox Christians '*Abiy tsom*' (Lent fasting) on metabolic syndrome indices and serum electrolytes** and submitted in partial fulfillment of the Degree of Masters of Science in Medical Biochemistry complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Acronyms

ASF:- Animal Source Foods

BMI: - Body Mass Index

CAD:- Coronary Arterial Diseases

CHD:- Coronary Heart Diseases

CNCD: - Chronic Non Communicable Diseases

COC: - Coptic Orthodox Christians

CR: - Caloric Restriction

CVD: - Cardio Vascular Diseases

GOC: - Greek Orthodox Christians

HC:- Hip Circumference

HDL: - High Density Lipoprotein

IDF:- International Diabetic Federation

LDL: - Low Density Lipoprotein

MetS: - Metabolic syndrome

NCD: - Non Communicable Diseases

NCE: ATPIII: - National Cholesterol Education Program's Adult Treatment Panel III

TG: - Triglycerides

T2DM: - Type 2 Diabetes Mellitus

WC:- Waist Circumference

WHR:- Waist Hip Ratio

WHO: - World Health Organization

ABSTRACT

Background: -Fasting, the voluntary abstention from all restricted foods, is a feature of many religions, and the putative health benefits have attracted both scientific and popular interest**.** There is clear understanding that religious fasting has great effects on metabolic syndrome. There are no available literatures that give information concerning Ethiopian Orthodox Christians fasting influence on metabolic syndrome indices and serum electrolytes to date.

Objective: -To determine effect of '*Abiy tsom'* (lent fasting) of Ethiopian Orthodox Christians on metabolic syndrome indices and serum electrolytes in Addis Ababa, Ethiopia

Methodology: - 88 Study subjects were included conveniently who were followers of Ethiopian orthodox Christianity faith and fasting "lent" from April 15 to June 15 and longitudinal cross-sectional study design was employed. Data were collected twice, the first during last week of the fasting months and the second during the last week of two months' time after returning to usual diet. The data and sample was collected, analyzed, interpreted and was displayed by using descriptive and analytical statistical methods.

Results: The study found that, Ethiopian Orthodox lent fasting had significant effects on Anthropometric measurements, systolic blood pressure, lipid profiles and Urea. It was also found that this fasting had significant effect on the levels of Calcium and Chloride ions while Serum sodium and potassium were influenced insignificantly.

Conclusion: Ethiopian Orthodox Christians lent fasting is beneficial for weight loss and fighting metabolic syndrome.

Recommendation: stakeholders are recommended to consider fasting as one strategy for prevention and treatment of metabolic syndrome.

1. Introduction

1.1 Background

Metabolic syndrome constitutes a group of clinical and laboratory diagnostic test abnormalities, which are associated with the increased risk of cardiovascular diseases (CVDs) and diabetes mellitus. Metabolic syndrome is not a disease by itself, but rather a set of undesirable conditions, rooted in one's poor lifestyle; it is also associated with the increased prevalence of obesity (1).

The common symptoms of metabolic syndrome include excessive accumulation of fat, especially in the abdomen, high blood pressure, and high levels of triglyceride (TG), blood glucose, and low-density lipoprotein-cholesterol (LDL-C), which can increase the risk of CVDs and diabetes mellitus. American Heart Association and the National Heart, Lung, and Blood Institute have defined metabolic syndrome as consisting of at least three of the above mentioned conditions (2, 3).

Metabolic syndrome is a cluster of metabolically related cardiovascular disease (CVD) risk factors that increases the risk of CVD by 2-folds and the risk of developing type 2 diabetes mellitus by 3folds. The cluster includes various combinations of obesity (total body obesity measured by body mass index, or central obesity measured by waist-to hip ratio or waist circumference), atherogenic dyslipidemia (increased triglycerides, decreased high-density lipoprotein cholesterol), elevated blood pressure (systolic and diastolic), abnormal glucose tolerance, an insulin resistance measured by the homeostasis model assessment (HOMAIR) or fasting insulin. The syndrome has been given different names such as the insulin resistance syndrome, or syndrome X and the deadly quartet, the most popular being metabolic syndrome (4).

The associated risk factors with metabolic syndrome can be divided into modifiable and non-modifiable types. The major modifiable types include high blood pressure, disturbances in sex hormones (e.g., polycystic ovary syndrome (POS), mental ill health, hyperandrogenism in pre- and postmenopausal women, energy excess (higher carbohydrate, high fat, low dietary fiber, high meat intake, family history (diabetes, hypertension, obesity , overweight) life style characteristics (tobacco use, alcohol consumption, physical inactivity, snoring and obstructive sleep apnea syndrome, psychosocial and personality factors (lower social class , difficulty in coping with stress

low socioeconomic status, alcohol) etc. On the other hand, the non-modifiable risk factors include age, sex, ethnicity, family history and previous stroke and heart attack (5).

Fasting, the voluntary abstention from all restricted foods, is a feature of many religions, and the putative health benefits have attracted both scientific and popular interest. Commonly, religious doctrines proscribe foods from animal sources permanently or for particular periods (6).

Greek Orthodox Christian holy books recommend a total of 180–200 days of fasting per year. The faithful are advised to avoid olive oil, meat, fish, milk and dairy products every Wednesday and Friday throughout the year. Additionally, there are three principal fasting periods per year: i) a total of 40 days preceding Christmas (meat, dairy products and eggs are not allowed, while fish and olive oil are allowed except on Wednesdays and Fridays), ii) a period of 48 days preceding Easter (Lent). During Lent fish is allowed only two days whereas meat, dairy products and eggs are not allowed. Olive oil consumption is allowed only at weekends, iii) a total of 15 days in August (the Assumption) when the same dietary rules apply as for Lent with the exception of fish consumption which is allowed only on August 6th. Seafood such as shrimps, squid, cuttlefish, octopus, lobsters, crabs as well as snails are allowed on all fasting days throughout the year (7).

The Greek Orthodox fasting practices can therefore be characterized as requiring a periodic vegetarian diet including fish and seafood. The variant of vegetarianism followed during fasting periods by Orthodox Christians, with a diet of vegetables, legumes, nuts, fruits, olives, bread, snails and seafood, is a type of the so-called Mediterranean diet (7,8).

The Coptic Orthodox Christian (COC) dietary regulations are an important component of the Mediterranean diet of Egypt, which is close to the Greek Orthodox Christian (GOC) diets, but low in some of its constituents, mainly olive oil and nuts, on the other hand, it is rich in whole-grain brown bread **(**Egyptian pita bread), beans (Fava beans) and sesame (as “tahini” and “helva” made from a paste of sesame seeds). The four major fasting periods are: Christmas (40 days, with sea food), lent (48 days, without sea food), apostles’ fast (varies from 15 to 49 days without sea food) and assumption (15 days, with sea food). The dietary pattern is unique in that it regularly interchanges between an omnivorous to

a vegetarian (with sea food) or vegan (without sea food) type of diet over the course of the ecclesiastical year (8).

These religious fasting due to their direct influence on food habits and life style of the concerned population, they are found to have effects on their health. However, a relatively small proportion of research studies conducted to date have explored the effect of religious fasting (9). Among religions those have been studied regarding their relation to health, Judaism (10-11), Islam (12-15), Seventh-Day-Adventists (16,17), and Greek Orthodox Christians (7).

Unlike the Greek, Ethiopian Orthodox Christians (EOC) in addition to animal source foods proscription, calorie restriction has also been practiced by most of the fasters. However, to the best of the researcher's knowledge, there is no study conducted on the role Ethiopian Orthodox Christians' fasting practice in relation to metabolic syndrome indices and level of serum electrolytes.

This study assesses the effects of Ethiopian Orthodox Christians Abiy tsom (lent fasting) on metabolic syndrome indices and level of serum electrolytes.

1.2. Statement of the problem

Metabolic syndromes are among the leading causes of morbidity and mortality worldwide (18). The prevalence of chronic diseases in general and metabolic syndrome in particular is increasing in alarming rate in Ethiopia (19). Unhealthy dietary behaviors like consumption of calorie-dense foods are among the responsible risk factors for the increased prevalence of metabolic syndrome (19).

The global statistics shows that approximately a quarter of adult populations suffer from this clinical entity. According to various studies the prevalence of MetS in general population in the United States, Saudi Arabia, and Turkey are 24%, 39.3%, and 33.4%, respectively. The literature also reveals that the prevalence of MS in Tehran is 30.1% while prevalence of MS in three major cities in center of Iran is 23.3%. A more interesting part of the MetS story in Iran is that 45% of adult the population in Khorasan (Northeast Iran) has MetS. Similarly, the prevalence of the metabolic syndrome according to the WHO definition in seven European countries was estimated to be 23%. In Canada, more than a quarter of the population between the ages of 35 to 75 years was affected by the metabolic syndrome based on the ATP III criteria. At least 12% of the population aged 25 years and above was found to have three or more risk factors in Australia (20-23).

The third National Health and Nutrition Examination Survey in the United States reported the prevalence of Met S is 24 per cent in healthy adults and found that cardiovascular and all-cause mortalities to be increased in men and risk of coronary disease increased in women. The men with Met S have been reported to be 2-4 times more likely to die of any cause than those without Met S, even after adjustment for conventional risk factors (23,25).

Metabolic syndrome is evolving into a pandemic, contributing to approximately 67% for all-cause mortality,12– 17% for cardiovascular disease, and 30–52% for diabetes in the population. In populations free of cardiovascular disease at baseline, cardiovascular morbidity and mortality increases 1.5- to 3fold in the presence of the metabolic syndrome. According to International Diabetes Federation (IDF) a quarter of the world's adults have metabolic syndrome. People with metabolic syndrome are twice as likely to die from, and three times as likely to have a heart attack or stroke compared with people without the syndrome. People with metabolic syndrome have a fivefold greater risk of developing type 2 diabetes mellitus. Up to 80% of the 200 million people with diabetes globally will

die of cardiovascular disease. This puts metabolic syndrome and diabetes way ahead of HIV/AIDS in morbidity and mortality terms yet the problem is not as well recognized. The main reason behind this is that the combination of MetS risk factors interacts synergistically to start or accelerate the progression of atherosclerosis (26).

In Africa, the first reported MetS study conducted in the mid-90s in Cameroon found a 1.5% and 1.3% prevalence of MetS among urban dwelling women and men using IDF criteria; however, the study did not measure HDL-C concentrations (27). A second study conducted in 2004 in Seychelles, found a high prevalence of MS where 25%–30% of their study population had the syndrome (28). A recent study involving adults in semi-urban and rural communities in Nigeria found a prevalence of MS to be 18% (29). A community based study conducted in Tanzania in 2009 reported a 38% prevalence of MetS (30). The prevalence of Met S in children and adolescents is relatively low (4%) when compared to the adult population (24%), except amongst overweight and obese adolescents where the prevalence of the metabolic syndrome has been reported as high as 29% (31).

Another important issue of MetS is that early diagnosis and efficient management of the disease will result in the reduced risk of future development of CAD. Previous research indicated that the risk for CHD and stroke was increased threefold in subjects with metabolic syndrome ($P<0.001$). Cardiovascular mortality was also markedly increased in subjects with metabolic syndrome (12.0 vs. 2.2%, $P < 0.001$). Another study showed that patients with even one or two metabolic syndrome components were at increased risk for mortality from CHD and CVD. Moreover, metabolic syndrome overall more strongly predicts CHD, CVD, and total mortality than its individual components. Similarly, the risk of incident CVD increased in conjunction with rising numbers of the components of metabolic syndrome; 2.5% of individuals with one component developed CVD, whereas 14.9% of those who had four or more components developed the disease (32).

In Ethiopia only single cross-sectional study among working adults conducted in Addis Ababa, revealed that the overall prevalence of MetS was 12.5% and 17.9% according to ATP III and IDF definitions respectively. Using ATP III criteria, the prevalence of MetS was 10.0% in men and 16.2% in women. Application of the IDF criteria resulted in a MS prevalence of 14.0% in men and 24.0% in women (33). But still no population based study has systematically evaluated the prevalence of MetS among Ethiopians.

Unlike the Greek, Ethiopian Orthodox Christians (EOC) in addition to animal source foods prescription, calorie restriction has also been practiced by most of the followers and the duration of lent fasting period is prolonged by one week. However, to the best of the researcher's knowledge, there is no study conducted on the role Ethiopian Orthodox Christians' fasting practice in relation to metabolic syndrome indices and level of serum electrolytes (34,35).

