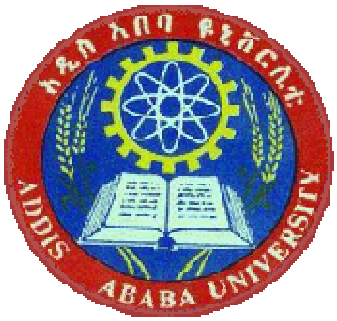

Addis Ababa University

School of Graduate studies

M. Sc. Thesis

Title: Coffee consumption association with insulin sensitivity and plasma lipid profile among non-diabetic individuals in Addis Ababa

By

Gizaw Mamo

Department of Biochemistry

Faculty of Medicine

July 2010

Addis Ababa University

Association of Coffee Consumption with Insulin Sensitivity and Plasma Lipid Profile among Non-Diabetic Individuals in Addis Ababa

A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfilment of the Requirement of the Degree of Master of Science in Biochemistry

By

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Acronyms

HOMA-IR	Homeostasis model assessment-insulin resistance
DM	Diabetes mellitus
ELISA	Enzyme Linked-Immuno-Sorbent Assay
CD36	cluster of Differentiation 36
ACO	acyl-coA oxidase
UCP2	uncoupling protein-2
OGTT	oral glucose tolerance test
HDL-C	high density lipoprotein-cholesterol
LDL-C	low density lipoprotein-cholesterol
BMI	body mass index
SBP	systolic blood pressure
DBP	diasystolic blood pressure
AAU	Addis Ababa University
WR	working reagent
TMP	1, 1, 3, 3-tetramethoxypropane
Acyl CS	acyl-coA synthetase
AMP	adenosine monophosphate
ACOD	acyl-coA oxidase
4-AA	4-aminoantipyrine
MEHA	3-Methyl-N-Ethyl-N-(B-hydroxyethyl)-Aniline
LPL	lipoprotein lipase
G3P	glycrol-3-phosphate
GPO	glycerol phosphate dehydrogenase
DHAP	dihydroxyacetone phosphate
4-AP	4- aminophenazone
POD	peroxidase
VLDL	very low density lipoprotein
TOOS	N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-Methylaniline

Abstract

Background: Coffee is the most consumed beverage in the world and research has attempted to make clear health benefits received from coffee drinking. Type 2 diabetes is one of the diseases that are largely determined by lifestyles factors. The role of coffee in relation to type 2 diabetes and other diseases such as cardiovascular disease, hypertension and neurological diseases in Ethiopia has not been well investigated thoroughly.

Objective: The aim of the present study is to investigate the relationship between coffee consumption and insulin sensitivity status among non-diabetic individuals in Addis Ababa.

Materials and Methods: A cross-sectional study was conducted in 70 non-diabetic and apparently health individuals in Addis Ababa. The participants were 35 coffee drinkers (16 males; 19 females) and 35 non-drinkers (15 males; 20 females). Data regarding coffee consumption was obtained by using questioners and laboratory analysis. Blood samples were collected for analysis of insulin sensitivity indicators, namely-; serum fasting and post loading glucose and insulin level and serum lipid profile (cholesterol, triglyceride, free fatty acid, high density and low density lipoproteins). Results were compared among coffee consumers and non consumers using appropriate statistical parameters

Results: Median of serum level of fasting and post-load glucose was 101.2 and 118.8 for coffee drinkers and 101.9 and 120.9 for non-drinkers. These results didn't show statistically significant ($P>0.05$) difference between the two groups. There was also no significance difference in the fasting (14.8 and 12.7) and post-loading (31.0 and 26.7) of blood insulin level between coffee drinkers and non-coffee drinkers. Similarly, blood level of fasting and post-loading glucose and insulin of non-coffee drinkers were not significantly difference from blood level of fasting and post-load glucose and insulin of the coffee drinkers of men, women, young and not-so young groups. Compared to non-coffee drinkers, coffee drinkers of men ($p<0.01$, $p<0.05$), young groups ($p<0.05$, $p<0.05$) and not-so young groups ($p<0.05$, $p<0.05$) showed significantly increased and decreased in blood concentration of free fatty acid and triglyceride respectively. The ratio of TG to HDL-C was significantly lowered in coffee drinkers. Other parameters were not significantly difference between coffee drinkers and non-drinkers.

Conclusion: Significantly higher of blood free fatty acid level in coffee drinkers may indicate that coffee reduces insulin sensitivity. However, non-significantly lower of blood level of triglyceride and LDL-C with higher HDL-C, significantly decrease of the ratio of TG to HDL-C and non-significantly lower HOMA analysis for fasting and post-loaded in coffee drinkers may indicate coffee improves insulin sensitivity and has antioxidant activities.

1. Introduction

Coffee is one of the most well known beverages, acting as a stimulant for people's psychological activity **(1)**. This biological effect of coffee has been mainly attributed to caffeine. Coffee also contains many other chemicals, such as chlorogenic acid, isoflavones and flavonoids which have a variety of physiological functions, such as antiviral, antibacterial, antifungal, antioxidant and antihyperglycemic capacities. Coffee has also an economical, social and political values and the consumption is a part of ceremony in Ethiopia society. Compounds in coffee other than caffeine have attracted clinicians and researchers to understand mechanisms regarding its positive or harmful effects on the human health. Effects of coffee and caffeine on type 2 diabetes, cardiovascular disease, hypertension, neurological diseases, different types of cancer, hormonal changes, gallstones, as well as renal stones have been studied through epidemiological, clinical, or experimental researches **(2-3)**. The present study tried to provide data for the protective effect of coffee consumption against the development of type 2 diabetes in Ethiopian subjects.

1.1. Chemical Composition of Coffee

More than 1,000 substances have been recognised in coffee by different techniques such as HPL chromatograph and NMR. Some of these components have been clearly identified and their biological functions defined **(4-5)**. Caffeine and chlorogenic acids are the most well known substances in coffee producing physiological effects in human being. Coffee also contains some essential nutrients which could be utilized by human body. Some important ingredients found in common types of coffee are shown in the Table 1.1.

Table 1.1 Composition of green or roasted coffee (expressed as a percentage of dry weight)

Component	Arabica		Robusta	
	Green	Roasted	Green	Roasted
Caffeine	0.9-1.2	0.9-1.0	1.6-2.4	1.9-2.0
Chlorogenic acids	5.5-8.0	1.2-2.3	7.0-10.0	3.9-4.6
Minerals	3.0-4.2	3.5-4.5	4.0-4.5	4.6-5.0
Lipids	12.0-18.0	14.5-20.0	9.0-13.0	11.0-16.0
Carbohydrates	56.0-63.0	24.0-42.5	42.0-54.0	0.0-3.5
Proteins	11.0-13.0	13.0-15.0	11.0-13.0	13.0-15.0

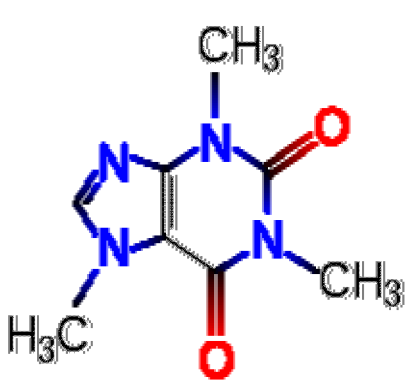
Caffeine was isolated in 1823 for the first time by Runge and Van Giese and is the most studied molecule in the coffee. Caffeine is a methylxanthine family, derived from purine (Figure 1.1) **(6)**. Caffeine becomes rapidly absorbed by the intestine and stomach within 45 minutes of being ingested, reaching its maximum concentration peak 15 to 20 minutes after being ingested. As it is a hydrophobic molecule, it passes cell barriers, including blood brain and foetal barriers in such a way that a foetus receives the same concentration of caffeine as its mother **(7)**.

Caffeine is metabolised in the liver where it undergoes successive demethylations and oxidations until being eliminated in the urine in the form of theophylline, theobromine and paraxanthine. Most researchers have argued that stimulant effect of caffeine on the nervous system is the result of adenosine A1 receptor inhibitory action. Adenosine A1 receptors are found in the brain and also in the heart, trachea, kidneys and adipose tissue **(7)**. These receptors intervene in cellular exchange of potassium and calcium and thus play a part in a process of regulating cell membrane polarity, excitability of the nerves, diuresis and secreting renin, partly explaining its mechanisms on the nervous system and polyuria **(8)**.

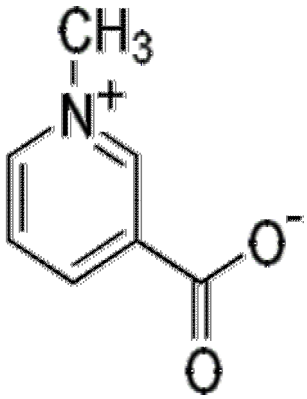
Caffeine also acts on phosphodiesterase, inducing relaxing of the trachea and bronchial tubes, and meaning that it has a favourable effect on asthma attacks. It also causes mobilisation of intracellular calcium inducing changes in nerve cell function **(9)**.

Nitrogenated bases other than caffeine are also found in coffee and divided into two large groups. There are roasted stable ones such as ammoniac, betaine and choline and some unstable ones such as trigonelline (Figure 1.1), which becomes decomposed to nicotinic acid or niacin **(10)**.

Figure 1.1 Molecular structures of caffeine and trigonelline



Caffeine



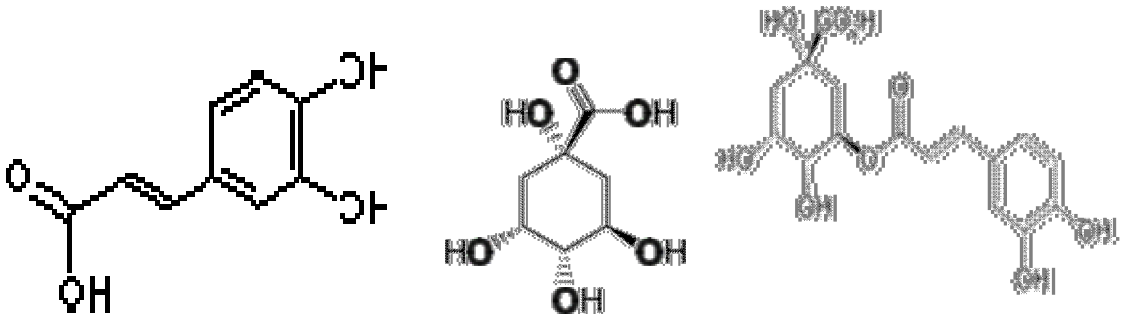
Trigonelline

Chlorogenic acids are other most common molecules in the coffee and they are hydrolyzed in to caffeic and quinic acids in human (figure 1.2). A prospective study performed in the Netherlands' and Japan reported that one or two cups of coffee stimulate gastric secretion and increases chlorhydric acid production as a result of chlorogenic acid action, while quinic acid has none of these activities **(11)**. Quinic and vinidinic acid content lying between 0.3% and 0.5% in green beans becomes increased during treatment, but from the biological point of view their derivatives are more important. n-Chlorogenic acid is one of these whose proportion and derivatives depends on the degree of roasting. A coffee's quality partly depends on relative mono and di-chlorogenic acid proportion **(12)**. A di-chlorogenic excess can lead to a coffee's metallic, bitter taste associated with coffee which has been kept hot for a long time resulting from an increase in quinic acid and a lowering of pyridine concentration. Table 1.2 shows different forms of chlorogenic acids in coffee Arabica and Robusta.