To date, only very few investigations have examined the health-related effects of these fasting periods. Those few studies in Greek again presented conflicting findings on blood pressure (36,37), similar inconsistent findings on lipid profile, blood glucose levels and anthropometric measurements (38-39) that additionally highlights that more work remains to be performed.

1.3. Significance of study

Metabolic syndrome (MetS) is a disease condition which is alarmingly rising both in developing and developed countries but there is very limited information and attention to it in Ethiopia, hence it is important to make a study and raise awareness regarding fasting and its benefits among people.

It is well established that dietary habits, caloric restrictions, and religious fasting have beneficial effect in prevention and treatment of metabolic syndrome. Most religions have long established fasting periods that convey religious identity; however, the importance of dietary rules and the degree to which they are observed by followers vary considerably over time, often in response to a changing environment and hence, it is important to investigate and document the role of religious fasting with respect to health. Therefore, study is needed to observe the effect of religious fasting on metabolic syndrome indices and level of serum electrolytes.

Moreover, the findings of the study will provide baseline data for elucidating the effect of Ethiopian Orthodox Christians fasting on metabolic syndrome indices and serum electrolytes that will serve as background information for future studies.

2. Literature review

2.1. Definition and pathogenesis of metabolic syndrome

Metabolic syndrome is a constellation of interrelated abnormalities (namely obesity, dyslipidemia, hyperglycemia, and hypertension) that increase the risk for cardiovascular disease and type 2 diabetes. This is a common metabolic disorder which increases in prevalence as the population becomes more obese. Metabolic syndrome was introduced as a diagnostic category to identify the individuals that satisfy arbitrary chosen criteria to initiate lifestyle changes, and drug treatment when needed, with the goal of decreasing risk of cardiovascular disease and type 2 DM (46). The diagnostic criteria have been established in various ways as indicated below:

a. World Health Organisation, 1998

Diabetes or impaired fasting glycaemia or impaired glucose tolerance or insulin resistance (hyperinsulinaemic, euglycaemic clamp-glucose uptake in lowest 25%) Plus any two of the following: Obesity: BMI > 30 or waist-to-hip ratio > 0.9 (male) or > 0.85 (female) Dyslipidaemia: triglycerides ≥ 1.7 mmol/L or HDL cholesterol < 0.9 (male) or < 1.0 (female) mmol/L Hypertension: blood pressure > 140/90 mm Hg Microalbuminuria: albumin excretion > 20 μg/min

b. National Cholesterol Education Program's Adult Treatment Panel III (NCEP: ATP III), 2001

Any 3 of the following: Central obesity: waist circumference > 102 cm (male), > 88 cm (female) Hypertriglyceridaemia: triglycerides ≥ 1.7 mmol/L Low HDL cholesterol: < 1.0 mmol/L (male), < 1.3 mmol/L (female) Hypertension: blood pressure ≥ 135/85 mmhg

c. International Diabetes Federation, 2005

Central obesity (defined as waist circumference ≥ 94 cm for Europoid men and ≥ 80 cm for Europoid women) Plus any two of the following: Raised triglycerides > 1.7 mmol/L, or specific treatment for this lipid abnormality Reduced HDL cholesterol: < 1.03 mmol/L in males, and 1.29 mmol/L in females, or specific treatment for this lipid abnormality Raised blood pressure: systolic blood pressure ≥ 130mmHg or diastolic blood pressure ≥ 85 mmHg

Raised fasting plasma glucose, or previously diagnosed diabetes mellitus

The pathogenesis of metabolic syndrome is complex with interactions between genetic and lifestyle factors. Overweight and abdominal obesity are principal and recurrent clinical characteristics which, together with insulin resistance in skeletal muscles, adipose tissue and liver, play a central role in the development of metabolic syndrome. A typical dyslipidemia with high levels of triglycerides, low HDL and high ApoB plus small, dense, oxidation prone and very atherogenic LDL particles is a common and important subcomponent of metabolic syndrome. Post-prandial (following food intake) hyperlipidemia and high levels of serum free fatty acids have also been found. Hypertension is another recurrent condition. Other subcomponents include a reduced fibrinolytic capacity, inflammatory activity, high levels of uric acid, a reduced endothelial function and fatty liver (41).

2.2. Factors Associated with Metabolic Syndrome

A majority of studies indicate a strong link between the level of physical activity or fitness and the prevalence of metabolic syndrome. A Swedish study of men and women aged 60 showed a strong dose-response relationship between reported physical activity in leisure time and metabolic syndrome. The relationship was not affected by factors such as gender, education, civil status, smoking or intake of fruit, vegetables and alcohol (42). Similar findings were made in other cross-sectional studies and prospective studies where an inactive lifestyle and/or poor fitness were closely linked to the existence of metabolic syndrome (43).

Many national and international reports indicate an increase in overweight and obesity among both children and adults. The waist circumference of children and adults has increased comparatively more than their weight. Abdominal obesity is closely linked to metabolic syndrome. Dietary factors have a major influence on metabolic syndrome. Indeed, nutritional imbalance due to high energy, fat and cholesterol intakes are considered to be a risk factor for the occurrence of this syndrome (44).

Increased consumption of energy dense and processed foods that are high in fat, sugar and salt is major reason for the raising prevalence of metabolic syndrome. This fast-paced lifestyle changes seems to be a major factor in the growing epidemic of non-communicable diseases in the world. Unhealthy diets are considered major causes of diseases, such as cardiovascular disease and type 2 diabetes mellitus, contributing substantially to the global burden of diseases and mortality in Middle-East countries. In Saudi Arabia, 66% of adult men and 71% of adult women are either overweight or obese. These countries spend nearly 5.6 billion USD on diabetes-related healthcare. The available data suggest that metabolic syndrome is an increasingly common problem in the Arab population, and the estimates of its prevalence vary from 20.8% to 40% (45,46).

A study by Al-Daghri and colleagues (45, 47) in Saudi adults observed that the prevalence of metabolic syndrome remains high, but has considerably decreased, reaching 37% as compared to the previously recorded 44.1%. However, among the metabolic syndrome components, low high-density lipoprotein (HDL)-cholesterol and hypertriglyceridemia were the most prevalent, affecting 88.6% and 34% of the subjects, respectively.

In 2005, Al-Nozha and colleagues reported that the prevalence of metabolic syndrome was almost 40% in Saudi Arabia. In addition, several epidemiological studies also demonstrated an increase in the prevalence of metabolic syndrome in other Middle-Eastern countries (46).

In addition to the findings of NCEP ATP III, several studies also suggest that lifestyle modifications could be an important element for reducing and managing metabolic syndrome risk factors. Several clinical and epidemiological studies suggest that among the therapeutic lifestyle changes, dietary factors could play a very important and beneficial role in combating several chronic diseases. It has also been shown that the various risk factors contributing to metabolic syndrome differ between genders and in different countries (40).

A study conducted in Addis Ababa Ethiopia revealed that the prevalence of MetS was 14.0% in men and 24.0% in women and showed consumption of calorie-dense foods, sedentary lifestyle, tobacco consumption, and use of antiretroviral medications as risk factors for MetS and concluded that the burden of metabolic syndrome and mortality from Chronic non-communicable diseases(CNCDs) that are linked to life styles changes is increasing in the country (48).

2.3. Religious Fasting and its effect on metabolic syndrome indices and level of serum electrolytes

2.3.1. Religious fasting

Fasting has been practiced for millennia, but studies have shed light on its role in adaptive cellular responses that reduce oxidative damage and inflammation, optimize energy metabolism and bolster cellular protection. In lower eukaryotes, chronic fasting extends longevity in part by reprogramming metabolic and stress resistance pathways. In rodents intermittent or periodic fasting protects against diabetes, cancers, heart disease and neurodegeneration, while in humans it helps to reduce obesity, hypertension, asthma and rheumatoid arthritis. Thus, fasting has the potential to delay aging and help prevent and treat chronic diseases while minimizing the side effects caused by chronic dietary interventions (49).

In humans, fasting is achieved by ingesting no or minimal amounts of food and caloric beverages for periods that typically range from 12 hours to three weeks. Many religious groups incorporate periods of fasting into their rituals including Muslims who fast from dawn until dusk during the month of Ramadan, and Christians, Jews, Buddhists and Hindus who traditionally fast on designated days of the week or calendar year. In many clinics, patients are now monitored by physicians while undergoing water only or very low calorie (less than 200 kcal/day) fasting periods lasting from 1 week or longer for weight management, and for disease prevention and treatment (6).

Fasting is distinct from caloric restriction (CR) in which the daily caloric intake is reduced chronically by 20–40%, but meal frequency is maintained. Starvation is instead a chronic nutritional insufficiency that is commonly used as a substitute for the word fasting, particularly in laboratory animals, but that is also used to define extreme forms of fasting,

which can result in degeneration and death. We now know that fasting results in ketogenesis that promotes potent changes in metabolic pathways and cellular processes such as stress resistance, lipolysis and autophagy, and can have medical applications that in some cases are as effective as those of approved drugs such as the dampening of seizures and seizure associated brain damage and the amelioration of rheumatoid arthritis (2).

Islamic Ramadan, the three principal fasting periods of Greek Orthodox Christianity, and the Daniel Fast each provide a unique and interesting vantage point for evaluating the effects of food restriction/modification. The majority of findings related to Ramadan fasting are mixed, and these discrepancies are most likely due to the differences in cultural norms - particularly dietary norms - of the groups studied (50). The three Greek Orthodox Christian fasting seasons appear to decrease body mass and lower both total and LDL cholesterol levels, although these fasts minimally affect the intake of most vitamins and minerals. The Daniel Fast is also associated with profound and favorable effects on a variety of markers related to human health, including blood pressure, serum lipids, insulin sensitivity, and biomarkers of oxidative stress (6,7).

Most studies on GOC have reported a decreased caloric intake during the fasting periods, which may result in lowered body mass. Percentage wise, carbohydrate intake appears to increase while both protein and fat intake decrease. Both saturated fat and trans-fatty acid consumption appear to decrease during fasting periods while both monounsaturated and polyunsaturated fat consumption do not change (7).

The intake of most vitamins and minerals do not appear to change during these periods, although riboflavin and calcium intake each appear to decrease, and magnesium intake appears to increase. Both riboflavin and calcium intake appear to decrease during fasting periods, while magnesium intake appears to increase. The intake of the following vitamins and minerals do not appear to change during fasting periods: vitamin A; thiamin; niacin; vitamin B12; vitamin C; vitamin E; phosphorus; potassium; and zinc. Mixed results have been recorded regarding the intake of both folate and sodium (51-53).

2.3.1.1. Religious fasting and anthropometric measurements

Religious fasts are partaken primarily for spiritual purposes; they also have the potential to greatly affect one's physical health. Regarding anthropometric outcomes, Greek

Orthodox Christian monks were observed to decrease significantly during fasting periods (35).

Anthropometric measures of adiposity such as body mass index (BMI), waist circumference (WC), and waist-to-hip ratio (WHR) have been shown to correlate differently with CVD risk. The risk of death from cardiovascular disease increases with excessive fat (54-58) and obesity is shown to adversely affect cardiac function, increases the risk factors for coronary heart disease, and is an independent risk factor for cardiovascular disease (59). Dyslipidemia, hypertension, and other CVD risk factors are highly correlated with increasing BMI (60,61) and excessive abdominal adiposity is also a strong independent predictor (62).

BMI is the most frequently used measure of adiposity in epidemiologic studies; however, some investigators have reported that using BMI alone is not the most accurate measure of increased CVD risk; instead, other studies argued that WC as a better predictors of future CVD risk (63,64).

BMI does not accurately reflect the degree of body fat and body fat distribution (65,66). There is a large body of evidence that suggests abdominal fat distribution measured by waist circumference (WC) may be more closely tied to metabolic risks than BMI (67,68). To this effect, the US National Institutes of Health has recommended combined measurements of WC and BMI as an assessment tool for CVD risk (69). Thresholds of BMI in accordance with WHO protocol (Underweight: <18.5 kg/m^2; Normal: 18.5–24.9 kg/m^2; Overweight: 25.0–29.9 kg/m^2; Obese ≥30 kg/m^2). Abdominal obesity is explained by having a waist circumference of ≥94 cm for men and ≥80 cm for women (48).