Table 1.2 Chlorogenic acids in the raw coffee (expressed as a percentage of dry weight)

Component	**Arabica**	**Robusta**
5-chlorogenic acid	3.0-5.6	4.4-6.5
4-chlorogenic acid	0.5-0.7	0.7-1.1
3-chlorogenic acid	0.3-0.7	0.6-1.0
Total	3.8-7.0	5.7-8.6
3,4-dicaffeoylquinic acid	0.1-0.2	0.5-0.7
3,5- dicaffeoylquinic acid	0.2-0.6	0.4-0.8
4,5- dicaffeoylquinic acid	0.2-0.4	0.6-1.0
Total	0.5-1.2	1.5-2.5
3-feruloylquinic acid	Traces	0.1
4-feruloylquinic acid	Traces	0.1
5-feruloylquinic acid	0.3	1.0
5-feruloyl,4-caffeoquinic acid	0	Traces
Total	1.2	1.2

Figure 2.2 Molecular structures of chlorogenic acid, caffeic acid and quinic acid



Caffeic acid Quinic acid Chlorogenic acid

Coffee contains nutrients, such as minerals, carbohydrates, lipids and proteins. It may be true that the composition of coffee represents an excellent source of nutrients for the human body. Sodium, potassium, calcium, phosphorous, iron, zinc and copper are common minerals in coffee **(13)**.

Chemical composition differences between Arabica and Robusta varieties are mainly concerning their carbohydrate content **(12)**. It is found that the Arabica variety's sucrose concentration is 2 to 3 times greater than that of the Robusta variety, an important factor in characteristics regarding taste (Table 1.3) **(14)**.

Table 1.3 Carbohydrate content of green coffee (% of dry weight)

Constituent	Arabica	Robusta
Monosaccharides	42.3-46.9	45.4-49.2
Sucrose	6.0-9.0	3.7
Polysaccharides	43.0-45.0	46.9-48.3

The protein content of both Arabica and Robusta is similar, being between 8.8% and 12.2% of green coffee on dry weight basis, but the free amino acid content is very poor ranging from 0.2% and 0.8%. About 20% to 40% proteins are lost during the roasting process as a result of denaturation. The proportion of some amino acids, such as alanine, aspartic acid, proline and tryptophan are increased during roasting processing. However, some amino acids such as arginine, glutamine, cysteine, serine and threonine are combined with other substances in coffee during roasting processing and so they are disappeared as a result of such processing **(15)**.

Main fatty acids in coffee are palmitic , linoleic , oleic , stearic and arachidonic acids , some of them are saturated and others are non-saturated. Roasted Arabica and Robusta contain higher lipids than their green forms **(16)**.

1.2. Biological Effects of Coffee in Human Health

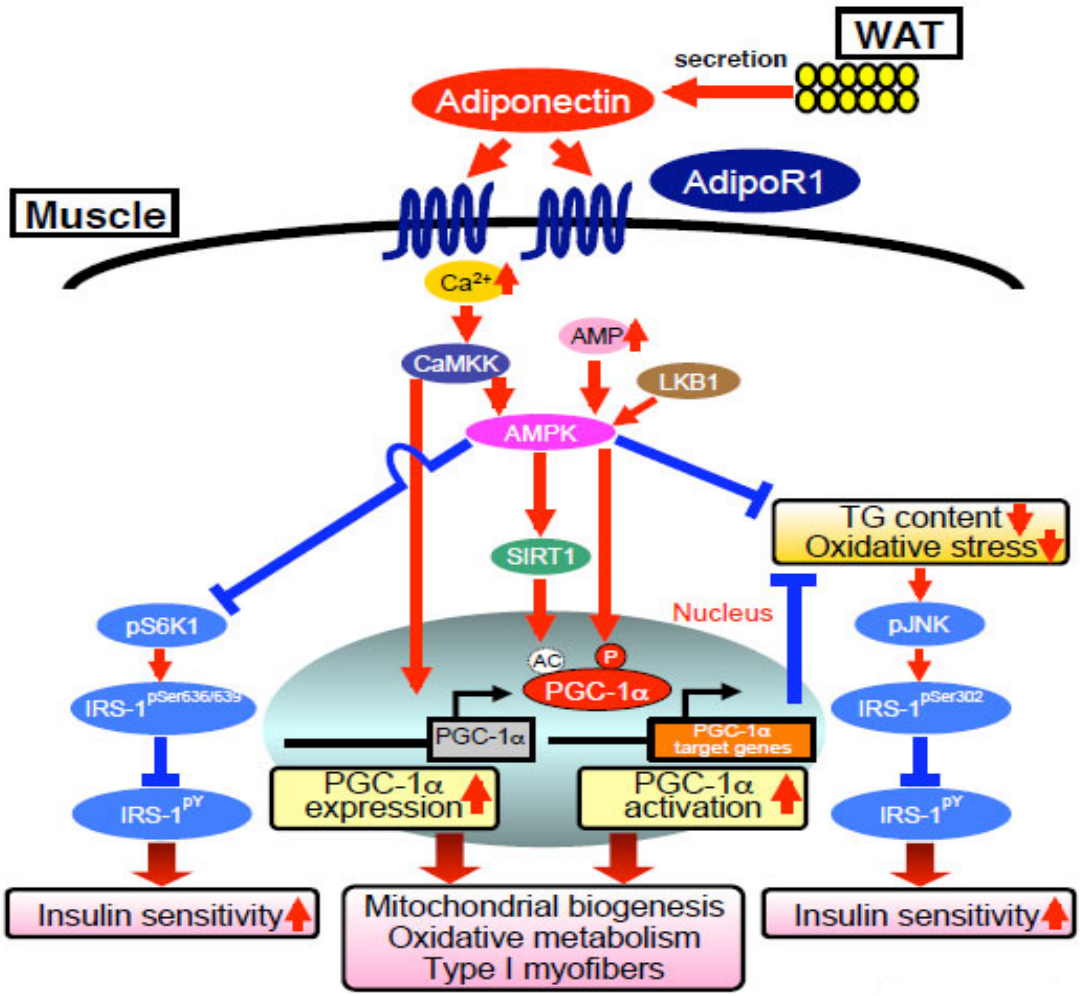
Several scientific studies now indicated that moderate consumption of coffee (between five and six cups a day) not only poses no risks for a normally healthy person but also has important benefits. A prospective study performed in the Netherland, England and Japan indicated that coffee has protective effects against type II diabetes. This appears to be due to metabolic effects of chemicals in coffee **(17)**. Many other studies have identified several positive effects of coffee, such as antioxidant and antitoxic properties of chlorogenic acids and polyphenols in coffee **(18)**, protective effects against degenerative brain diseases like Alzheimer's and Parkinson's **(19)**, protective effects against colon and skin cancers **(20)** and anti-inflammatory properties **(21)**. Recent studies in Finland, USA and India added other positive effects of coffee consumption to health: it reduces the risk of hepatic cirrhosis and prevents the formation of gallstones, increases the level of alertness, improves short-term memory and permits better use of the prefrontal cerebral cortex due to rapid and short period action of caffeine in coffee. They also reported that coffee helps to alleviate asthma symptoms, because the metabolic product of caffeine called theophylline induces relaxing of the trachea and bronchial tubes by counteracting with adenosine (**22-24)**.

1.2.1. Coffee consumption and type 2 diabetes

Type II diabetes has become a global burden; worldwide, the number of people with type II diabetes was approximately 171 million in 2000, and this figure is predicated to rise above 366 million by 2030 **(25)**. In Ethiopia, A large-scale survey of the prevalence of diabetes has not been performed. However, in sub-Saharan Africa, it is estimated that nearly 10.8 million individuals suffer from type 2 diabetes in 2006, which is 5% of the total affected individuals in the World wide **(26)**. Of the lifestyle factors associated with the risk of type II diabetes, obesity and physical inactivity are the two most important contributory factors in the development of the disease **(27)**. Several recently published coherent studies have suggested that higher coffee consumption was associated with higher insulin sensitivity and a lower risk for type II diabetes in diverse population, and that adiponectin may be involved in the mechanism **(28-29)**. Adiponectin were found to be higher in individuals who were consuming coffee daily bases than the non-consumer or those consuming lesser amount.

Adiponectin is 224 amino acids long polypeptide, which is exclusively secreted by adipose tissue into the blood stream and is very abundant in the plasma relative to many hormones. It modulates a number of metabolic processes including glucose regulation and fatty acid catabolism in the skeletal muscles and the liver. In skeletal muscle, low doses of adiponectin increased expression of molecules involved in fatty-acid transporter (CD36) **(30)**, combustion (acyl-CoA oxidase) **(31)** and energy dissipation such as uncoupling protein (UCP2) **(32)**. In turn, these processes lead to decreased tissue triglyceride content in skeletal muscle. In the liver, in contrast to skeletal muscle, low doses of adiponectin decreased the expression of molecules involved in fatty-acid transport into tissues such as CD36, thereby reducing fatty-acid influx into the liver, which might lead to decreased hepatic triglyceride content. Thus, even though triglyceride content was decreased in both muscle and liver, the mechanisms involved are apparently quite different in the two tissues. Adiponectin acts primarily on skeletal muscle to increase influx and combustion of FFA, thereby reducing muscle and plasma triglyceride content **(33)**. As a consequence of decreased serum FFA and triglyceride levels, hepatic triglyceride content is decreased. Increased tissue triglyceride content has been reported to interfere with insulin-stimulated activation of phosphatidyl inositol-3-kinase and subsequent translocation of glucose-transporter 4 and uptake of glucose, which leads to insulin resistance (Figure 1.1) **(29)**. Thus, decreased triglyceride content in muscle and serum might contribute to the improved insulin signal transduction, as demonstrated by increases in insulin-induced tyrosine phosphorylation of insulin receptor and insulin-receptor substrate-1. Similar results have been found with decaffeinated coffee **(34)**. Some studies in USA hypothesized that caffeinated coffee reduces insulin sensitivity. This may be due to the high level catecholamine and as result of blocking adenosine-mediated stimulation of peripheral glucose uptake **(29).** In addition, short term metabolic studies showed that caffeinated coffee intake can acutely lower insulin sensitivity and increase glucose concentrations. However, other studies in the USA and studies in Finland, Scotland and England indicated that habitual consumption of caffeinated or decaffeinated coffee was associated with higher insulin sensitivity and a lower risk for type II diabetes **(35)**.

Figure 1.3 Effects of adiponectin on insulin sensitivity



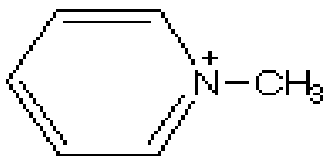
AdipoR1=Adiponectin receptor, AMP=Adenosine monophosphate, CaMKK= Calcium-calmodulin depedent kinase kinase, PGC-1•=Peroxisome proliferator-activated receptor gamma coactivator-1•, IRS-1= insulin receptor substrate-1 and SIRT1=Sirtuin

1.2.2. Anticancer Activity of Coffee

Recent studies have shown that coffee contains various food components, which have anticancer activities **(13)**. Cancer cells are believed to arise out of normal cells through three phases called initiation, promotion and progression **(36)**. The best policy to eliminate cancer is to prevent its occurrence **(37)**. Most evidence on coffee suggests that coffee drinking has no any cancer risks, such as breast **(38)**, stomach **(39)**, pancreatic **(40)**, renal cancer and prostate cancer **(41)**. Coffee drinking has also been reported to decrease colorectal cancer and liver cancer by protecting DNA damage caused by free radicals (36)

In contrast, high consumption of coffee is mentioned to possibly increase the risk of bladder cancer at high levels of intake, but is probably not associated with risk at consumption below five cups/day **(**42). Coffee drinking has been reported to prevent oral, pharyngeal and esophageal cancer by limiting free-radical formation and protecting the body from harmful effects of free-radical **(43)**. Very recently, a new anti-cancer compound called methylpyridinium (Figure 1.4) has been found in coffee, and it is formed during the roasting process from its chemical precursor, trigonelline, which is common in raw coffee bean **(44-45)**. Methylpyridinium is found to increase activities of detoxifying enzymes in rats, suggesting that this compound is one of anticancer agents present in coffee **(46)**. Polyphenols are other chemicals in the coffee that possess the highest ability to neutralize free radical forms of oxygen by donating an electron to them. They are also the inducer of apoptosis in the cancer cells of different origin.