Waist to hip ratio (i.e. the waist circumference divided by hip circumference) suggested as an additional measure of body distribution. This ratio can be measured more precisely than skin fold and it provides an index for both subcutaneous and intra-abdominal adipose tissue. The cutoff point for risk of metabolic complication, WHR >0.90 for males and >0.85 for females (68).

2.3.1.2. Religious fasting and Blood glucose and Blood pressure

Cardiovascular diseases due to hypertension and diabetes mellitus or elevated glucose level are among the top causes (69). Hypertension is a growing public health problem,

with remarkable contribution to cardiovascular diseases morbidity (70) and it is major risk factor of causing heart attack (71).

Cardiovascular complications are now the leading causes of diabetes-related morbidity and mortality. The public health impact of cardiovascular disease in patients with diabetes is already enormous and is increasing. The adverse influence of diabetes extends to all components of the cardiovascular system: the microvasculature, the larger arteries, and the heart, as well as the kidneys (73). Cut off values with mean systolic blood pressure (SBP) ≥ 140 mmHg (millimeters of mercury) or diastolic blood pressure (DBP) ≥ 90 mmHg are considered to be hypertensive, while fasting blood sugar level >110mg/dL are considered as hyperglycemic (74).

Greek Orthodox Christian fasting appears to have no effect on blood glucose levels, although fiber intake increases during fasting periods (7).

There are conflicting findings on the effects of Greek Orthodox Christian fasting on blood pressure. One study found that systolic blood pressure increased during fasting periods (75), while another study found no change in systolic blood pressure when fasters were compared with non-fasters (76). One study reported that non-fasters' diastolic blood pressure decreased significantly during fasting periods when compared to the changes in fasters' diastolic blood pressure (76), while another study reported that fasters' diastolic blood pressure did not change during fasting period (75).

More research remains to be performed on hematological variables and blood pressure during fasting periods due to both the lack of previous research and the inconclusive findings. Also, future studies should examine each of the three principal fasting periods both separately and aggregately, because each fasting period has unique food prescriptions and durations (76,77,78).

2.3.1.3. Lipid profiles and religious fasting

Lipids are characterized by their insolubility in water and include a family of compounds; triglycerides, phospholipids, and sterols. Sterols are compounds with a multiple ring structure; the most common sterol is cholesterol (74).

Cholesterol is an essential substance involved in many functions, such as maintaining cell membranes where it is mainly incorporated in to the plasma membranes of cells and

regulates membrane fluidity conferring a higher degree of rigidity. Furthermore, it is the precursor for the synthesis of fat soluble vitamins, all steroid hormones, bile acid and help cell connections in the brain (79). While in circulation, cholesterol, being a lipid, requires a transport vesicle to shield it from the aqueous nature of plasma. Complex, micelle-like join up of various proteins and lipids achieve cholesterol transport through the vascular system. These particles are known as lipoproteins (79).

Lipoproteins are heterogeneous in size, shape, composition, function, and perhaps most importantly, their contribution to vascular disease. All types of lipoproteins carry all classes of lipids: triacylglycerides, cholesterol, phospholipids and amphipathic proteins called apolipoproteins (79). Lipoproteins can be differentiated on the basis of their density, as depicted in Figure 1, the degree of lipid in a lipoprotein affects its density—the lower the density of a lipoprotein, the more lipid it contains relative to protein. The four major types of lipoproteins are chylomicrons, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) (74,79).

Chylomicrons and VLDL: These two lipoproteins are rich in triglyceride. Chylomicrons are synthesized by enterocytes from lipids absorbed in the small intestine and VLDL is synthesized in the liver. Their function is to deliver energy-rich triacylglycerol (TAG) to cells in the body. TAG is stripped from chylomicrons and VLDL through the action of lipoprotein lipase, an enzyme that is found on the surface of endothelial cells. This enzyme digests the TAG to fatty acids and monoglycerides, which can then diffuse into the cell to be oxidized, or in the case of an adipose cell, to be re-synthesized into TAG and stored in the cell (74,79).

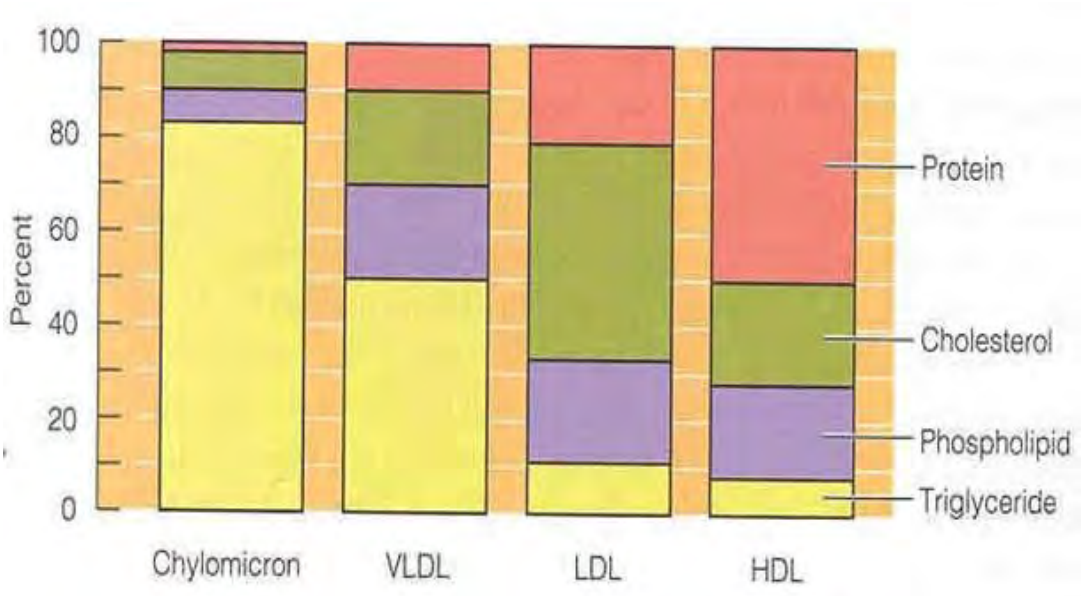


Figure 1: Compositions of lipoproteins (74).

Low Density Lipoproteins (LDL): As VLDL particles are stripped of triacylglycerol, they become denser and these particles are remodeled at the liver and transformed into LDL. The function of LDL is to deliver cholesterol to cells, where it is used in membranes, or for the synthesis of steroid hormones. Cells take up cholesterol by receptor-mediated endocytosis when LDL binds to its receptor and internalized in an endocytic vesicle. Receptors are recycled to the cell surface, while hydrolysis in an endolysosome releases cholesterol for use in the cell (74,79). High levels of LDL cholesterol (the so-called “bad cholesterol”) greatly increase the risk for atherosclerosis because LDL particles contribute to the formation of atherosclerotic plaques (79).

High Density Lipoprotein (HDL): Excess cholesterol is eliminated from the body via the liver, as excess cholesterol from cells is brought back to the liver by HDL in a process known as reverse cholesterol transport. HDL is synthesized and secreted by the liver and it travels in the circulation where it gathers cholesterol to form mature HDL, which then returns the cholesterol to the liver via various pathways (79). Cholesterol delivered to the liver via HDL enters the bile acid synthesis pathway also known as the cholesterol catabolic pathway (79). Low HDL levels ("good cholesterol") are an independent risk factor, because reverse cholesterol transport works to prevent plaque formation, or even cause regression of plaques once they have formed. HDL may also have anti-inflammatory properties that help reduce the risk of atherosclerosis and thereby promote vascular health (79).

Liver is central to the regulation of cholesterol levels in the body; the liver is not only synthesizing cholesterol for export to other cells, but it also removes cholesterol from the body by converting it to bile salts and putting it into the bile where it can be eliminated in the feces. Furthermore, the liver synthesizes the various lipoproteins involved in transporting cholesterol and lipids throughout the body. Cholesterol synthesis in hepatocytes is under negative feedback regulation; increased cholesterol in the cell decreases the activity of 3-hydroxy-3-methyl glutaryl-coenzyme A (HMG-CoA reductase), the rate-limiting enzyme in cholesterol synthesis (44). When cholesterol levels rise in the blood, they can, however, have dangerous consequences. Homeostasis of cholesterol is centered on the metabolism of lipoproteins, which mediate transport of the lipid to and from tissues (79). Figure 2 below summarizes the fates of lipoproteins produced by the liver.

Abnormal levels of these lipoproteins in blood are linked to increase risk of atherosclerosis. Atherosclerosis is a cardiovascular disease in which lipids and inflammatory cells accumulate in plaques within the walls of blood vessels. As a result, vessel walls are narrowed and clots may form, impeding blood flow and oxygen delivery and causing tissue injury. Heart disease occurs because the coronary arteries supplying the heart are a major site where atherosclerotic plaques form. Although atherogenesis is a multifactorial process, abnormalities in lipoprotein metabolism are one of the key factors, representing around 50% of the population-attributable risk of developing cardiovascular disease (80).

Serum lipid profile is measured for cardiovascular risk prediction and has now become almost a routine test including four basic parameters: total cholesterol (TC), high density lipoproteins (HDL) cholesterol, low density lipoproteins (LDL) cholesterol and triglycerides (16). However, lipoproteins ratios, TC/HDL cholesterol and LDL/HDL cholesterol, are being used as risk indicators with greater predictive value than isolated parameters used independently, particularly the former. These two indices can be regarded as similar; since two thirds of plasma cholesterol is found in LDL, total and LDL cholesterol are closely correlated. Moreover, an increased in the level of the denominator, HDL cholesterol is more prevalently associated with plaque regression, while a decrease in LDL cholesterol would slow down progression. Both predict greater cardiovascular risk for a wide range of cholesterol concentrations. However, when there

is no reliable determination of LDL cholesterol, as in cases of hypertriglyceridemia, it is preferable to use the total/HDL cholesterol ratio (80).

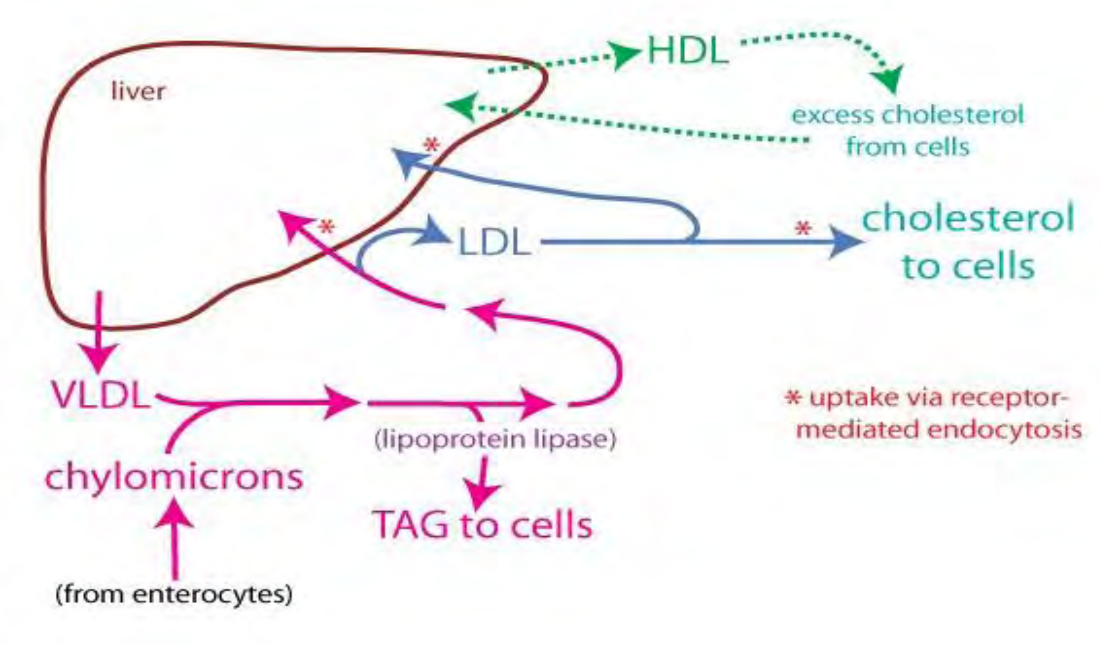


Figure 2: Summary of the fates of lipoproteins produced by the liver: VLDL and chylomicrons (74).

In the presence of infection or inflammation, the level of total cholesterol and HDL decreases and triglyceride level increases (81) whereas the ratio of total cholesterol to HDL (TC/HDL) remains relatively stable (82). This ratio may therefore serve as a more reliable measure of serum lipid level (83). Individuals with a high TC/HDL or LDL/HDL ratio have greater cardiovascular risk owing to the imbalance between the cholesterol carried by atherogenic and protective lipoproteins; this may be due to an increase in the atherogenic component contained in the numerator, a decrease in the anti-atherosclerotic trait of the denominator, or both. TC/HDL ratio is a more sensitive and specific index of cardiovascular risk than total cholesterol; for this reason, the ratio is also known as atherogenic or Castelli index (80).

Lipid profile measurement is usually done in fasting blood specimen, fasting refers to 12–14 h overnight complete dietary restriction with the exception of water and medication. This may hold true due to two main reasons: the first being postprandial triglycerides

remain elevated for several hours (84), and secondly, most reference values for serum lipids are established on fasting blood specimen (81).

Desirable blood lipid profiles are total cholesterol <200mg/dL, LDL cholesterol <100mg/dL, HDL cholesterol ≥60mg/dL, and triglycrides <150mg/dL. Low HDL-C was defined to be <40 mg/dL in men and <50 mg/dL in women (3, 79, 46). Lipoprotein ratios therapeutic target cut-off values are < 3.7 for LDL/HDL cholesterol and < 5 for TC/HDL cholesterol ratio (80).

A study conducted on Greek Christian Orthodox Church fasting found the decreased level of LDL and LDL to HDL ratio and Total Cholesterol. In various literatures, results for Triglycerides is conflicting (7).

2.3.1.4. Effects of religious fasting on Urea and Total protein

Most of the studies conducted on Greek Orthodox Christians, Ramadan, and Coptic Christians fasting revealed the fact that, urea level is affected significantly by religious fasting while none attributed Total protein level alteration to religious fasting (7, 8).

2.3.1.5. Effects of religious fasting on Serum electrolytes

Studies underwent during Ramadan fasting on electrolyte imbalance reported contradictory results for Sodium, some showed normal, and others decreased amount of total Na^+ excretion throughout the fasting and their justification was decreased intake of food. The results for K^+ were also conflicting. Some of them showed normal level and in other studies increased value during fasting and reasoned that common practice of drinking large volumes of fruit juices, eating dates and dried fruits as well as reduced potassium excretion. Literatures indicated increased level during Ramadan fasting for Ca^{+2} (63,64). In Greek Orthodox Christians decreased level of Ca^{+2} and unchanged level of Cl^- during fasting was reported (85).

2.4. Fasting in Ethiopian Orthodox Christianity

Ethiopian Orthodox Church has seven fasting periods to be observed by all believers and during those periods, the believers are not allowed to eat any animal or dairy source foods unless exempted from these fasts because of serious sickness, breastfeeding mothers, and children less than seven years of age (86). Greek Orthodox Christians fast for a total of 180 - 200 days each year (6), while Ethiopian Orthodox Christians fast for 250 days each year, of which about 180 are obligatory for all, and the rest are only for special groups in the church (priests, monks, and nuns) and their main fasting periods are the Fast of Prophets (*Tsome Nebiyat* or *Gena* 40 days prior to Christmas), the Great Lent (55 days prior to Easter), the fasting of Salivation (all Wednesdays and Fridays, except for the fifty days after Easter) and the Assumption of the Virgin Mary (*Tsome Filseta*, 15 days in August) (87).

The Great Lent or *Abye Tsome*, also called *Hudade* is the longest fasting period and the major fast of the church. This Lent is observed as a remembrance of the 40 days and nights fasting of Lord Jesus Christ after His baptism. Later the Ethiopian church added 15 more days to it and made it 55 days (87).

The effect of Ethiopian Orthodox Christian fasting on metabolic syndrome indices and level of serum electrolytes had not been studied. So the aim of this Study is to assess the effect of the longest fasting period of Ethiopian orthodox Christians, "*Abiy tsom*" (lent fasting) on the metabolic syndrome indices and level of serum electrolytes.

3. Hypothesis and objectives

3.1. Hypothesis

Ethiopian Orthodox Christian '*Abiy tsom*' (lent Fasting) alters Biochemical parameters related to metabolic syndrome and serum electrolytes.

3.2. Objectives

3.2.1. General objective

To determine effect of '*Abiy tsom*' (lent fasting) of Ethiopian Orthodox Christians on metabolic syndrome indices and serum electrolytes in residents of Addis Ababa, Ethiopia

3.2.2. Specific objectives

To compare anthropometric measurements during fasting and non-fasting periods.

To compare Systolic/Diastolic blood pressure and pulse rate during fasting and non-fasting periods.

To determine effect of lent fasting of Ethiopian Orthodox Christians on Lipid profiles.

To compare Blood Glucose level between two months fasting and non-fasting periods.

To determine the effect of Ethiopian Orthodox Christian lent fasting on level of Total protein and Urea level.

To assess the effect of Ethiopian Orthodox Christians lent fasting on serum electrolytes.

4. Materials and methods

4.1. Study area and period

Study was conducted at Addis Ababa University, college of health sciences, from March 15 to June 15, 2017. It is located in the City of Addis Ababa, capital city of Federal Republic of Ethiopia and Head Quarter of Africa Union. According to the Ethiopian National Population and Housing Census of 2007 (29), Addis Ababa has a total population of 2,737,551. About 23% of the total urban population of Ethiopia lives in Addis Ababa and with respect to religion 74.7% are Orthodox Christians, 16.2% Islam and 7.8% are Protestants (29).

4.2. Study design

Community based Longitudinal comparative cross sectional study was employed. Different measurements and data of voluntarily selected participants from eligible population were taken twice; the first during the last week of fasting period and the second during last week of the second month counted from Easter (last day of lent fasting). A major advantage of this, within subject, study design is that participants serve as their own controls, thus reducing the error variance and increasing the statistical power of the test with considerably fewer participants (89-91). Measurements include fasting blood collection for biochemical tests, anthropometric and blood pressure measurements, and the completion of questionnaires.

4.3. Study populations and subjects

Study populations were Orthodox Christians living in Addis Ababa and were chosen using convenience sampling technique on the basis of their willingness to participate and satisfying the inclusion criteria of the study. Employees and students of College of Health Sciences, Addis Ababa University and who are Ethiopian orthodox Christians believers and fasting '*Abiy tsom*'(lent) were selected as study subjects purposively for they are easily available around for the data and sample collection.

4.4. Sample size Determination and sampling technique

To determine sample size, the study used GPowerVersion3.1.9.2 as a tool, this software provides sample size and power analyses for tests that use F, t, chi-square, or z distributions and various distributions for nonparametric applications. GPower is one of the software packages that performs sample size calculations like Minitab and Epi-info

covering a wider range of study designs (92,93). As an input GPower requires selecting appropriate test family (t-test in our case), type of statistical test within test family (dependent sample t-test), specifying α error probability, power (1-β error probability), and determining effect size (94).

An effect size is the difference between two means (e.g., treatment minus control) divided by the standard deviation of the two conditions. It is the division by the standard deviation that enables us to compare effect sizes across experiments (95). Effect size can be used at planning stage to find the sample size required for sufficient power for study and for the purpose of calculating a reasonable sample size and effect size can be estimated by pilot study results, similar work published by others, or the minimum difference that would be considered important by experts (96). To calculate effect sizes from similar published research articles, a simplified methodology by Thalheimer and Cook (95) was used, employing equations 1 and 2;

$$d = \frac{\overline{x}_t - \overline{x}_c}{s_{pooled}} \quad \text{Eq. 1}$$

d= Cohen's **d** effect size

X = mean (average of treatment or comparison conditions)

S = standard deviation

Subscripts: **t** refers to the treatment condition and **c** refers to the comparison condition (or control condition).

$$s_{pooled} = \sqrt{\frac{(n_t - 1)s_t^{\,2} + (n_c - 1)s_c^{\,2}}{n_t + n_c}} \quad \text{Eq. 2}$$

Where: ***S*** = standard deviation

n = number of subjects

Subscripts: **t** refers to the treatment condition and **c** refers to the comparison condition ……. (or control condition).

From the study done by Sarie *and their colleagues* (7), fasters, as compared to their pre-fasting status, have showed a decreased levels of end total cholesterol, LDL-c and BMI as presented in Table 1. From that study, calculating for effect size among the three variables using equations 1 and 2 indicated that BMI (with mean difference of 0.4 and pooled standard deviation of 0.7) had relatively smaller or least detected difference with Cohen's **d** effect size of 0.56.

Table 1: Paired samples T-test with mean pre-fasting values compared to mean end-fasting results from Sarie *and their colleagues (*7).**

Variables	Mean	Stan Dev	Mean	Stan Dev	P-value	Pre-Fasting (n=43) End-Fasting (n=43) Effect Size (*d*)
Total cholesterol (mmol/L)	5.6	0.15	5.1	0.14	<0.001	3.40
LDL cholesterol (mmol/L)	3.3	0.1	3.2	0.1	<0.001	0.61
BMI (kg/m^2)	28	0.7	27.6	0.7	<0.001	0.56

Generally, effect sizes of 0.20 are considered small, 0.50 are medium, 0.80 are large and 1.3 are very large (96,97), these known benchmarks enable us to compare the above calculated effect size (0.56) to be categorized around medium effect size. Therefore, the present study considers medium effect size (0.5); power (1-β) of the study (0.85) and α-error probability (0.05) to have a sufficient sample size so as to detect differences that might present in the study variables; since sample size increases with increase in power, with a decrease in effect size and with decreasing level of significance.

Incorporating the above assumptions for sample size calculation using GPower software, sample size was found to be 73. Adding 20% for non-respondent rate the final sample size will be 88.

Similar previous studies conducted to evaluate the effect of religious fasting on biochemical and anthropometric variables used comparable sample sizes, to mention some: study by Mansi (14), used a total of 70 study subjects; Maislos *and their colleagues* (12), used 24 subjects; Thannoun & Mahmoud (13), used 31 subjects; Sarri and their colleagues (7), used 40 fasters and 31 controls; Sarraf-Zadegan *and their colleagues* (98), used a total of 50 study subjects and again Sarri *and their colleagues* (101), used 38 fasters and 29 matched controls. In repeated measures design fewer participants are often involved because of subject differences are minimized and hence reduces the error variability (90). Ha and Ha (99) suggests that if dependent variables of the study are either an interval or ratio scale, the sample size per group should be greater or equal to 30 ($n \geq 30$).

4.5. Inclusion and exclusion criteria

4.5.1. Inclusion criteria

All employees of College of Health Sciences, Addis Ababa University and who were Ethiopian orthodox Christians believers and fasting '*Abiy tsom*'(lent).

4.5.2. Exclusion criteria

Physical deformity (kyphosis, scoliosis…)

Pregnant women

Seriously ill and diagnosed of any chronic diseases

4.6. Measurements / variables

4.6.1. Dependent variable

Body mass index**,** Blood pressure, Fasting glucose (mg/dL), HDL cholesterol (mg/dL), LDL cholesterol (mg/dL), Triglycerides (mg/dL), Weight, Height, Waist circumference (cm), Hip circumference (cm), Waist Hip Ratio,level of urea and total protein, serum Na^{+}, K^{+}, Ca^{+2}, and Cl^{-} level (mmol/L).

4.6.2. Independent variable

Socio demographic characteristics

Age, Sex, Education status, Marital status

Life style Characteristics and Dietary Practices

Fasting animal source food**,** Smoking status/ Tobacco use, coffee drinking, Alcohol consumption, Khat chewing

4.7. Data Collection and Instruments for Data Collection

The instruments used for data collection were adapted mainly from the WHO's stepwise (STEPs) approach for non-communicable disease surveillance (102) and partly from Sarri *and their colleagues* (7). STEPs is the WHO-recommended surveillance tool for chronic disease risk factors and chronic disease-specific morbidity and mortality which is intended to serve as an entry point for low and middle-income countries into surveillance of chronic diseases and their risk factors (102). This approach is characterized by the use of questionnaires to gain information on risk factors, simple physical measurements (anthropometric and blood pressure measurements) and biochemical measurements (lipid profile and glucose level).