Figure 1.4 Molecular structure of methylpyridinium



1-Methylpyridinium

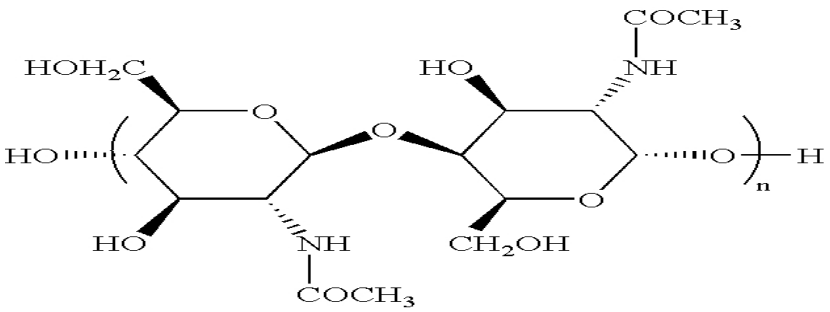
1.2.3. Antioxidant Activity of Coffee

Coffee contains several phenolic components, other than tocopherols, endowed with antioxidant capacity, and the total polyphenols amount ranges from 200 to 550 mg per cup **(47)**. Polyphenols have been reported to exert a variety of biological actions, such as free radical scavenging, metal chelation, modulation of enzymatic activity and, more recently, signal transduction, activation of transcription factors and gene expression **(48-51)**. Some epidemiological studies have suggested associations between the consumption of polyphenol rich food and the prevention of range of human diseases **(52)**.

The phenolic compounds identified from coffee include - chlorogenic acids, a family of esters formed between quinic acid and several cinnamic acids such as caffeic, ferulic and p-coumaric acid, caffeoylquinic acid being by far the most abundant **(53)**. Caffeic acid is endowed with strong antioxidant activity in vitro and in vivo **(54)**. As caffeic acid is present in human plasma at µmolar concentration after coffee drinking **(55)**, it can be assumed that it is, at least in part, responsible for

the increase in plasma antioxidant capacity. Melanoidins (Figure 1.5) are also other molecules in coffee, which exhibit strong antioxidant activity, and inhibited significantly lipid oxidation. These are formed during roasting of coffee beans **(53)**.

Figure 1.5 molecular structure of melanoidin



Melanoidin

1.2.4. Coffee's effect against Alzheimer's and Parkinson's diseases

Alzheimer's and Parkinson's disease are two of the most common causes of dementia, in the world **(56)**. Both Alzheimer's and Parkinson's disease are neurodegenerative diseases and a lower incidence for the two has been reported amongst coffee drinkers **(57)**. In fact, rodent experiments have demonstrated that caffeine may improve memory performance **(58)**. Many Neurologists advice patients to reduce the amount of coffee they drink, however the overwhelming weight of evidence shows that, in moderation, coffee is perfectly safe and can be enjoyed as part of a healthy lifestyle **(59)**. Further studies are needed to determine whether caffeine consumption could have an affect on the development of Alzheimer's disease or age-related cognitive decline, but for the moment it may said that the moderate coffee consumption of 4 -5 cups of coffee per day is safe for patients and may even confer certain health benefits including protecting against the risk of developing Alzheimer's and Parkinson's **(60)**.

1.2.5.Coffee and Heart Disease

Many studies have been conducted concerning the relationship between drinking coffee and heart disease. Many of the test results were conflicting **(61-63)**. Several earlier reports stated that there was direct connection between drinking coffee and heart disease **(64)**. On the other hand, some reports have refuted these claims and stated that there was no evidence of the correlation. One of the problems with coffee is that it contains caffeine, which is a CNS stimulant. Some recent prospective cohort studies have showed that habitual consumption of boiled coffee decreases low density-lipoprotein-cholesterol concentration, so it may reduce the risks of heart disease **(65)**. Further

experimental studies are warranted to elucidate the underlying mechanisms and possibly identify the components in coffee that are responsible for such positive effects.

1.3. *Significance of the study*

Coffee is among the most widely consumed beverage in the world including Ethiopia. Thus knowledge of both the positive and negative health effects of coffee is important to empower individuals to make informed choice regarding coffee consumption.

This study is planned to provide the data in Ethiopia on the effects of coffee consumption on the development or severity of the type 2 diabetes. The information obtained may also provide valuable information for the management of type 2 diabetes patients.

1.4. *Hypothesis*

Coffee consumption may prevent postprandial hyperglycaemia, hyperinsulinemia, and may change plasma lipid profile and thereby provide protection against the development of type 2 diabetes.

1.5. *Objective of the Study*

1.5.1. General objective

The aim of this study is to investigate the relationship between coffee consumption and insulin sensitivity status among non-diabetic individuals in Addis Ababa.

1.5.2. Specific objective

1. To assess fasting and postprandial plasma glucose level among coffee drinkers and non-drinkers
2. To assess fasting an postprandial plasma insulin level among coffee drinkers and non-drinkers
3. To assess plasma lipid profile (Triglycerides, Total Cholesterol, HDL-Cholesterol, LDL-Cholesterol and Free fatty acids) among coffee drinkers and non-drinkers
4. To compare the fasting and postprandial plasma glucose level of participants who do not consume coffee on a daily basis (Group 1) with those who drink more than 1 cup per day (Group II).
5. To compare the fasting and postprandial plasma insulin level of participants in the two groups to determine the insulin sensitivity of the participants.
6. To compare the plasma lipid profile (Triglycerides, Total Cholesterol, HDL-Cholesterol, LDL-Cholesterol and Free fatty acids), as risk factors for the development of Type II diabetes mellitus in the participants in the two groups
7. To assess the correlation between plasma lipid profile and type 2 diabetes among coffee drinkers and non-drinkers

2. MATERIAL AND METHODS

2.1. *Study Design*

A cross sectional study with comparative nature was conducting on apparently healthy individuals residing in Addis Ababa.

2.2. *Study area and subjects*

The study was conducted in Addis Ababa, in the subcity of Lancha. The study population was collage students and staff members of the Central University Collage, Lancha branch. Participants with 18 years or more were randomly selected from students and staff members, until the sample size was reached. The intention of the study was explained to each potential participant and informed consent was taken (Annex-II). The questionnaire attached (Annex-I) was administered by the researcher.

2.3. *Sample size*

Research conducted with title coffee consumption and glucose tolerance status in middle aged Japanese men showed that coffee consumer had lower serum glucose than non consumer **(25).** We used information from this data to calculate the sample for the present study. Mean and Standard deviations of serum glucose of coffee drinkers and non-drinkers were selected; the sample size was estimated as follows Sample size was calculated by the following formula (Daniel, 1995).

$N_1=N_2= [(1+1/r) (Z_{/2} + Z)^2 (^2)]/ \mu$

Where:

N1-number of coffee drinkers

N_2-number of non-coffee drinkers

$r = N_1/N_2$

$Z_{/2}$ =1.96 at 5% type I error

Z =0.84 at 80% po

N-Sample size $=N_1+N_2 = 70$

$N_1=N_2=35$

2.4. *Inclusion and Exclusion criteria*

Participants with Liver disease, thyroid disease, Cushing's syndrome, chronic alcoholics, those receiving treatment with lipid lowering drugs and estrogens, suffering from gastrointestinal disorders, diabetes, cardiovascular disease, arthritis, hypertensive and pregnant women or habits likely affect insulin Sensitivity status were excluded. The volunteers of less than 18 years of age were not included in the study. Apparently health Volunteers who gave informed consent and who were living in Addis Ababa were included in the study.

2.5. *Sample collection and handling*

Information about the study was explained to both the students and the staff of the collage. They were asked to fill the questioner which was enquired about their general health status and any history of chronic disease. A drop of blood from their finger was assessed for blood glucose by glucometer to select volunteers. They were instructed for an overnight fasting.

A standard mercury sphygmomanometer was used to measure blood pressure after participants were allowed to sit for at least 5min. Then, about 5ml of blood was drawn, by venipuncture from the medial cubital vein, after an overnight fast. The subjects were given to drink 75g of glucose dissolved in water and another blood sample was drawn after 2hr. All the blood samples were taken by a trained laboratory technician using universal precautions. Blood samples were left to clot and serum was separated within 2 hours of collection by centrifugation for 10 minutes at 3000 rpm. The aliquotted serum samples (250µLof each) were stored frozen at – 70 ^{o}C until analysis.

2.6. *Ethical Issues*

The study was approved by the research and publication committee of Faculty of Medicine, Addis Ababa University (AAU).

2.7. *Methods of Epidemiological and Biochemical Assays*

2.7.1. Anthropometric measurements

Waist and hip circumference was measured using a measuring tape and the ratio was calculated.

Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters and categorized as under weight (<18kg/m^2), normal (18-25kg/m^2), over weight (25.0 – 29.9kg/m^2), or obese (•30.0 kg/m^2).

2.7.2. Blood pressure

A standard mercury sphygmomanometer was used to measure blood pressure after participants were allowed to sit for at least 5min.

Hypertension is classified according to the seventh report of the US joint national committee on prevention, detection, evaluation and treatment of high blood pressure (66). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) are used to categorize participants. Blood pressure as normal (SBP<120mmHg and DBP<80mmHg), pre hypertensive (SBP 130-139mmHg or DBP 80-89 mmHg), or hypertensive (SBP • 140mmHg or DBP •90mmHg). In addition, individual taking antihypertensive medication were categorized as hypertensive.

2.7.3. Assessment of coffee and tea consumption and other life style characteristics

A self administered questionnaire was used to ascertain age, sex, physical activity, coffee and tea consumption, alcohol use and other life style characteristics.

Average consumption of coffee and tea on a daily basis was reported by the number of cups of each participants consumed per day. An average serving of one cup of coffee is estimated to be equal to 100ml (25). Participants were asked about the number of cups of coffee and tea they drink per day and categorized as none, 1-2 cups, 3-4 cups and •5 cups.

2.7.4. Methods of Biochemical assays

Serum samples were analyzed for fasting and 2 hours loaded plasma glucose and insulin, fasting plasma triglycerides, free fatty acids, total cholesterol, HDL_C and LDL_C in the Biochemistry Laboratory, Department of Biochemistry, Faculty of Medicine, AAU. All reagents used for the assay were prepared in the Biochemistry Laboratory, except ready-made kits for enzyme Colorimetric and insulin immunoassay. Colorimetric determinations for all parameters were conducted by microplate reader 2001 (Anthos Labtec instruments; Austria).

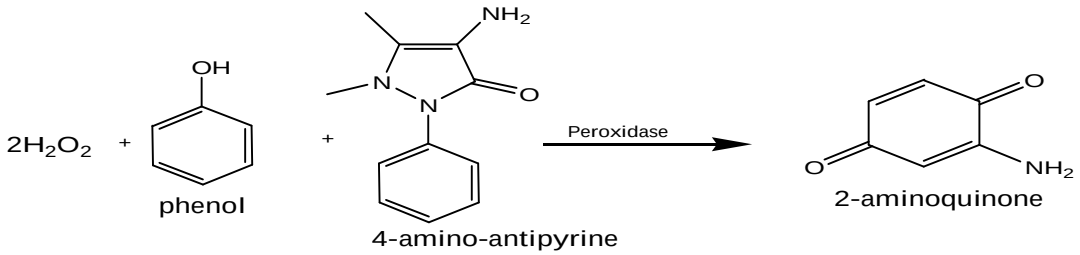
2.7.4.1. Determination of Fasting and 2 hours Post-load Serum Glucose Level

Fasting and 2 hr post-load plasma level were determined by enzymatic glucose oxidase method using commercial reagents (SPINREACT, SPAIN).

Principle:

Glucose oxidase is an enzyme that catalyses the oxidation of glucose to gluconic acid. The hydrogen peroxide (H_2O_2) generated in the reaction is detected by a chromogenic oxygen acceptor, phenol-aminophenazone in the presence of peroxidase :

Figure 2.1 Principle of serum glucose estimation



The intensity of the colour formed is proportional to the glucose concentration in the sample.

Reagents:

Reagent 1 (R1):

TRIS, pH 7.4	92mmol/L
Phenol	0.3 mmol/L
Glucose Oxidase	150000 U/L
Peroxidase	1000 U/L
4-Aminophenazole	2.6 mmol/L

Reagent 2 (R2): Standard Glucose 100 mg/dl

Reagent preparation:

Both R1 and R2 were ready for use

Procedure:

1. Samples and reagents were brought to room temperature and each serum was properly homogenized properly using vortex. Into each of labelled tubes reagents were pipette as the follows

Table 2.1 Glucose reaction mix and procedure steps

Additions	Reagent Blank	standard	Sample
Reagent 1(mL)	1.0	1.0	1.0
Standard (µL)	-	10	-
Sample (µL)	-	-	10

2. Reagents were mixed and incubated for 15 min. at room temperature

3. Absorption (A) of the samples and Standard, against the Blank was read at 546nm

Calculation:

Concentration of glucose in mg/ml in the samples were calculated by the following formula

(A) Sample

(A) standard x 100 mg/dl = mg/dl of glucose in the Sample

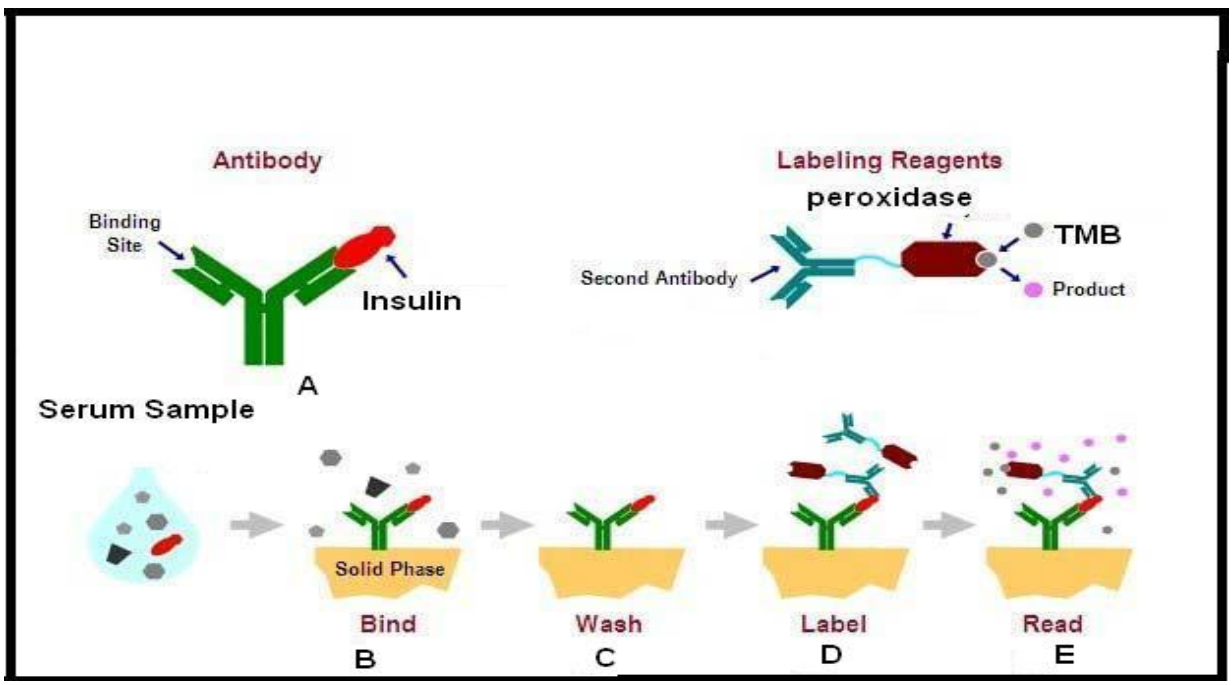
2.7.4.2. Fasting and 2 hours Post load Plasma Insulin level

Plasma level of insulin was determined by sandwich Enzyme Linked-Immuno-Sorbant Assay (ELISA) technique using commercial kit (Catalog No.Sp-401, United Biotech incorporation,UBI Magiwel, USA) and procedure presented by them.

Principle:

The Insulin ELISA was based on the sandwich immunoassay principle. The Insulin in the serum is sandwiched between the anti-insulin antibody coated on the wells and enzyme labelled anti-insulin antibodies. When the samples, control and standards of known insulin content were incubated in the well with an enzyme conjugate (antibodies linked to horseradish peroxidase) a sandwich complex is formed bound to the well. Unbound conjugate are then washed off with wash buffer. The amount of bounded peroxidase is proportional to the concentration of the insulin present in the sample. Upon addition of TMP substrate, the intensity of colour will develop in proportional to the concentration of insulin in the samples (figure 2.1).

Figure 2.2 Principle of insulin immunoassay



Reagents:

1. Microwell strips: Monoclonal Anti-insulin Antibody coated 96 wells.
2. Enzyme conjugates: 11ml anti-insulin antibodies conjugated to horseradish peroxidase.

3. Standards: Six 0.70ml human insulin standards at concentration of 0, 5, 25,50,100 and 200uIU/ml

4. TMB solution: 11ml buffer solution containing hydrogen peroxide and TMB.

5. Concentrated wash buffer: 10mLwash buffer

6. Stop solution: 11ml of 2N HCl

Reagent preparation:

Working wash solution: 10ml of wash buffer was diluted with 990ml of distilled water

Procedure:

All serum samples and insulin standards were brought to room temperature

1. 25ul of serum samples and standards were added into the assigned wells

2. 100ul of enzyme conjugate was added into each well and mixed for 5 seconds.

3. Incubated for 30 minutes at 25ºC.

4. Incubation mixture was removed and the wells were rinsed five times with washing buffer

5. 100ul of TMB solution was added into each well.

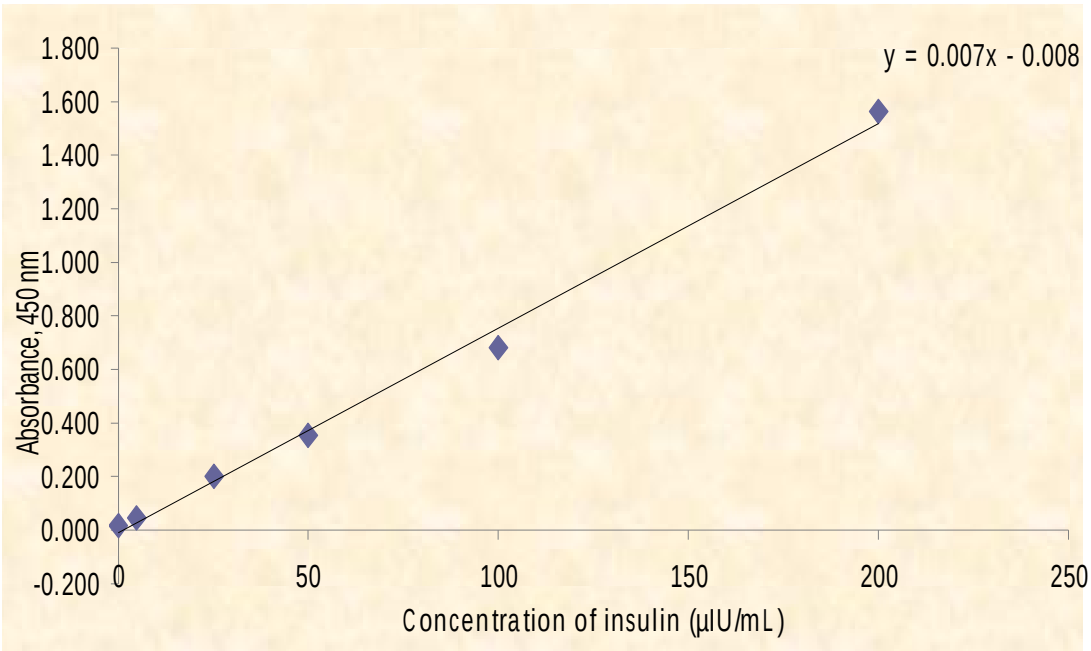
6. Incubated for 15 minutes at 25ºC.

7. Reaction was stopped by adding stop solution to each well and Optical density was read at 450nm by microplate reader 2001 (Anthos Labtec instruments; Austria).

Calculation:

The insulin values of the samples were obtained with reference to the standard curve.

Figure 2.3 Standard curve of Insulin



The question Y= 0.007x- 0.008 is obtained from the curve shown above.

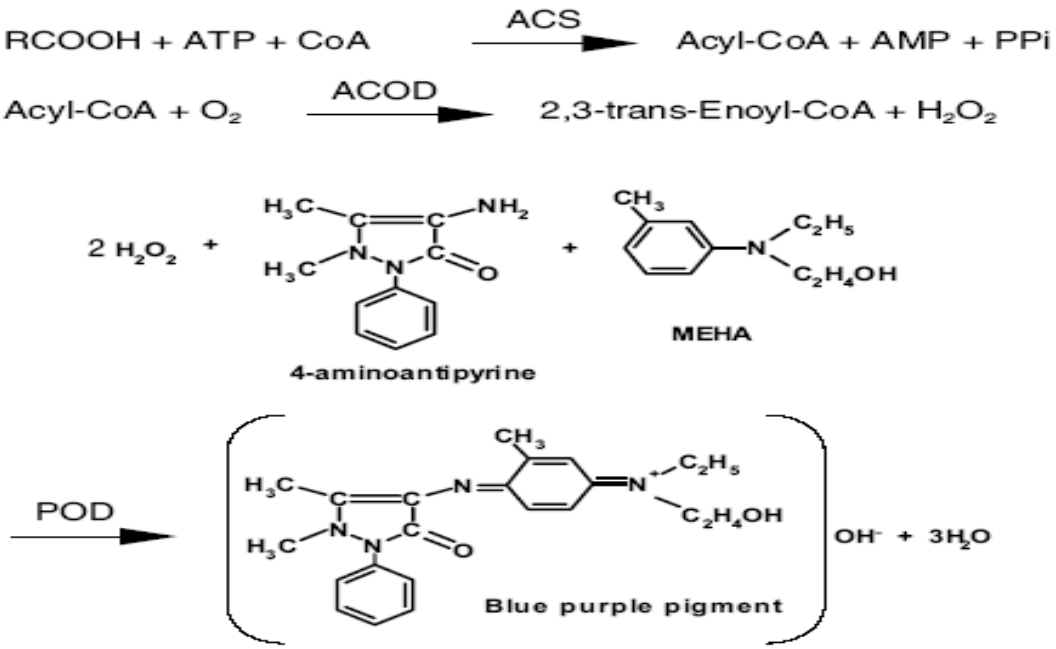
2.7.4.3. Determination of Serum Free Fatty Acid Level

Serum free fatty acid was determined by enzyme coupled assay using commercial kit obtained from Spinreact, Spain.

Principle:

In the presence of the enzyme acyl-CoA synthetase (Acyl CS) and adenosine-5'-triphosphate (ATP), free fatty acids are converted into acyl-coenzyme A (acyl-CoA). Acyl-CoA reacts with oxygen (O_2) in the presence of acyl-CoA oxidase (ACOA) to form 2,3-enoyl-coenzyme A (enoyl-CoA). The resulting hydrogen peroxide (H_2O_2), 3-Methyl-N-Ethyl--N-(B-Hydroxyethyl)-Aniline (MEHA) and 4-aminoantipyrine (4-AA) are converted to a blue purple dye in the presence of the peroxidase (POD). The dye is measured in the visible spectrophotometer at 546 nm.