Data and Samples were collected twice, one at the last week of two months fasting period and the second at the end week of the second month after fasters returned to usual diet. After overnight fasting, sample were collected in the morning between 8 AM and 10 AM (7).

4.8. Anthropometry measurements

Body weight was measured two times at pre-stage and end of fasting by a digital scale (Seca, Hamburg, Germany) to the nearest 100g, placed in flat surface. Subjects were weighed barefoot in very light clothing. Standing height was measured with an adjustable wooden measuring board once, without shoes to the nearest 0.1 cm with the shoulders in relaxed position, arms hanging freely, feet together, heels against the back board and knees straight. Body Mass Index (BMI) was calculated by dividing weight (kg) by height squared (m^2) (7).

Waist circumference was measured at the midpoint between the lower margin of the least palpable rib and the top of the hip or minimal waist using stretch-resistant tape. Hip circumference was measured around the widest portion of the buttocks, with the tape parallel to the floor. For both measurements, the subject stand with feet close together thereby body weight evenly distributed, arms at the side and wearing light clothing. When the subject become at relaxed state measurement was taken at the end of normal expiration and these measurements were done in a private place (102). The cut-off points for waist to hip ratio above 0.90 for males and above 0.85 for females used to indicate CVD risk (102).

4.9. Physical/ Clinical examination

Blood pressure was measured digitally (Microlife BP A50, Microlife AG, Switzerland) The BP was taken using a mercury sphygmomanometer from the right upper arm after the subject was seated quietly for 5 min. Pulse rate was counted from radial artery and the count per minute was registered (102).

4.10. Laboratory Test

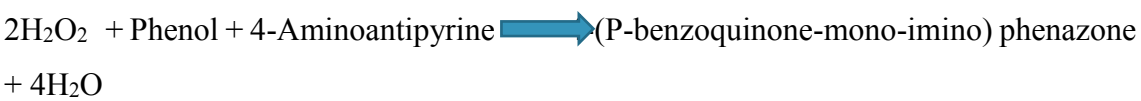
4.10.1. Specimen Collection and Handling

10ml of blood was drawn from fasting individuals using serum separator tube. The drawn sample stayed for 30 minutes and then centrifuged at a speed of 4000 rpm for 10 minutes. Then serum was taken and stored under -80^{0}C till the time of biochemical analysis. The serum levels of glucose, TC, HDL-c, LDL-c and TG were measured using COBAS INTEGRA 400 (Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim Germany) random access full automated auto analyzer.

4.10.2. DETERMINATION OF FASTING BLOOD GLUCOSE (GOD-PAP METHOD)

Test principle

Enzymatic colorimetric determination of Glucose according to the following reactions:



Glucose is oxidized by Glucose Oxidase, Phenol reacts with 4-Aminoantipyrine and Hydrogen Peroxide to produce a quinonimin dye, the intensity of color produced measured at 505nm, is proportional to the concentration of glucose in the sample.

Procedure

Mix, incubate for 8 min. at 37 °C or 12 min. at 20 - 25 °C. Read the absorbance (A). The final color is stable for 1 hour.

Table 2. Reaction mixture for determination of blood glucose

	Blank	Calibrator / Standard	Sample
Distilled water	10 □l	--	--
Calibrator/ Standard	--	10 □ l	--
Sample	--	--	10 □l
Reagent	1000 □l	1000 □l	1000 □l

4.10.3. DETERMINATION OF LIPID PROFILE

After all the specimens are collected the **serum is packed with ice** and transported for the biochemical analysis and the lipid profile tests were done by **spectrophotometer.**

Lipid profiles were analyzed by experienced hand and quality was assured. Samples were analyzed using fully automated biochemistry analyzers by the direct end point enzymatic method.

4.10.4. TOTAL CHOLESTEROL

Cholesterol is measured enzymatically in serum or plasma in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. One of the reaction by products H_2O_2 is measured quantitatively in a peroxidase catalyzed reaction that produces a color. Absorbance is measured at 500 nm. The color intensity is proportional to cholesterol concentration. The reaction sequence is as follows:

Cholesteryl ester + H_2O ---------->cholesterol + fatty acid

Cholesterol + O_2 ----------> cholest-4-en-3-one +H_2O_2

$2H_2O_2$+ 4-aminophenazone + phenol -------> 4-(p-benzoquinone- monoimino)-phenazone + $4H_2O$

Desirable cholesterol levels are considered to be those below 200 mg/dL in adults and below 170 mg/dL in children.

4.10.5. TRIGLYCERIDES

Triglycerides are measured enzymatically in serum or plasma using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol. Glycerol is then oxidized using glycerol oxidase, and H_2O_2, one of the reaction products, is measured as described above for cholesterol. Absorbance is measured at 500 nm. The reaction sequence is as follows:

Triglycerides + $3H_2O$ ----------> glycerol + fatty acids

Glycerol + ATP ----------> glycerol-3-phosphate + ADP

Glycerol-3-phosphate + O_2 ----------> dihydroxyacetone phosphate + $H_2O_2H_2O_2$ + 4-aminophenazone + 4-chlorophenol ---------> 4-(p-benzoquinone-monoimino)-phenazone + $2H_2O$ + HCl.

Desirable fasting triglyceride levels are considered to be those below 200 mg/dL, and are further categorized as Borderline, 200-400 mg/dL; High, 400-1,000 mg/dL; and Very High (> 1000 mg/dL). Triglycerides are also measured because the value is used to calculate low density lipoprotein (LDL)-cholesterol concentrations.

4.10.6. HIGH DENSITY LIPOPROTEIN (HDL) CHOLESTEROL

HDL is measured directly in serum. The basic principle of the method is as follows. The apoB containing lipoproteins in the specimen are reacted with a blocking reagent that renders them non-reactive with the enzymatic cholesterol reagent under conditions of the assay. The apoB containing lipoproteins are thus effectively excluded from the assay and only HDL-chol is detected under the assay conditions. The method uses sulfated alpha-cyclodextrin in the presence of Mg+2, which forms complexes with apoB containing lipoproteins, and polyethylene glycol-coupled cholesteryl esterase and cholesterol oxidase for the HDL-cholesterol measurement. The reactions are as follows:

(1) ApoB containing lipoproteins + α-cyclodextrin + Mg+2 + dextran SO4 ---> soluble non-reactive complexes with apoB-containing lipoproteins

(2) HDL-cholesteryl esters ----------> HDL-unesterified cholesterol + fatty acid

(3) Unesterified chol + O_2 ----------> cholestenone + H_2O_2

(4) H_2O_2 + 5-aminophenazone + N-ethyl-N-(3-methylphenyl)-N'_succinyl ethylene diamine + H_2O+ H+ ----------> qunoneimine dye + H_2O

Absorbance is measured at 600 nm.

4.10.7. LDL-CHOLESTEROL

Most of the circulating cholesterol is found in three major lipoprotein fractions: very low density lipoproteins (VLDL), LDL and HDL.

[Total chol] = [VLDL-chol] + [LDL-chol] + [HDL-chol]

LDL-cholesterol is calculated from measured values of total cholesterol, triglycerides and HDL cholesterol according to the relationship: [LDL-chol] = [total chol] - [HDL-chol] - [TG]/5 where [TG]/5 is an estimate of VLDL-cholesterol and all values are expressed in mg/dL.

Desirable levels of LDL-chol are those below 130 mg/dL in adults and 110 mg/dL in children.

4.10.8. DETERMINATION OF TOTAL PROTEIN BY BIURET METHOD

Principle:

Peptide bonds react with Cu^{2+} ions in alkaline solution to form a colored product. A colored chelate is formed between the Cu^{2+} ion, the carbonyl oxygen and amide hydrogen atoms (Fig 3). One Cu^{2+}ion is linked to 6 peptide bonds. Tripeptides, oligopeptides, and polypeptides react to yield pink to reddish-violet products.

The intensity of the color is proportional to the number of peptide bonds that are reacting and therefore to the amount of protein present in the medium, the absorbance of which is measured spectrophotometrically at 546 nm.

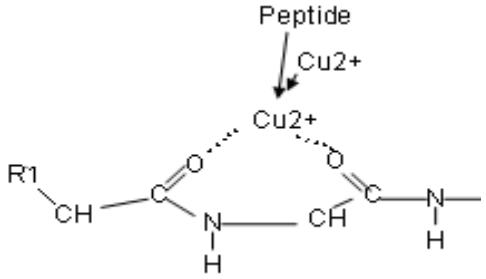


Figure 3: Schematic representation of Cu2+- Peptide complex

Reagents:

A. Biuret reagent:

I. Stock reagent

12mM $CuSO_4.5H_2O$, 32mMPotassium-Sodium tartarate dihydroxide, 30mM KI, 100ml of 6M NaOH

II. Working Reagent: The above stock reagent is dissolved in water until the volume reaches 1 liter.

B. Biuret Blank:

I. Stock reagent: 32mMPotassium-Sodium tartarate4H_2O, 30mM KI, 100mlof 6M NaOH

II. Working reagent: The above stock reagent is dissolved in water until the volume reaches 1 liter.

Procedure

To the labeled test tubes, 10 µL of serum sample or standard and 2.5 ml of working reagent was added (table 2). After mixing and incubation for 20 minutes at room temperature, concentration was measured at 546 nm.

Table 3. Reaction mixture for determination of Total Protein.

	Blank	**Standard**	**Sample**
Standard	---	10 µL	---
Sample	---	---	10µL
Working Reagent	2.5 ml	2.5 ml	2.5 ml
	Incubation:	20 minutes at room temperature.	
	OD reading:	546 nm.	

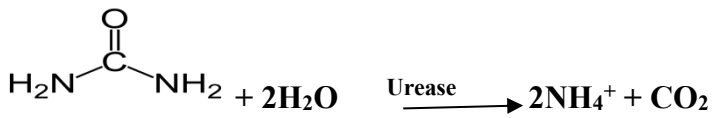
Normal Value

Adults: 6.3-8.6 g/dL Children: 4.6-7.0 g/dL

4.10.9. UREA ASSAY

Principle

Urea in the sample is hydrolyzed enzymatically into ammonia (NH_4^+) and carbon dioxide (CO_2). Ammonium ion formed reacts with salicylate and sodium hypochlorite (NaOCl), in the presence of nitroprusside, to form a green indophenol. The intensity of the color formed is proportional to the urea concentration in the sample.



Urea

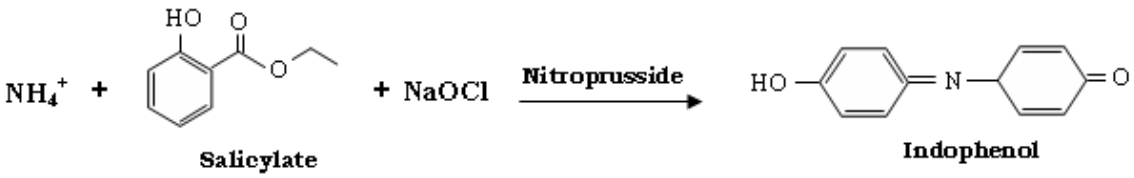


Figure 4: Principle of determination of urea level in the serum.

Reagents

I. R_1/Buffer: 50 mmol/L Phosphate buffer pH 6.7, 2 mmol/L EDTA, 60 mmol/L Sodium salicylate, 3.2 mmol/L Sodium nitroprusside

II. R_2 /NaOCl: 140 mmol/L Sodium hypochlorite (NaClO), 150 mmol/L Sodium hydroxide

III. R_3: 30000 IU/L Urease

IV. R4: 50 mg/dl urea standard.

Procedure: To the labeled test tubes, 1.0 ml of R1 was mixed with 10 µl of sample or standard. After incubation for 5 minute at 37^0C, 1 ml of R2 was added to all test tubes and incubated for 5 minutes at 37 ^{0}C. After adjusting the instrument to zero with distilled water, the absorbance was measured against the blank at 546 nm.

Table 4: Reaction mixture for the determination of urea

	Blank	Standard	Sample
R_1(ml)	1.0	1.0	1.0
Standard (µl)	--	10	--
Sample (µl)	--	--	10
Incubation	: 37 ºC, 5 min.		
R_2 (ml)	1.0	1.0	1.0
Incubation	: 37 ºC , 5 min.		