Figure 2.3 Principle of fatty acid estimation



Reagents:

Commercial kits contained the following-

Reagent1 (R1):

A. Colour A (R1a): is a mixture of ACS (o.53U/L), COA(0.31mmol/L), ATP (0.43mmol/L), 4-AA (1.5mmol/L), Sodium azide (0.055%) and AOD (2.6U/L) R1(50ml).

B. Solvent A (R1b): is a mixture of Phosphate buffer, PH 7.0 (50mmol/L) and Sodium azide (0.055%).

Reagent 2 (R2):

A. Color B (R2a): is a mixture of ACOD (12U/L) and POD (14 U/L)

B. Solvent B (R2b): contains MEHA.

Reagent 3 (R3): Standard free fatty acid 28 mg/dl

Reagent preparation:

1. **R1**: Prepared by mixing one bottle of R1a and R1b.
2. **R2**: Prepared by mixing one bottle of R2a and R2b.

Procedure:

1. Samples and reagents were brought to room temperature and each serum was vortexed. Into each of labelled tubes reagents were pipette as the follows

Table 2.2 Free fatty acid reaction mix and producer steps

Additions	Sample/Standard Blank	Standard	Sample
R1 (mL)	3.0	3.0	3.0
Standard (µL)	7.0	7.0	-
Sample (µL)	7.0	-	7.0
R2 (mL)	-	1.5	1.5

2. Reagents were mixed and incubated for 15 min. at room temperature
3. Absorption (A) of the samples and Standard, against the Sample blank and standard black respectively was read at 546nm

Calculation:

Concentration of free fatty acid in mg/ml of the samples was calculated by the following formula

$$\frac{(A)\ \text{Sample}}{(A)\ \text{standard}} \times 28\ \text{mg/dl} = \text{mg/dl of free fatty acid in the Sample}$$

2.7.4.4. Determination of Serum Triacylglycerol Level

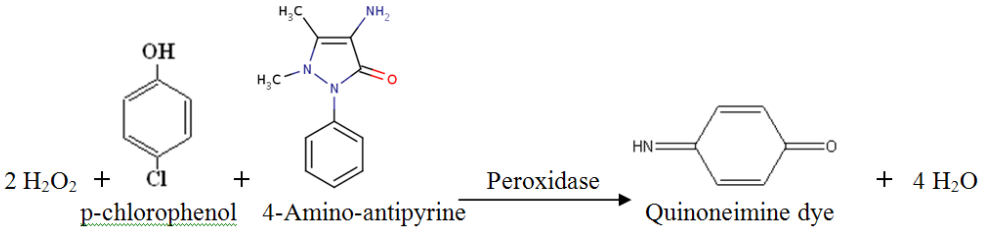
Serum Triacylglycerol Level was determined by procedure presented by commercial kit supplied from Spinreact, Spain.

Principle:

Sample triglycerides incubated with lipoprotein lipase (LPL), liberate glycerol and free fatty acids. Glycerol is converted to glycerol-3-phosphate (G3P) and adenosine-5-diphosphate (ADP) by glycerol kinase and ATP. Glycerol-3-phosphate (G3P) is then converted by glycerol phosphate dehydrogenase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H_2O_2). In the last reaction, hydrogen peroxide (H_2O_2) reacts with 4-aminophenazone (4-AP) and p-chlorophenol

in presence of peroxidase (POD) to give a red colored dye. The intensity of the color formed is proportional to the triglycerides concentration in the sample.

Figure 2.4 Principle of serum triglyceride estimation



Reagents:

Buffer (R1): Contains Good PH 7.5 (50mmol/L) and p-Chlorophenol (2mmol/L)

Enzymes (R2): Contains lipoprotein lipase (150000U/L), glycerol kinase (500U/L), glycerol-3-oxidase (2500U/L),peroxidase (440 U/L),4-aminophenazone (0.1mmol/L) and ATP (0.1mmol/L).

Reagent 3(R2): Standard triglyceride 200mg/dl

Reagent preparation:

Working reagent (WR): Prepared by dissolving R2 Enzyme into one bottle of R1 Buffer

Procedure:

1. Samples and reagents were brought to room temperature and each serum was vortexed. Into each of labelled tubes reagents were pipette as the follows

Table 2.3 Triglyceride reaction mix and procedure steps

Reagent	Reagent Blank	Standard	Sample
WR (mL)	1.0	1.0	1.0
Standard (µL)	-	10	-
Sample (µL)	-	-	10

2. The reagents were mixed and incubated for 15 min. at room temperature
3. Absorption (A) of the samples and Standard, against the Blank was read at 546nm

Calculation:

Concentration of triglyceride in mg/ml of the samples were calculated by the following formula

$$\frac{(A)\ \text{Sample}}{(A)\ \text{standard}} \times 200\ \text{mg/dl} = \text{mg/dl of triglyceride in the Sample}$$

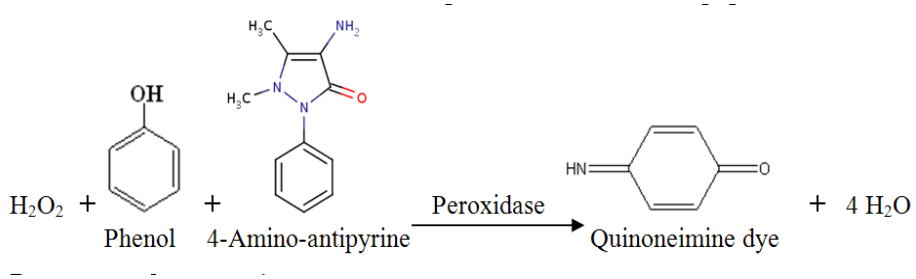
2.7.4.5. Determination of Serum Cholesterol Level

Sample cholesterol was determined by procedure presented by commercial kit supplied from Spinreact, Spain.

Principle:

Enzymatic hydrolysis of cholesterol by cholesterol esterase and oxidation by cholesterol oxidase produce cholestenone . Finally, the indicator quinoneimine is formed from hydrogen peroxide and 4-aminophenazone in the presence of phenol and peroxidase. The chromogen is determined photometrically at 546 nm. The intensity of the color formed is proportional to the cholesterol concentration in the sample.

Figure 2.5 Principle of serum cholesterol estimation



Reagents:

Buffer (R1): Contains PEPIS PH 6.9 (90 mmol/L) and phenol (26 mmol/L)

Enzymes (R2): Contains cholesterol estrase (300U/L), cholesterol oxidase(300U/L), peroxidase (1250 U/L) and 4-amino-antipyrine (0.4mmol/L).

Reagent 3(R2): Standard cholesterol 200mg/dl

Reagent preparation:

Working reagent (WR): Prepared by dissolving R2 Enzyme into one bottle of R1 Buffer

Procedure:

1. Samples and reagents were brought to room temperature and each serum was vortexed. Into each of labelled tubes reagents were pipette as the follows

Table 2.4 Cholesterol reaction mix and procedural steps

Reagent	Reagent Blank	Standard	Sample
WR (mL)	1.0	1.0	1.0
Standard (µL)	-	10	-
Sample (µL)	-	-	10

2. The reagents were mixed and incubated for 15 min. at room temperature

3. Absorption (A) of the samples and Standard, against the blank was read at 546nm

Calculation:

Concentration of Cholesterol in mg/ml of the samples was calculated by the following formula

$$\frac{\text{(A) Sample}}{\text{(A) standard}} \times 200 \text{ mg/dl} = \text{mg/dl of Cholesterol in the Sample}$$

2.7.4.6. Determination of Serum HDL-C Level

Serum HDL-Cholesterol was estimated by enzymatic colorimetric method by using commercial kit supplied from Spinreact, Spain.

Principle:

Chylomicrone, VLDL and LDL-C are precipitated by adding Phosphotungstic acid and Magnesium ions to the sample. Centrifugation leaves in the clear supernatant containing high density lipoprotein, which is used for determination of HDL-C.

Reagents:

Reagent 1(R1):

Phosphotungstic acid 14mmol/L

Magnesium chloride 2mmol/L

Reagent 2 (R2): Standard cholesterol 50mg/L

Reagent preparation:

Precipitants: One reagents strip was immersed in one bottle of buffer solution and used to stir the bottle content for 10 second. Then allowed to stand in buffer solution for 5 minutes, stirred once again for 10 seconds and then the reagent strip was discarded.

Procedure:

A. Precipitation

Samples and reagents were brought to room temperature and each serum was vortexed. Then, 0.2ml serums and standard was added to 0.1ml of precipitant in labelled Eppendorf's microtubes and vortexed for 10 seconds. The reaction mixture was incubated at room temperature for 10 minutes and then centrifuge for 10 minutes at 6000rpm. After centrifugation, the clear supernatant was separated and the cholesterol content was determined by the CHOD-PAP method.

B. Cholesterol Assay

1. Reagents were added into each of labelled tubes as the follows

Table 2.5 HDL-C reaction mix and procedure steps

Reagent	Reagent Blank	Standard	Sample
Precipitant (mL)	1.0	1.0	1.0
Standard (µL)	-	50	-
Supernatant (µL)	-	-	50

2. The reagent solutions were mixed and incubated at room temperature for 5 minutes.

3. Absorption (A) was read against the blank at 546nm.

Calculation:

HDL-C concentration of the samples was determined by the following formula

$$\frac{(A)\text{Supernatant}}{(A)\text{ standard}} \times 50 \text{ mg/dl} = \text{mg/dl of HDL-C in the Sample}$$

2.7.4.7. Determination of Serum LDL-C Level

Serum LDL-Cholesterol was estimated by enzymatic colorimetric method by using commercial kit supplied from Spinreact, Spain.

Principle:

LDL-C is precipitated by adding polyvinyl sulphate and Polyethylene glycol to the sample. Centrifugation leaves the clear supernatant containing VLDL and HDL-C. LDL-C is calculated by subtracting the supernatant cholesterol fractions from the total cholesterol of the sample.

Reagents:

Reagent 1(R1):

Polyvinylsulfate 1g/L

Polyethylene glycol 170g/L

Reagent 2 (R2): Standard LDL-C 50mg/L

Reagent preparation:

Precipitants: They were ready to use

Procedure:

A. Precipitation

Samples and reagents were brought to room temperature and each serum was vortexed. Then, 0.2ml serums and standard was added to 0.1ml of precipitant in labelled Eppindorff's microtubes and vortexed for 10 seconds. The reaction mixture was incubated at room temperature for 10 minutes and then centrifuge for 10 minutes at 6000rpm. After centrifugation, the clear supernatant was separated and the cholesterol content was determined by the CHOD-PAP method.

B. Cholesterol Assay

1. Reagents were added into each of labelled tubes as the follows

Table 2.5 LDL-C reaction mix and procedure steps

Reagent	Reagent Blank	Standard	Sample
Precipitant (mL)	1.0	1.0	1.0
Standard (µL)	-	50	-
Supernatant (µL)	-	-	50

2. The reagent solutions were mixed and incubated at room temperature for 5 minutes.

3. Absorption (A) was read against the blank at 546nm.

Calculation:

LDL-C concentration of the samples was determined by the following formula

$$\frac{(A)\text{Supernatant}}{(A)\text{ Standard}} \times 50 \text{ mg/dl} = \text{mg/dl of Cholesterol in the supernatant}$$

Total Serum Cholesterol (mg/dl) - Cholesterol in the supernatant (mg/dl) = Serum LDL-C

2.8. Statistical Analysis:

Prism 3.0 statistical package (Graph Pad Software, Inc, San Diego, CA, USA) and SPSS version 13.0 (Chicago, USA) were used to analyze data from questionnaire and laboratory analyses. The data was expressed as median, standard error of the mean (SEM), interquantile range and p-value whenever necessary. Correlation among the parameters in each group was made by appropriate statistical test. The level of significance was set at p• 0.05.

3. Results and Analysis

A total of 75 subjects were recruited to participate in the study, but five had to be excluded because two did not report empty stomach and three did not tolerate the oral glucose well and had to discontinue due to onset of vomiting. Among those who completed the study, 55.7% were women, and 44.3% were men. The mean (±SD) age of the women was 26 ± 5 years and of the men was 31 ± 10 years.

The participants were classified on the basis of their daily coffee consumption: A) **Low drinkers** (n=20) individuals who were consuming 1-2 cups of coffee per day, B) **Moderate drinkers** (n=12) individuals who were consuming 3-4 cups of coffee per day, C) **High drinkers** (n=3) individuals who were consuming five and more cups of coffee per day and D) **Non-drinkers** (n=35) individuals who didn't consume or consuming less than one cup coffee per day.

3.1. Characteristics of the study population

Age and Gender distribution

The majority of the study participants were within the age group of 20-25 years accounting for 38.6% followed by 26-30 years accounting for 27% and the least were in the age group greater than 35 years accounting for 14% (**Table 3.1**)

Table 3.1 Age and gender distribution in the coffee drinkers and non-drinkers

Age group (year)	Gender		Participant		Total
	F	M	Non-coffee drinker	Coffee drinker	
20 to 25	19(27.1%)	8(11.5%)	13(18.6%)	14(20%)	27(38.6%)
26 to 30	13(18.6%)	6(8.5%)	11(15.7%)	8(11.4%)	19(27.1%)
31 to 35	6(8.5%)	8(11.4%)	7(10.0%)	7(10%)	14(20.0%)
36 and above	1(1.4%)	9(12.9%)	4(5.7%)	6(8.6%)	10(14.3%)
Total	39(55.7%)	31(44.3%)	35(50%)	35(50%)	70(100%)

The highest proportions of coffee drinkers and non-drinkers were also observed within the age group of 20-25 years but there were no significant difference between the two groups, 40% and 37% for the coffee consumers and non-consumers respectively.

Table 3.2 Gender distribution in different groups of coffee drinkers

Participant	Gender		Total
	Female	**Male**	**Total**
Coffee drinker	19(27%)	16(22.9%)	35(50%)
Low-drinker	12(17.1%)	8(11.5%)	20(28.6%)
Moderate-drinker	5(7.1%)	7(10%)	12(17.1%)
High-drinker	2(2.9%)	1(1.4%)	3(4.3%)
Non-coffee drinker	20(28.6%)	15(21.4%)	35(50%)
Total	39(55.7%)	31(44.3%)	70(100%)

Table 3.2 shows that there were non-significant different between the coffee drinkers and non-drinkers in gender distribution, 51.3% of the total non-coffee drinkers and 48.5% of the total coffee drinkers were females and the rest were males for both groups. Among the coffee drinkers, the highest proportion were in the category of low coffee drinkers while the least proportion were in heavy drinkers accounted only 5.1% and 3.2% of the total females and males.

Body mass index (BMI) for the two groups

The body mass index (BMI) of the coffee drinkers and non-drinkers participants in the study was 20.6 ± 2.3 and 20.9 ± 2.4 respectively (**Table 3.3**). It found with a range of 17.0 to 27.0 in coffee drinkers and 17.0 to 25.0 in non-coffee drinkers. Only 2 individuals in coffee drinkers showed BMI >25 (data not shown). There was no significantly different with regard to body mass index between coffee drinkers and non-drinkers (P>0.05).

Table 3.3 ***Body mass index (BMI) for the two groups***

Grades of coffee consumption	BMI		
	Mean ± SD	**Range**	**P value**
Coffee drinkers (n=35)	20.6 ± 2.3	17.0-27.0	ns
Low-drinker (n = 20)	20.5 ± 1.9	17.0-24.0	
Moderate-drinker (n = 12)	20.0 ± 2.4	17.0-24.0	
High-drinker (n = 3)	22.6 ± 3.5	20.0-25.0	
Non-coffee drinkers (n=35)	20.9 ± 2.4	17.0-25.0	

There was no statistically significance difference between mean of BMI (P>0.05) of different coffee drinker groups. Other characteristics of the study population documented from the self-assessed questionnaire included tea consumption, family history of diabetes, age; cigarette smoking and alcohol intake were common confounding factors for the study. All participants in coffee drinkers

and non-drinkers were non smoker, didn't drink alcohol, had no family history of diabetes and in average drank 2.5 cups of tea per day.

3.2. Biochemical analysis for coffee drinkers and non-coffee drinkers

3.2.1 Blood glucose and Insulin concentration of coffee drinkers and non-coffee drinkers

Blood Glucose

The median level of fasting serum glucose concentration was similar and within the normal range for coffee drinkers and non-drinkers. Similarly there was no difference in the median level of post-loaded serum glucose concentration (118.8 vs. 120.8) (**Table 3.4**).

Table 3.4 Fasting and Post-loading blood glucose level in the two groups

Variable	Coffee drinkers (n=35)	Non-coffee drinkers (n=35)	P value
Fasting glucose level, mg/dl			
Median (1^{st}-3^{rd} quartile)	101.2 (87.2-112.9)	101.9 (86.9-116.9)	ns
Range	63.3 - 123.9	71.0 - 134.9	
Post-load glucose level, mg/dl			
Median (1^{st}-3^{rd} quartile)	118.8 (108.9-124.0)	120.8 (109.0-127.3)	ns
Range	98.0 - 131.7	71.0 - 134.9	

Serum Insulin Levels

The median value of fasting insulin level was 14.8 and 12.2 for coffee drinkers and non-drinkers respectively which was not significant. Similarly, there was no significant difference in the post-loaded of insulin level between coffee drinker and non-coffee drinker. However, the insulin concentration level was doubled after post-loaded sugar (14.8 vs. 31.0 and 12.7 vs. 26.7) both in coffee drinkers and non-drinkers (**Table 3.5**).

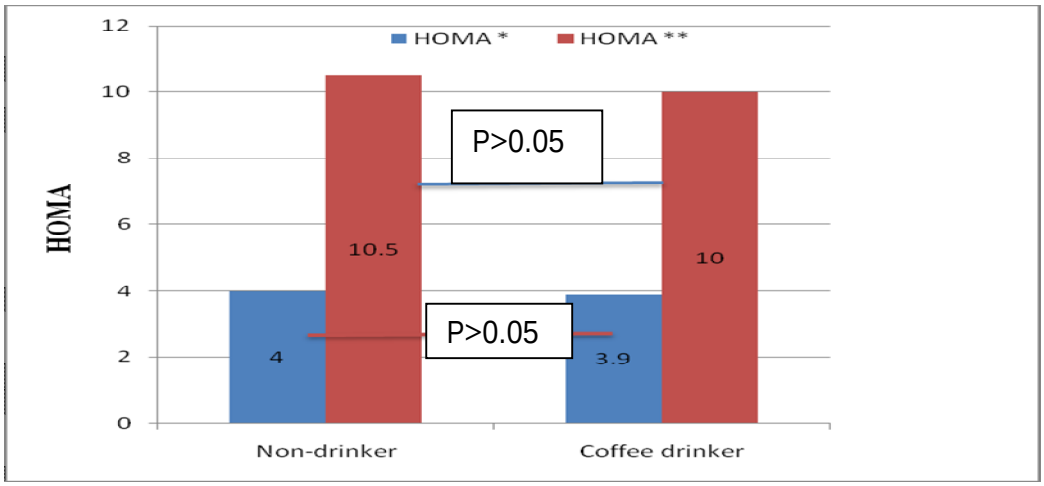
Table 3.5 Fasting insulin and Post-load insulin level in the two groups

Variable	Coffee drinkers (n=35)	Non-coffee drinkers (n=35)	P value
Fasting insulin level, µU/l			
Median(1^{st}-3^{rd} quartile)	14.8 (10.5-19.1)	12.7 (10.5-16.2)	ns
Range	6.3 - 39.9	8.9 - 54.4	
Post-load insulin level, µU/l			
Median (1^{st}-3^{rd} quartile)	31.0 (21.5-43.0)	26.7 (19.0-45.0)	ns
Range	12.6 - 104.4	12.50 - 102.5	

Homeostasis model assessment for insulin resistance (HOMA-IR)

Homeostasis model assessment analysis for fasting insulin and glucose (P>0.05) showed that there was no statistically significant difference between non-coffee drinkers and drinkers. The same result was observed for HOMA analysis for post-loading glucose and insulin (P>0.05). These results shown in Figure 3.1

Figure 3.1 Homeostasis model assessments value for coffee drinker and non-drinker



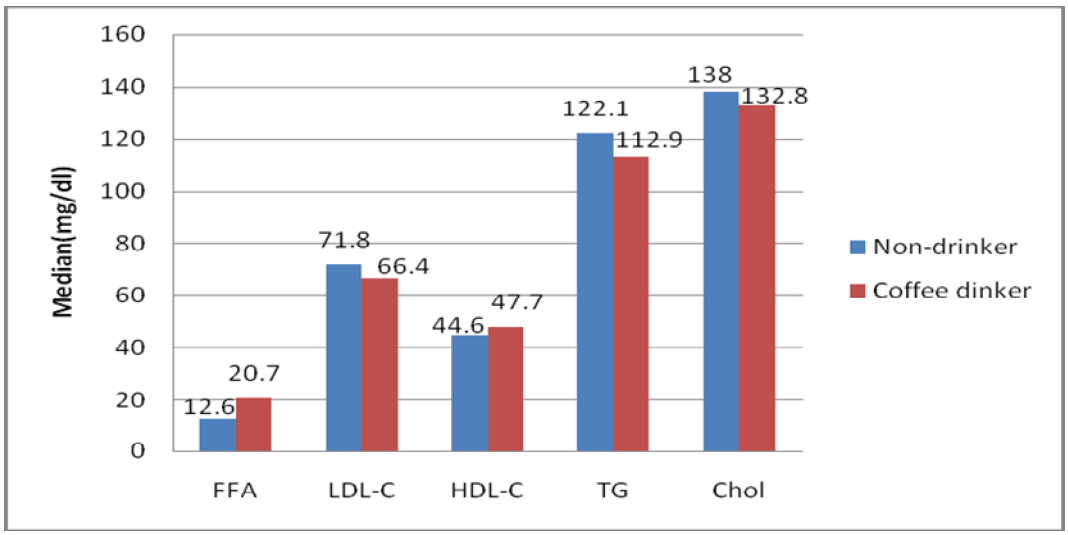
HOMA*= Homeostasis model assessment for fasting insulin and glucose

HOMA**= Homeostasis model assessment for post-loading insulin and glucose

3.2.2 Plasma lipid profile of coffee drinkers and non-coffee drinkers

Blood concentration of free fatty acid (FFA), triglyceride (TG), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein (HDL-C) and cholesterol (CHOL) of coffee drinkers and non-drinkers had a range of 4.0-33.0 vs. 4.8-43.1, 60.0-150.0 vs. 54.2-161.8, 131.6-120.9 vs. 133.9-125.8, 34.0-73.1 vs. 31.5-78.1 and 101.0-192.0 vs. 115.3-187.6 respectively (**Figure 3.2**). The graph was shown in median (mg/dl).