Calculation (Urea concentration)

Urea (mg / dL) = (ΔA_{sample} / $\Delta A_{standard}$) X Concentration of the standard

Normal Values: 15-45 mg/dl

4.10.10. ASSAY OF ELECTROLYTES (Na^+, K^+, Ca^{2+} and Cl^-)

Principle-

Analysis methodology is based on ion-selective electrode. There are six different electrodes used in the analyzer: Na^+, K^+, Cl^-, Ca^{2+}, Li^+, and a reference electrode. Each electrode has an ion-selective membrane that interacts specifically with the corresponding ions contained in the sample. The membrane is an ion exchanger, reacting to the electrical charge generating membrane potential, or voltage. A difference in ion concentrations between the inner electrolyte and the sample causes formation of electrochemical potential across the membrane of the active electrode. The potential is transferred by a highly conductive, inner electrode to an amplifier. The reference electrode is connected to ground as well as the amplifier. The ion concentration in the sample is then determined by using a calibration curve determined by standard curve generated using standards (64).

Reagents

Ion selective electrode (ISE) SnapPak™: containing150 mmol/L Na^{2+}, 5.0 mmol/L K+, 115 mmol/L Cl^-, 0.9 mmol/L Ca^{2+}, 0.3 mmol/L Li^+.

Procedure

After switching on the instrument, a brief count down began. After countdown was completed, the prompt 'Open Sample Door Introduce Sample' was displayed. The door was lifted and 100µl of sample was introduced in to the probe and closed sample door. The pump started to aspirate and the electrolyte results were displayed.

Reference range

Na^+:136-145 mmol/L, K^+:3.5-5.1 mmol/L, **Cl^-** : 97-111 mmol/L, **Ca^{2+}:**0.8-1.1 mmol/L for adults and 0.9 -1.2 mmol/L for children.

4.11. Ethical Consideration

Before starting the research ethical review committee of Department of Medical Biochemistry approved this research project and a letter of supports and ethical clearance was obtained from the ethical board with protocol Number **MSC 4/2017**. The nature of the study was fully explained to the study participant. After getting permission from the study participant, written consent was obtained from each study participant. Each study participant was informed about the research, their right to abandon the involvement at any time and confidentiality of information was maintained during data collection, analysis, interpretation and publication of results.

4.12. Data Quality Control

The principal investigator was with data and specimen collectors, directly involved, and controlled any kind of procedures and processes that may affect the result. The specimen was collected, stored and transported according to the guideline and the suspected specimen in terms of poor quality were rejected automatically. Working and acceptable commercial kits were used. The daily performances were reported to supervisors and checked and cross checked timely.

Anthropometric measuring instruments and biochemical analyzers were calibrated by their respective reference materials. Blood pressure was measured using gold standard method for measuring blood pressure, mercury sphygmomanometer.

Two assistant data collectors were involved one Health Officer for BP measurement and one laboratory professional for drawing blood specimen and anthropometric measurement. Two days training on the contents of the questionnaire, data collection techniques, and research ethics was given for those assistants and any doubts/question in the method they were going to undertake was clarified.

Pretest of the questionnaire was conducted in 10 volunteer subjects (~10% study sample size) for validation of questionnaire two weeks prior to actual data collection and some adjustment on additional preparations was made. During the actual data collection period, the questionnaire was checked for completeness every night after data collection. Feedback on previous day activities was given for the assistant data collectors before the

next day data collection and the overall coordination was made by the principal investigator.

4. 13. Data Management

Data was checked for completeness and entered into SPSS version 21 computer software package. After complete entry of all the questionnaires, soft copy was checked with its hard copy to see the consistency. After cross checking, cleaning was made to avoid missing values, outliers and inconsistencies were removed before analysis. Study participant who could not complete their end status measurement because of various reasons were also excluded from analysis.

4.14. Data Analysis and Report

Data obtained from laboratory results and anthropometry measurements were entered to Epi data version 3.1 and exported to SPSS version 21 for statistical analysis. Normality of continuous variables was checked by using graphic methods (Histograms with normality curves and QQ plots). Models were selected based on the type of variables and to compare the data during fasting period and non-fasting period, Paired sample t test was used and P value ≤ 0.05 was considered significant. Mean average of data was calculated and used for comparison.

4.15. Dissemination of the result

The result of this study will be disseminated to different concerned bodies including Addis Ababa University, College of Health Sciences and will be available as baseline informative document. Also the principal investigator will do his best with supervisors to publish the result on respective scientific journals and will make available for all online users and will consider presentation at national and international scientific conferences.

5. RESULTS

5.1. Socio demographic Characteristics of the Study participants

A total of 88 study subjects were enrolled and 40 were female and 48 were male. According to Age distribution most of them are found in age group of 21-30(61.3%). Regarding Educational status, most of them fall under the category of Above secondary education (79.7%). None of them responded yes for Chat chewing, alcohol use and cigarette smoking (Table 5).

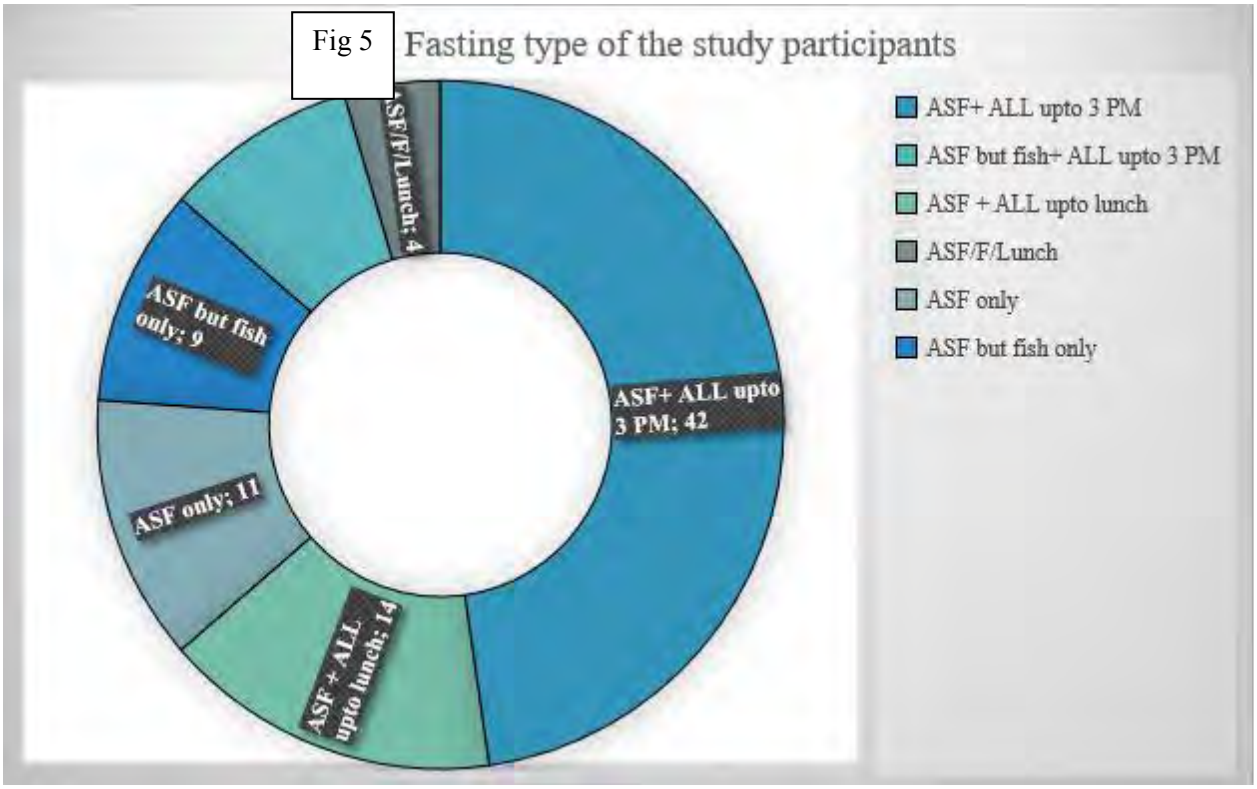
Table 5: - Socio demographic characteristic of the study participants

s.n	Characteristics	Frequency	Percent
1	**Sex**		
	Male	48	**54.5**
	Female	40	45.5
2	**Marital status**		
	Single	62	**70.4**
	Married	26	29.6
	Divorce		
	Widow		
3	**Age**		
	21-30	54	**61.3**
	31-40	30	34
	>40	4	4.7
4	**Educational status**		
	Cannot read and write		
	Primary Education	8	9
	Secondary Education	10	11.3
	Above secondary education	70	**79.7**
5	**Smoking cigarette**		
	Yes	0	0
	No	88	100
6	**Alcohol use**		
	Yes	0	0
	No	88	100
7	**Chat chewing**		
	Yes	0	0
	No	88	100

5.2. Fasting type of study participants

Fasting of Ethiopian Orthodox church may vary per individuals, so that type of the fasting study participants was assessed by interviewing them. Accordingly, most of our study subjects fast all animal source foods up to 3:00 PM (42 persons). 14 of them fast all animal source foods up to lunch (1:00PM) while 8 persons fast all animal source foods but not fish plus all foods up to 3:00 PM. Eleven of them fast all animal source foods only and 9 of them fast all animal sources except fish only (Fig. 5).

Fig 5



5.3. Comparison of Anthropometric Measurements During fasting and Non- Fasting

Anthropometric measurements of our study participants were taken twice, one during fasting period and the other non-fasting period, and comparison of the two measurements was done. The mean Weight of the study participants was increased from 60.39kg to 61.36kg after they returned to usual diet and their BMI was decreased from 20.04 kg/m^2 to 21.6 kg/m2. Waist Circumference was increased from 67.76cm to 74.63cm; Hip Circumference from 84.65cm to 92cm and Waist to Hip Ratio from 0.796 to 0.816 after they returned to their usual diet (Fig. 6).

Accordingly, the Weight, Body Mass Index, Waist and Hip Circumference were significantly increased during non-fasting period with P value of 0.037, 0.022, 0.001, and 0.001 respectively by paired sample t test. Hip to Waist ratio was found to be increased with non-significant change with p value of greater than 0.05(0.225) (Table 6).

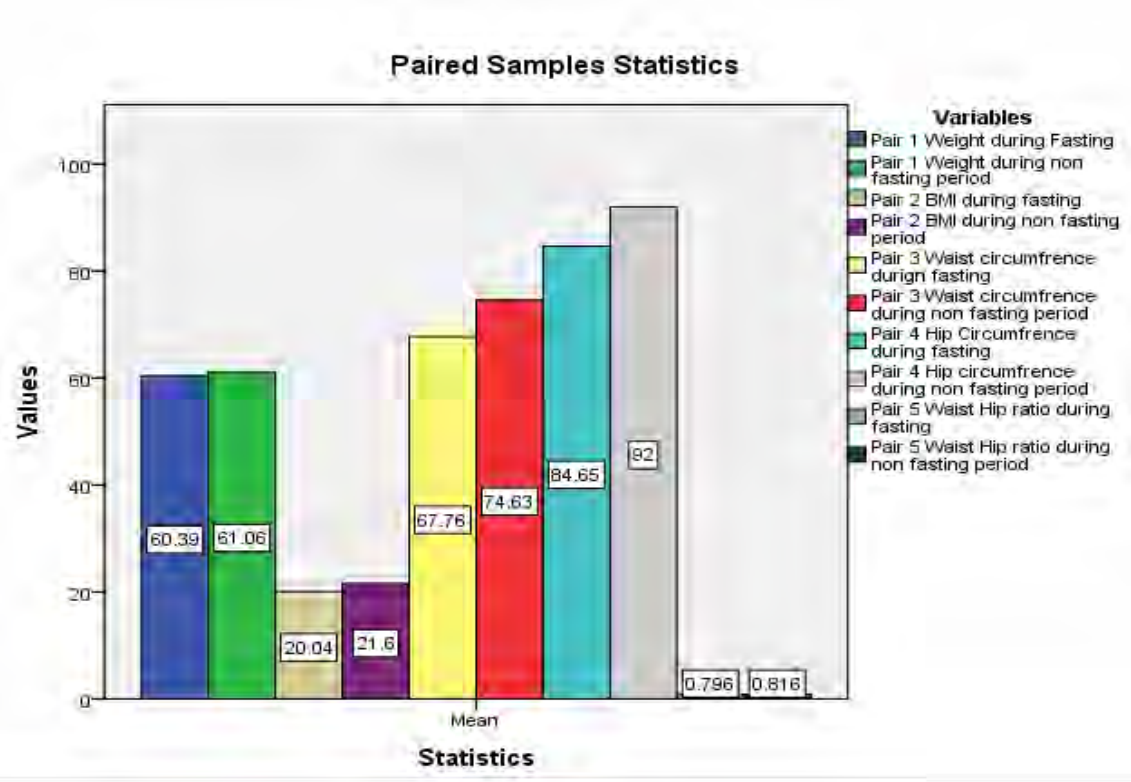


Figure 6: comparison of anthropometric means during fasting and non-fasting periods