Figure 3.2 Plasma lipid profiles of coffee drinkers and non-drinkers

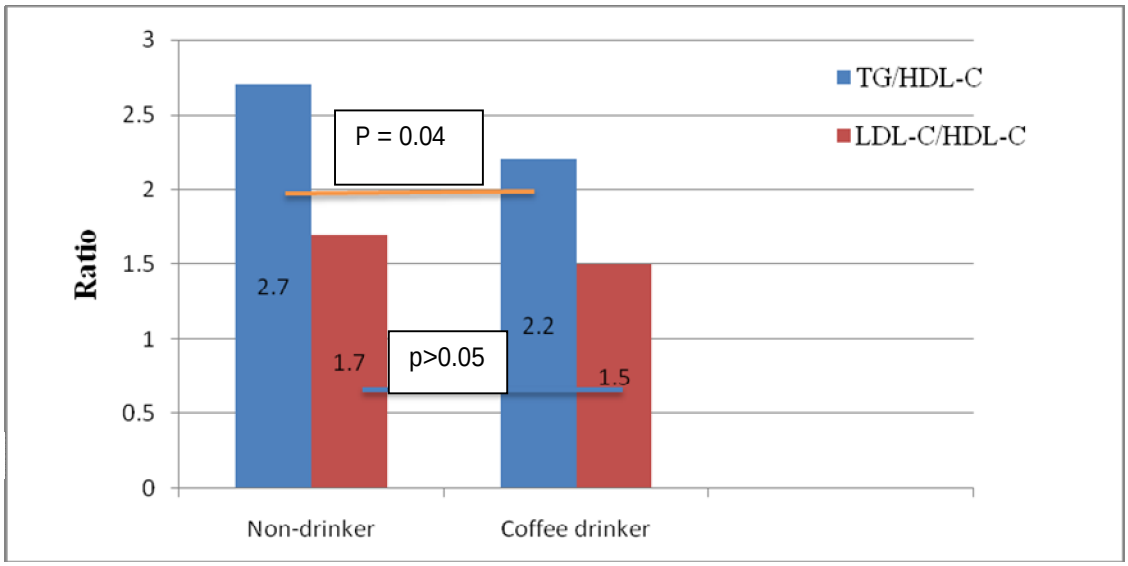


As compared to non-coffee drinkers, coffee drinkers showed significantly higher serum free fatty acids level (20.7 vs. 12.6, P=0.004) but significantly lower serum triglyceride level (112.9 vs. 122.1, P=0.04). The median serum level of LDL-C (64.6 vs.71.8, p>0.05) and cholesterol (132.8 vs.138.0 p>0.05) were lower for coffee drinkers compared with non-coffee drinkers and the difference was not statistically significant. Serum level of HDL-C (47.7 vs.44.6, p>0.05) was non-significantly higher for coffee drinkers.

3.2.3 Lipid ratios for coffee drinkers and non-drinkers

The fasting serum lipid profile (TG, LDL-C and HDL-C) is used to calculate lipid ratios (LDL-C/HDL-C and TG/HDL-C) that allow better predication for analysis of insulin sensitivity compare with single lipid markers. There was no statistically significance difference between mean ratio of LDL-C to HDL-C (p>0.05) for coffee drinkers and non-coffee drinkers. However, mean ratio of TG to HDL-C (p= 0.04) was lowered for coffee drinkers than non-coffee drinkers (**Figure 3.3**).

Figure 3.3 Lipid ratios of coffee drinkers and non-drinkers

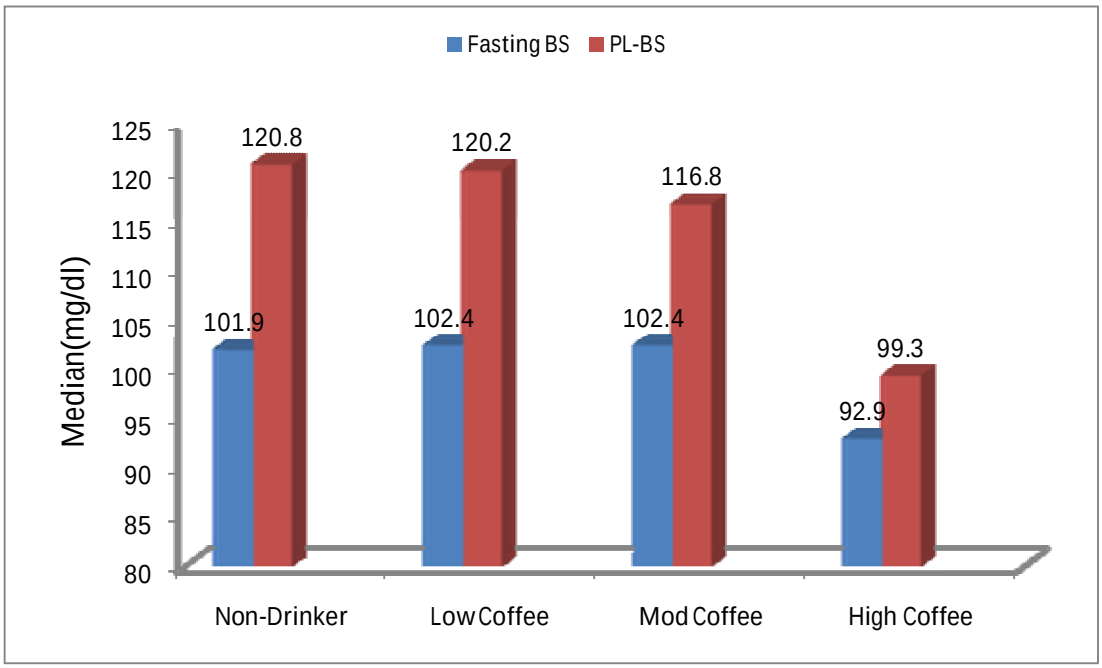


3. 3 Analysis with respect to grades of coffee consumption

3. 3.1 Concentration of serum fasting and post-loading glucose in coffee consuming groups

Blood concentration of fasting and post loaded glucose of low-coffee drinkers, moderate-drinkers, high-drinkers and non-drinkers had a range 71.3-123.8 and 99.0-131.7, 63.4- 114.5 and 98.0-127.2, 73.3-118.9 and 99.0-120.6, 71.0-134.8 and 99.0-138.6 respectively (**Figure 3.4**).

Figure 3.4 Fasting and post-loading glucose level in coffee consuming groups



Fasting BS= Fasting blood sugar PL-BS= Post-loading blood sugar

There was no statistically significance difference between median of both fasting (P>0.05) and post load serum glucose level (P>0.0.05) of different coffee groups. Median of serum level of post load glucose was lowered according to grades of coffee consumption than fasting glucose. Heavy coffee drinkers appeared to have low fasting and post-loading glucose than other groups.

3.2.3 Concentration of serum fasting and post-load insulin for coffee consuming groups

There was no statistically significance difference between median of fasting ((P>0.05) and post-loading serum insulin level (P>0.05) of different coffee groups. Moderate-coffee drinkers showed non-significance higher serum fasting insulin level, where as heavy coffee drinkers appeared to have low post-loading insulin than other groups (**Table 3.6**).

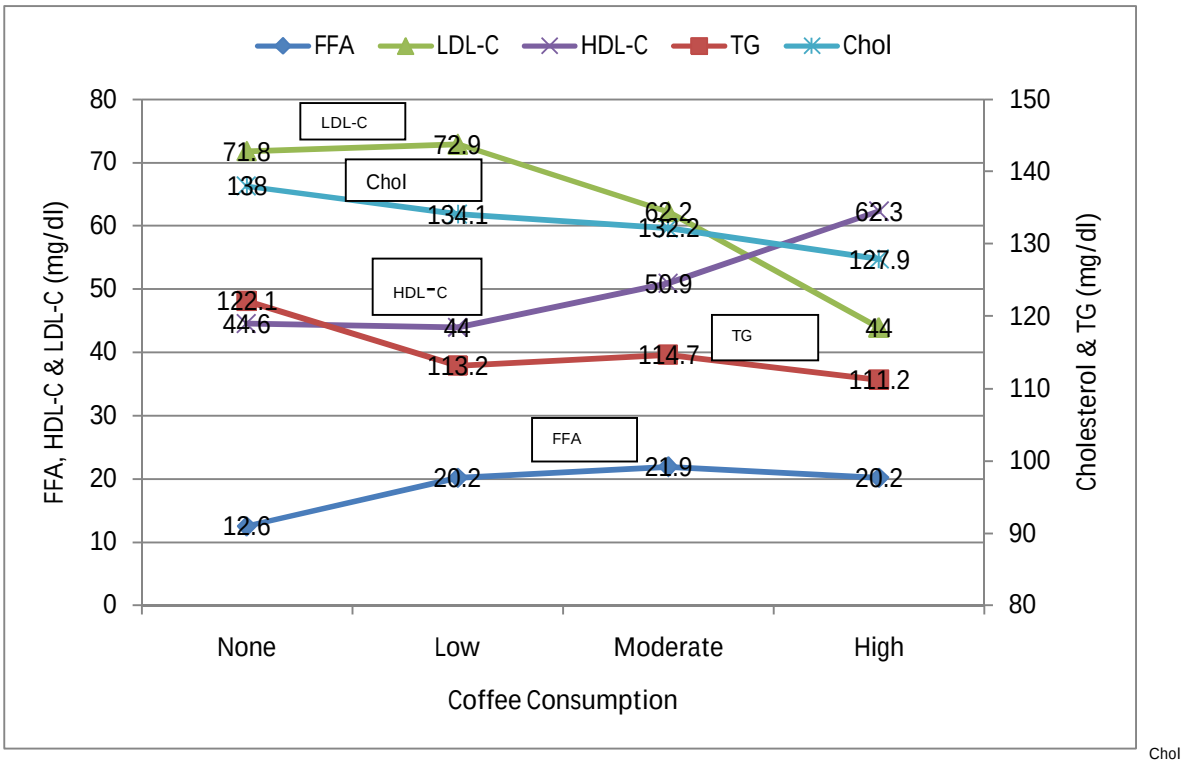
Table 3.6 Concentration of serum fasting and post-load insulin for coffee consuming groups

Variable	Low-coffee drinker (n=20)	Moderate-drinker (n=12)	High-drinkers (n=3)	Non-drinker (n=35)
Fasting insulin, µU/l				
Median(1^{st}-3^{rd} quartile)	15.2(10.4-19.0)	13.9(11.0-20.5)	15.0 (9.8-?)	12.7(10.5-16.2)
Range	8.9-26.6	6.3-40.1	9.8-17.5	8.9-54.4
P value	ns	-	-	-
Post load insulin, µU/l				
Median(1^{st}-3^{rd} quartile)	28.6 (22.6-36.4)	41.5 (27.1-55.9)	19.8 (12.6-?)	26.7(19.0-45.0)
Range	14.8-65.3	20.4-104.4	12.6-50.2	12.5-102.5
P value	ns	-	-	-

3.2.3 Lipid profile for different coffee consuming groups

Serum concentration of free fatty acids, triglyceride, LDL-C, cholesterol and HDL-C was varied according to grades of coffee consumption. Median of serum level of LDL-C (P>0.05), cholesterol (P>0.05) and triglyceride (P>0.05) were non-significantly lower in heavy coffee drinkers. However, median of free fatty acid (P=0.031) was significantly but HDL-C (P>0.05) was non-significantly higher in moderate-coffee drinkers and high- drinkers respectively (**Figure 3.5**).

Figure 3.5 Plasma lipid profile of coffee consuming groups



Chol

The effect of coffee consumption on cholesterol, LDL-C and HDL-C was quite clear.

3.3 Analysis for young groups (age= < 30 years) and older groups (age>30 years)

Serum level of glucose, insulin and different groups of lipid may vary depends on age and gender. So, comparing serum level of those parameters according to their age groups may important to clarify effects of coffee consumption on insulin sensitivity.

3.3.2 Concentration of serum fasting and post-load glucose in the two age groups

Medians of fasting and post-loading serum glucose was varied between non-coffee drinkers of young (age= < 30 years) and older (age> 30 years) groups. The same result was also observed between coffee drinkers of the two groups. Relatively serum sugars were higher in the non- coffee drinkers and drinkers of older groups (age> 30 years) (**Table 3.7**).