Table 6: Paired sample t test Comparison of means of Anthropometric measurements of the study participants

Variables	Mean average during fasting period	Mean average during non-fasting period	t	df	p-value
WT	60.386	61.06	-2.119	76	0.037
BMI	20.04	21.6	-2.343	83	0.022
WC	67.76	74.62	-3.434	79	0.001
HC	84.65	92	-3.555	79	0.001
WHR	0.796	0.816	-1.224	79	0.225

5.4. Comparison of Systolic/Diastolic blood pressure and Pulse rate of the study participants

Paired sample t test analysis of comparison of means of systolic blood pressure, diastolic blood pressure and pulse rate indicated that systolic blood pressure was significantly changed with P value of 0.001 while the rest were not affected (Table 7)

Table 7: Paired sample t test Comparison of means of Blood pressure and pulse rate of the study participants

Variables	Mean average during fasting period	Mean average during non-fasting period	t	df	p-value
SBP	91.15	109.13	-3.379	65	0.001
DBP	76.0	75.24	0.455	53	0.651
PR	79.18	78.41	0.576	78	0.566

5.5. Comparison of Lipid Profile of the study subjects

Means of Lipid profiles were compared and changes were observed. Total cholesterol was decreased from 156.8mg/dl during fasting period to 144.4mg/dl after returning to usual diet. Triglyceride level was increased from 104.4mg/dl during fasting period to 105.1mg/dl after returning to usual meal. LDL cholesterol level was increased from 107.9mg/dl during fasting to 109.9mg/dl during non-fasting and HDL cholesterol level increased to 42.22mg/dl to 45.36mg/dl after fasting gone (Fig. 7).

Statistical Analysis of lipid profiles of the study participants by paired sample test indicates that, Total cholesterol, High Density Lipoprotein, LDL to HDL ratio and Total Cholesterol to HDL ratio were significantly changed with P value of 0.044, 0.0001, 0.015 and 0.035 respectively. Low density lipoprotein and Triglycerides level were not significantly affected (Table 8).

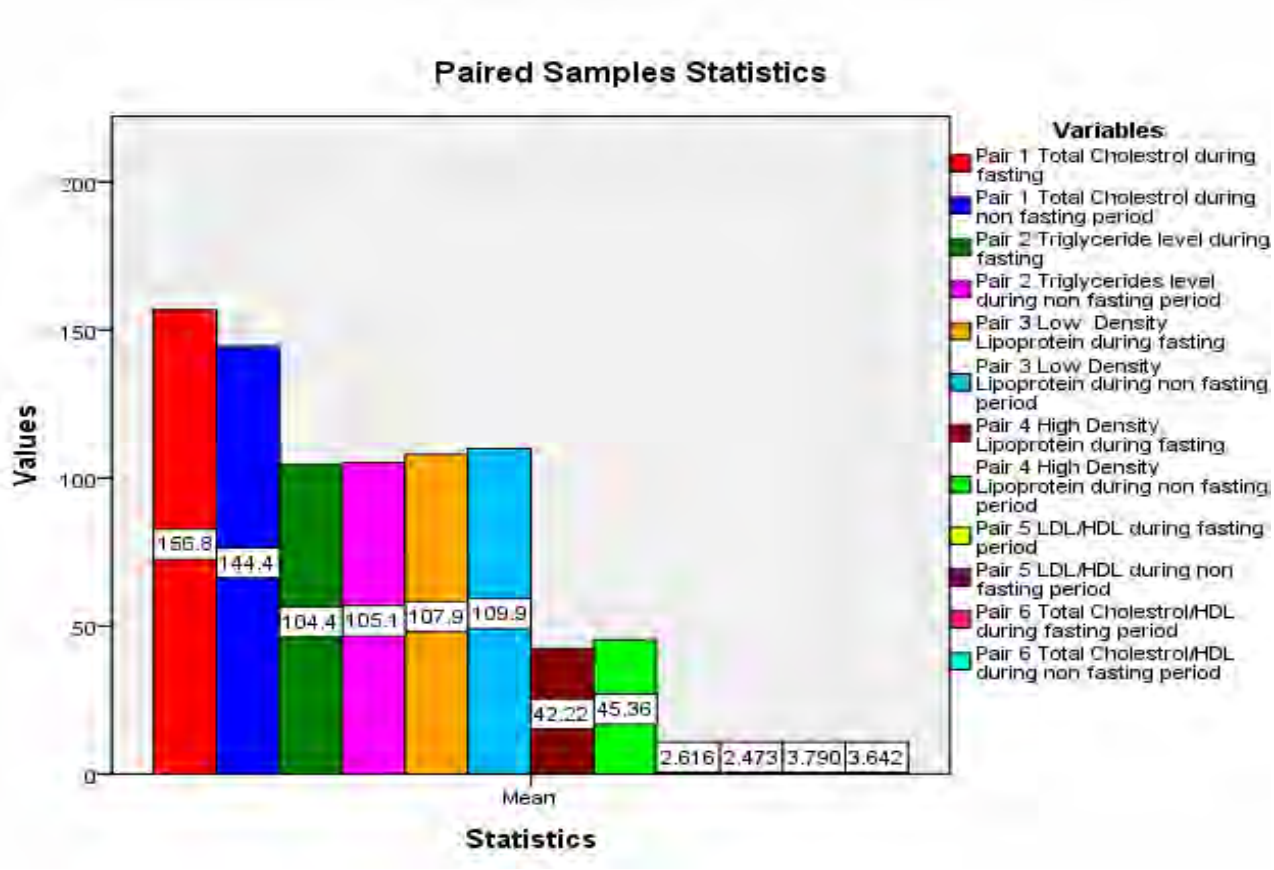


Figure 7: comparison of means of lipid profiles during fasting and non-fasting period

Table 8: Paired sample t test comparison of means of lipid profiles of the study participants

Variables	Mean average during fasting period	Mean average during non-fasting period	t	df	p-value
TC	156.8	144.4	1.88	83	0.044
TRI	104.4	105.1	0.115	73	0.909
LDL	107.9	109.9	-0.67	73	0.503
HDL	42.22	45.36	-4.0	73	0.0001
LDL/HDL	2.616	2.473	2.49	73	0.015
TC/HDL	3.79	3.642	2.15	73	0.035

5.6. Comparison of Urea level and total protein of the study participants

As the table below clearly shows, Urea level of the study subjects was significantly affected during two periods with P value of 0.028 while Total Protein remained unaffected (Table 9).

Table 9: paired sample t test comparison of means of Total protein and Urea level of study participants

Variables	Mean average during fasting period	Mean average during non-fasting period	t	df	p-value
UREA	18.89	20.31	-2.236	73	0.028
TOTAL PROTEIN	7.82	7.74	0.878	73	0.383

Comparison of Blood glucose of the study subjects

As shown below, the analysis made, revealed that Blood glucose level was not significantly affected after the fasting ceased for two months (Table 10)

Table 10: Paired sample t test comparison of blood glucose of participants

Variables	**Mean average during fasting period**	**Mean average during non-fasting period**	t	df	p-value
FBS	91.59	93.15	-0.830	73	0.409

5.7. The effect of Ethiopian Orthodox Christians fasting on the levels of serum electrolytes (Na^+, K^+, Ca^{+2} and Cl^-)

The analysis made to determine the effect of fasting on the levels of serum electrolytes indicated that, the average means of Sodium and Potassium ions were slightly higher during fasting period than the non- fasting period (142 mmol/L, 141 mmol/L) and (4.508 mmol/L, 4.426 mmol/L). It was also found that serum calcium and chloride ions were sharply decreased after the study participants ceased the fasting and returned to usual diet (1.510 mmol/L,1.047 mmol/L) and (107.7 mmol/L, 105.9 mmol/L) (Fig. 8).

Paired samples t test was done to check whether this effect was significant and showed that the effect on calcium and chloride ions were significant with P value 0.015 and 0.033 respectively (Table 11).

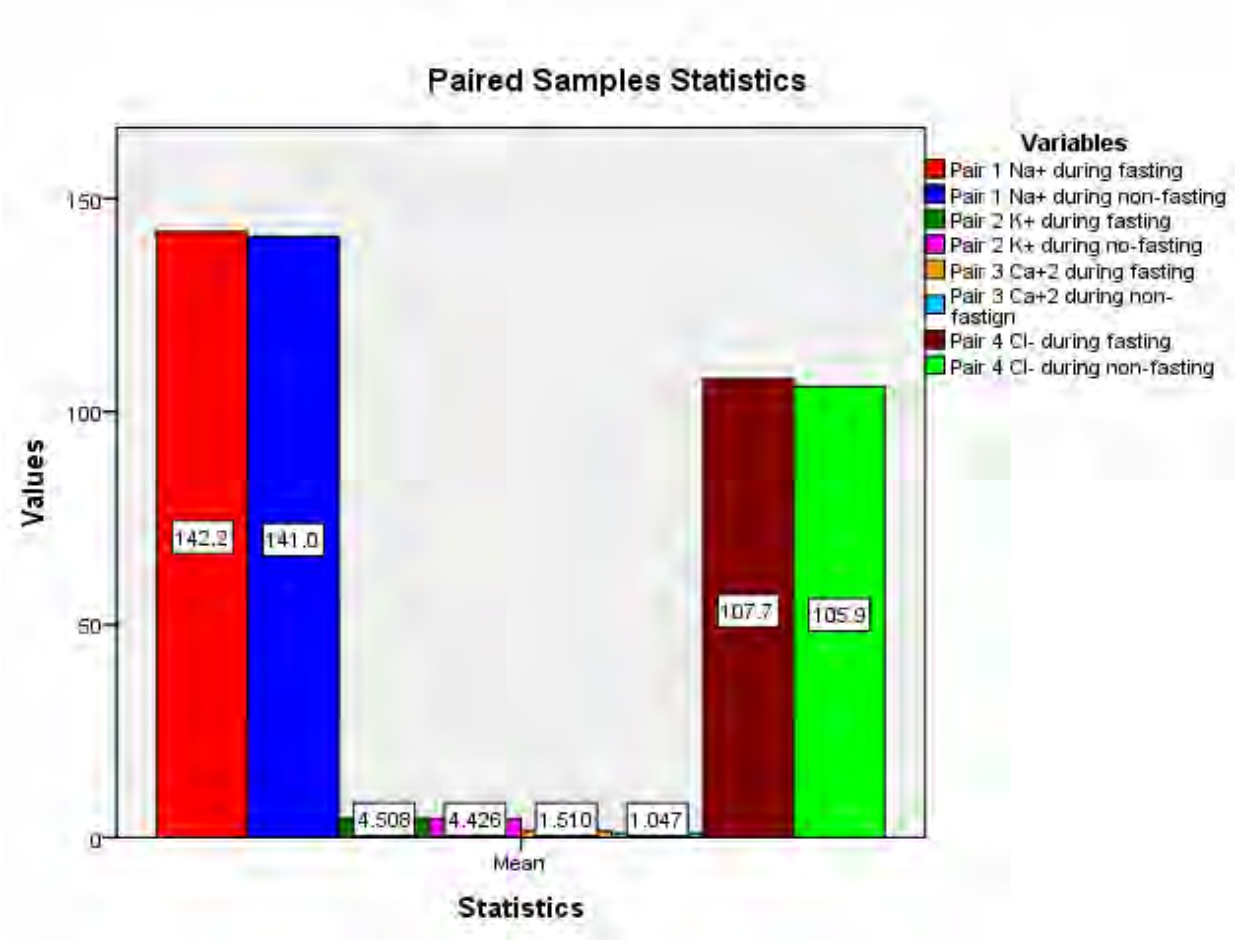


Figure 8. Comparison of mean levels of serum electrolytes during fasting and non-fasting.

Table 11: paired samples t test comparison of levels of serum electrolytes of study participants

Variables	**Mean average during fasting period**	**Mean average during non-fasting period**	t	df	p-value
Na^+	142.2	141.0	1.545	80	0.126
K^+	4.508	4.426	1.678	80	0.097
Ca^{+2}	1.51	1.047	2.478	80	0.015
Cl^-	107.7	105.9	2.165	80	0.033

6. DISCUSSION

This study found that Ethiopian orthodox Christian fasting significantly decreased anthropometric measurements like weight, Body Mass Index, Waist and Hip Circumference. These findings are supported by various studies. A study undergone to assess effects of Greek Orthodox Christian church fasting revealed that Body mass index was decreased during fasting period (7). Another interventional study conducted on a strict vegetarian diet for five weeks on 35 women found significantly decreased Body Mass Index (76). Other researchers that investigated seventh day Adventist who were strict vegetarians, found decreased weight, Body mass index and Waist to Hip ratio of the subjects (74,78). This significant change of Weight, Body Mass Index, Waist circumference and Hip circumference can be attributed to decreased protein intake and caloric restriction of Ethiopian Orthodox Christians fasters and insignificant influence on Waist and Hip ratio may be due to increment of both waist and Hip circumferences simultaneously keeping the ratio almost constant.

Regarding the effect of Ethiopian orthodox Christian fasting on systolic blood pressure, diastolic blood pressure and pulse rate, we found that the fasting significantly affected systolic blood pressure while diastolic blood pressure and Pulse rate remain unchanged. A literature reported that Ramadan fasting had no significant effect on all of these three parameters (68). In contrary, other study found that Ramadan fasting had significantly affected systolic blood pressure and the latter two remain unaltered which supports our finding (70). Similar to that of Ramadan, the results from studies conducted on Greek Orthodox Christians are conflicting. A study found that systolic blood pressure increased during fasting periods (69), while another study found no change in blood pressure when fasters were compared with non-fasters (7). One study reported that non-fasters' diastolic blood pressure decreased significantly during fasting periods when compared to the changes in fasters' diastolic blood pressure (7), while another study reported that fasters' diastolic blood pressure did not change during fasting periods (69). But all of them revealed that Pulse rate at rest was not affected significantly.

The comparison of the lipid profile of the fasters during period of two months fasting and non-fasting period indicates that most of the parameters were affected significantly. Specifically, our study showed that Total cholesterol, High density lipoprotein, Total

cholesterol to HDL ratio and LDL to HDL ratio were significantly influenced by fasting with P value 0.044, 0.001, 0.015 and 0.035 respectively. However, the level of Low density lipoprotein and triglycerides were not decreased significantly during the fasting period.

A study conducted on Greek orthodox Christians fasting also found the decreased level of LDL and LDL to HDL ratio. But in this study after the fasters returned to usual diet, sharp increment in LDL and Total Cholesterol was witnessed. The reduction in HDL we found is the common finding with low fat and vegetarian diet. Conflicting with our study, a study reported the ratio of Total cholesterol to HDL and LDL to HDL were remain unchanged (76). Another literature also found decreased Lipid profile parameters in their study on low fat and vegan group for 12 weeks (77). The disparity may be due to different study setting, where they studied by intervention diet and also the population samples differ with possible genetic variation.

Concerning total cholesterol, a group of researchers reported decreased level during Greek Orthodox fasting and also in catholic Christian during lent. The difference may be due to the fasting style of Catholics which is different from that of orthodox (7,66). In various literatures, results for Triglycerides is conflicting (77). In our case, we didn't get significant change. This may be because during fasting the body may synthesize Triglycerides from carbon skeletons of amino acids and metabolites from carbohydrate metabolism.

The other crucial effect we observed is that urea level which was significantly increased during Fasting period. This is well supported by a study carried out in district of Maharashtra, India that underwent among Judo athletes (71,72). Many dietitians consider the diet of plant origin consumed by vegans to be "lighter" and "more healthful" for the kidneys than the diet of both plant and animal origin consumed by omnivores (71). The present findings agree with these conclusions.

Additionally, significant changes of Total protein and blood glucose were not observed. This is also similar finding of various studies among different fasting season of religious groups during Ramadan fasting and in Greek orthodox Christian church fasting (7,73). Normally this is expected result because Ethiopian Orthodox Christian fasters do not restrict protein from plant sources and in the same way the carbohydrates, except that of animal sources.

This study observed the effect of Ethiopian Orthodox Christians fasting on serum electrolytes and found that, Serum Sodium and Potassium ions were insignificantly increased while Calcium and Chloride ions were significantly raised during fasting season with P value of 0.015 and 0.035 respectively.

Studies underwent during Ramadan fasting on electrolyte imbalance reported contradictory results for Sodium i.e., some showed normal, and others decreased value of total Na^+ excretion throughout the fasting and reasoned decreased intake of food. The results for K^+ were also conflicting. Some of the studies showed normal level while others studies increased value during fasting and reasoned that common practice of drinking large volumes of fruit juices, eating dates and dried fruits as well as reduced potassium excretion. Literatures indicated increased level during Ramadan fasting for Ca^{+2} and Cl^- which is similar to our findings (81,82)

In Greek Orthodox Christians decreased level of Ca^{+2} and unchanged level of Cl^- during fasting was reported. This may be due to difference of the study setting. In line with our findings, the level of Na^+ and K^+ were not affected significantly (83).

Generally, lent fasting of Ethiopian Orthodox Christians affected several parameters significantly. The variation of our findings from previous studies conducted among Greek Christians fasting, Ramadan fasting, Adventists fasting and catholic fasting may be attributed to differences in study setting, the type and period of fasting, type of food avoided during fasting period and environmental variations.

7. CONCLUSION

This study was an effort to assess effect of Ethiopian orthodox Christians fasting of two months' period on some metabolic syndrome indices.

Ethiopian orthodox Christians fasting has a considerable effect on those metabolic indices changing various parameters. It is observed that, anthropometric parameters like weight, Body mass index, Waist circumference and Hip circumference were significantly decreased during fasting season indicating that fasting is beneficial for weight loss and combating against metabolic syndrome.

Systolic blood pressure was decreased during fasting period while diastolic blood pressure and pulse rate were remained unchanged.

The fasting also caused significant change in lipid profiles decreasing multiple biochemical parameters which again shines green light on using fasting as a strategy to fight disorders caused by increased consumption of fats specifically animal fats.

Fasting affected the serum electrolytes which was not significant in Na^{+} and K+, and significant in Ca^{+2} and Cl^{-}.

In general, Ethiopian orthodox Christian fasting was found to causes significant alterations of biochemical parameters and our study findings were very congruent to findings of different studies carried among Greek orthodox Christians.

8. Strengths and Limitations of the study

The study assessed the effect of religious fasting to metabolic syndrome indices and level of serum electrolytes and therefore will serve as a baseline for future studies.

The study used participants selected through non-probability sampling method, and thus may affect the external validity of the findings. However, the study used a within-subject design that helped see changes before and after fasting. This study didn't check for the geographical and seasonal factors that may affect the results. Data were collected during different season one in autumn (April) and the other summer (June) and thus, the result may be affected by environmental variations (weather condition).

9. RECOMMENDATION

This study found that Ethiopian Orthodox Christians fasting has considerable effect on Metabolic syndrome indices and level of serum electrolytes and therefore:

- Ethiopian Orthodox Christians Church is recommended to intensify teaching its believers the benefit of fasting regarding health in addition to that of spiritual relevance.
- Researchers are recommended to undertake this kind of study with wide ranges of parameters.
- All stake holders are also recommended to carry out extensive behavioral change communication and life style modification promotion; including decreasing the consumption of animal source foods.

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Annex I: - CONSENT FORM

Code of study subject _____

I have been informed about a study plan that is entitled with "the effect of Ethiopian Orthodox Christians "*Abiy tsom"* (lent fasting) on metabolic syndrome indices and level of serum electrolytes" and for this purpose some information and blood sample will be taken from me. The aims of this study were explained to me. Collection of the sample would follow the usual procedure for laboratory investigation but there might be some pain that is associated with the blood collection.

I am also informed that all the information contained within the questionnaire is to be kept confidential. Moreover, I have also been well informed of my right to keep hold of information, decline to cooperate and make myself withdraw from the study. I have been informed that laboratory results will be disclosed to me whenever the result is ready and incase the result had any pathological indication, I am told that I will be linked to appropriate place for further diagnosis and treatment.

It is therefore with full understanding of the situation that I gave the informed consent voluntarily to the researcher to use the specimen taken from me for the investigation. Moreover, I have had the opportunity to ask questions about it and received clarification to my satisfaction.

Signature (participant)_____ Signature(investigator)_____

Witness name_____ Signature _____

Annex III: Questionnaire

I. Socio- demographic characteristics

1. Study participant identification number_____
2. Date of interview_____
3. Place of interview_____
4. Age (in year) _____
5. Gender 1. Male ☐ 2. Female ☐
6. Educational Status _____
7. Marital Status _____
8. Address _____
9. Household wealth income_____

II. Previous medical related conditions

1) Have you diagnosed with any of the following diseases previously?
   - A. Diabetes ☐
   - B. Hypertension ☐
   - C. Cardiac vascular diseases ☐
   - D. Renal diseases ☐
   - E. Liver disease ☐
   - F. Other, specify_____

2) Have you any previous surgical history?
   - A. YES ☐
   - B. NO ☐

3) Have you taken any medications recently? If yes, specify_____

III. Dietary and behavioral related questions

1. Would the person use the following items, if so how frequent?

a. Alcohol Frequently ☐ Sometimes ☐ Never ☐

b. Smoking Frequently ☐ Sometimes ☐ Never ☐

c. cigarette Frequently ☐ Sometimes ☐ Never ☐

d. Chat Frequently ☐ Sometimes ☐ Never ☐

2. What was your most utilized food type during this fasting period? Specify_____

3. Which of the following resembles your fasting situation of this period?

   A. Abandon all type of animal source foods since the beginning of fasting period and daily consumed non animal source foods as usual.

   B. Abandon ☐ type of animal source foods and restrict all food daily up to 6:00 local time ☐

   C. Abandon all type of animal source foods and restrict diet daily up to 9:00 local time ☐

   D. Other, specify. _____

IV. Anthropometric measurements

A) Body weight_____

B) Height_____

C) Body mass index_____

D) Waist circumference _____

E) Hip circumference_____

F) Systolic blood pressure_____

G) Diastolic blood pressure_____

Annex III: - Laboratory test result

1. Fasting glucose_____
2. High density lipoprotein_____
3. Low density lipoprotein_____
4. Total cholesterol_____
5. Triglyceride_____
6. Total protein_____
7. Urea_____
8. Na^{+} _____
9. K^{+}_____
10. Ca^{+2} _____
11. Cl^{-}_____

Annex IV. DECLARATION

I, the undersigned, declare that this thesis is my original work and that all sources of materials used for the thesis have been dully acknowledged.

Name: **Chala Kenenisa**

Signature: _____ Date: _____

Annex V: Ethical Clearance

BIOCHEMISTRY DEPARTMENT
SCHOOL OF MEDICINE
ADDIS ABABA UNIVERSITY

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Ref. No. SOM/BCHM/ 147/2009
Date: 24/5/2017

epartment Ethics and Research Committee (DRERC) Decision

eeting No. DRERC 01/17 Date: 09/03/2017

otocol number: M.Sc. 4/17

otocol Title: **Comparison of Metabolic Syndrome Indices between two months Fasting and on-Fasting Period among Ethiopian Orthodox Christians**		
ncipal Investigator	**Chala Kenenisa**	
titute:	Department of Biochemistry, School of Medicine. Addis Ababa University	
ments reviewed	Attached	
ision of the meeting:	Approved Resubmission	√**Approved with Recommendation** Disapproved

igation of the PI:-

Should comply with national and international scientific and ethical guidelines
All amendments and changes made in protocol and consent needs DRERC approval

DOB, DRERC approval period from: 24/5/2017

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