Table 3.7 Fasting and post-loading blood glucose level in the two age groups

Coffee groups	age= < 30 years			age> 30 years		
	Range	**Median (IQR)**	**n**	**Range**	**Median (IQR)**	**n**
	Fasting Blood Sugar					
Non-Drinker	71.0-134.9	100.4 (85.2-124.8)	26	73.3-124.8	109.1(92.1-112.6)	9
Coffee-drinker	63.4-123.9	101.9 (86.7-124.4)	25	71.3-118.9	100.6(86.2-113.3)	10
p-value	ns	-		ns	-	
	Post-loading blood sugar					
Non-Drinker	99.0-138.6	118.8(106.5-120.4)	26	108.9- 128.7	123.5(110.0- 14.1)	9
Coffee-drinker	98.0-131.7	118.1(108.7-124.4)	25	99.0-126.7	118.3(111.1-120.7)	10
P-value	ns	-		ns	-	

IQR= Interquantile Range

Coffee drinkers had non-significantly lower median of serum post-load glucose level both in young (p>0.0.05) older (p>0.05) groups but non-coffee drinkers had non-significantly lower median of serum fasting glucose level in young groups. Effect of coffee consumption was quit clear in serum post-load glucose than fasting glucose in both groups.

3.3.3 Serum level of fasting and post-loading insulin in the two age groups

Older (age> 30 years) groups showed relatively lower serum level of fasting insulin than young (age= < 30 years) groups between the same coffee groups. Similarly, non-coffee drinkers in so-young groups had lower median of post-loading insulin serum level than the same group inYoung groups. However, median of serum level of post-loading insulin of coffee drinkers in young groups was lower than that of the same group in older groups (**Table 3.8**).

Table 3.8 Fasting and post-loading insulin level in the two age groups

Coffee groups	age= < 30 years			age> 30 years		
	Range	**Median (IQR)**	**n**	**Range**	**Median(IQR)**	**n**
	Fasting Blood insulin					
Non-Drinkers	9.4-54.4	12.8(12.8-16.5)	26	8.9-54.4	12.7 (9.8-18.2)	9
Coffee-drinkers	8.9-39.9	17.5(10.6-20.4)	25	7.9-19.1	12.0 (9.8-14.8)	10
P-value	ns	-		ns	-	
	Post-loading blood insulin					
Non-Drinker	12.5-102.2	25.1(19.0-18.6)	26	15.2-66.7	28.6 (46.9-44.4)	9
Coffee-drinker	12.6-65.3	31.4 (24.3-43.5)	25	14.8-104.4	29.7 (20.3-45.3)	10
P-value	ns	-		ns	-	

As shown above, there was non-significant difference in both serum level of fasting and post-load insulin between two groups in young and older groups.

3.3.4 Plasma level of lipids in the two age groups

Non-coffee drinkers in older (age> 30 years) groups had relatively higher median of serum level of free fatty acid, triglyceride, LDL-C and cholesterol but lower HDL-C than that of the same group in young (age= < 30 years) groups. Medians of serum level of free fatty acid, triglyceride and HDL-C were lowered but LDL-C and cholesterol were higher in the coffee drinkers of older (age> 30 years) groups. These laboratory results may support the evidence that age is one of the major factors for plasma lipid profile (**Table 3.9**).

Table 3.9 Plasma level of lipids in the two age groups

Coffee groups	age= < 30 years			age> 30 years		
	Range	**Median** (IQR**)**	**n**	**Range**	**Median** (IQR**)**	**n**
	Free fatty acid					
Non-Drinker	4.8-43.1	12.3(9.0-15.5)	26	6.4-21.4	13.3(10.6-16.2)	9
Coffee-drinker	6.0-33.0	20.7(10.8-26.9)	25	4.0-28.0	20.9 (14.7-23.7)	10
P-value	0.03	-		0.034	-	
	HDL-C					
Non-Drinker	31.5-78.1	44.8 (38.4-39.8)	26	32.2-56.9	42.1(54.8-50.0)	9
Coffee-drinker	34.0-66.0	45.2 (42.0-56.6)	25	37.6-73.1	49.1(41.5-61.6)	10
P-value	ns	-		ns	-	
	Triglyceride					
Non-Drinker	54.2-161.8	121.5 (100.8-150.5)	26	63.7-154.0	131.3 (113.5-131.5)	9
Coffee-drinker	60.0-150.0	112.1(90.5-123.5)	25	89.1-126.0	112.9(97.4-120.3)	10
P-value	0.05	-		0.042	-	
	LDL-C					
Non-Drinker	33.9-109.1	69.6 (53.8-86.4)	26	56.2-125.8	73.0(59.1-92.9)	9
Coffee-drinker	32.8-120.9	68.1 (52.6-88.0)	25	31.6-86.1	57.0 (43.7-70.5)	10
P-value	ns	-		0.041	-	
	Cholesterol					
Non-Drinker	115.3-184.6	136.0 (122.7-126.2)	26	121.1-187.6	140.9 (160.8-167.0)	9
Coffee-drinker	101.0-192.0	134.6 (120.6-156.0)	25	118.6-150.2	124.8 (124.0-131.2)	10
P-value	ns	-		0.045	-	

IQR= Interquantile range

Medians of free fatty and HDL-C were significantly and non-significantly higher in coffee drinkers compared with non-coffee drinkers of the young and not so-young group. However, medians of LDL-C, triglyceride and cholesterol were significantly reduced in coffee drinkers of older groups but they were non-significantly lowered in coffee drinkers of the young groups.

3.4 Effect of coffee drinking in relation to sex

3.4.2 Fasting and post-loading plasma glucose for coffee consuming groups in male and female

Serum level of fasting and post-load glucose was different between two genders of the same group. Coffee drinkers and non-drinkers in men showed lower median of fasting insulin level than that of the same group in the women. However, non-coffee drinkers and coffee drinkers in the men had low and high median of post-loading insulin than the same group in the women respectively **(Table 3.10).**

Table 3.10 Fasting and post-loading glucose level in male and female

Coffee groups	Male			Female		
	Range	**Median** (IQR**)**	**n**	**Range**	**Median**(IQR**)**	**n**
	Fasting Blood Sugar					
Non-Drinker	71.0-134.9	101.9 (86.1-114.9)	15	73.3-133.1	103.9 (86.9-122.8)	20
Drinkers	64.4-123.9	98.9(81.2-107.9)	16	63.4-123.7	103.6(91.2-119.1)	19
p-value	ns	-		ns	-	
	Post-Loading Blood Sugar					
Non-Drinker	99.0-136.6	111.7(102.0-127.3)	15	99.0-138.6	120.9(101.0-128.0)	20
Drinkers	99.0-128.7	119.0(105.4-124.9)	16	98.0-131.7	118.8(109.8-123.1)	19
p-value	ns	-		ns	-	

IQR= Interquantile Range

Median of fasting serum glucose level was non-significantly lower in the coffee drinkers of men (P>0.05) and females (P>0.05). Where as, median of post-loading serum glucose was non-significantly lower in the coffee drinkers of females (P>0.05) but non-significantly lower in the non-coffee drinkers (P>0.0.05) of men.

3.4.3 Serum fasting and post-load insulin for coffee consuming groups in male and female

Coffee drinkers in men showed higher median of serum level of post-loading insulin (36.1vs. 28.7) but lower fasting insulin (12.9 vs. 15.9) than that of same group in the females. Non-coffee drinkers in men had higher in serum median of fasting (13.0 vs. 12.6) and post-loading (30.0 vs. 22.3) insulin than that of the same group in females (**Table 3.12**).

Table 3.11 Serum level of fasting and post-load glucose in the two sexes

Coffee groups	Male			Female		
	Range	**Median** (IQR)	**n**	**Range**	**Median**(IQR)	**n**
	Fasting Blood insulin					
Non-Drinker	9.5-54.4	13.0 (10.5-17.6)	15	8.9-27.1	12.6 (10.4-16.2)	20
Drinkers	6.3-39.9	12.9(10.3-17.3)	16	8.9-26.6	15.9 (10.6-20.3)	19
p-value	ns	-		ns	-	
	Post-Loading Blood insulin					
Non-Drinker	12.5-101.2	30.0 (21.4-66.7)	15	13.5-102.5	22.3(18.9-33.4)	20
Drinkers	15.4-104.4	36.1(27.7-53.4)	16	12.6-65.3	28.7 (20.2-35.4)	19
p-value	ns	-		ns	-	

IQR= Interquantile Range

Median of serum level of fasting insulin was non-significantly lower but post-loading insulin was non-significantly higher in coffee drinkers in men. However, coffee drinkers in females showed non-significantly higher both fasting and post-loading insulin than that of non-coffee drinkers.

3.4.4 Serum level of lipids in relation to sex

Serum level (Median) of free fatty acid (13.3 vs. 10.7), LDL-C (72.4 vs. 62.0), cholesterol (148.2 vs. 129.0) and HDL-C (46.9 vs. 44.6) was higher but triglyceride (120.3vs. 123.0) was lower in non-coffee drinkers of females than that of the same group in men. Whereas, serum level of LDL-C (60.6 vs. 68.1) and HDL-C (47.0 vs. 47.4) was lower but free fatty acid (21.2 vs. 13.6), cholesterol (133.2 vs. 131.5) and triglyceride (115.3 vs. 110.0) was higher in coffee drinkers of men than that of the same group in the females (**Table 3.12**).

In females, serum level (median) of cholesterol, LDL-C and triglyceride was non-significantly lower but free fatty acid and HDL-C was not significantly higher in coffee drinkers than that of non-coffee drinkers. In men, serum level (median) of cholesterol, LDL-C was not significantly but triglyceride (p=0.04) was significantly lower in coffee drinkers than that of non-coffee drinkers. However, free fatty acid (P= 0.003) was significantly but HDL-C was not significantly higher in coffee drinkers than that of non-coffee drinkers in men.

Table 3.12 Serum level of lipids for each category of coffee groups in the two sexes

Coffee groups	Male			Female		
	Range	**Median(IQR)**	**n**	**Range**	**Median(IQR)**	**n**
	Free fatty acid					
Non-Drinker	4.8-21.4	10.7(8.8-15.8)	15	6.2-43.1	13.3(11.0-15.4)	20
Coffee-drinker	4.0-33.0	21.2(12.8-24.5)	16	6.0-31.1	13.6(11.7-26.9)	19
P-value	0.003	-		ns	-	
	HDL-C					
Non-Drinker	31.5-60.2	44.6 (40.3-46.0)	15	32.2-78.1	46.9(36.8-55.4)	20
Coffee-drinker	34.0-73.1	47.0 (41.0-58.3)	16	36.0-66.0	47.4(42.9-58.3)	19
P-value	ns	-		ns	-	
	Triglyceride					
Non-Drinker	71.5-161.8	123.0 (113.1-149.7)	15	54.2-157.1	120.3 (100.3-148.5)	20
Coffee-drinker	78.1-131.2	115.3 (96.4-118.9)	16	60.0-150.0	110.0 (90.7-126.5)	19
P-value	0.04	-		ns	-	
	LDL-C					
Non-Drinker	44.0-96.4	62.0 (56.2-85.0)	15	33.9-125.8	72.4(58.6-97.2)	20
Coffee-drinker	31.6-106.8	60.6(55.2-86,6)	16	32.8-120.9	68.1(43.5-82.6)	19
P-value	ns			ns	-	
	Cholesterol					
Non-Drinker	121.1-184.6	129.0 (121.6-149.3)	15	115.3-187.6	148.2 (127.8-167.3)	20
Coffee-drinker	118.6-157.2	133.2 (124.4-141.8)	16	101.0-192.0	131.5 (114.6-155.8)	19
P-value	ns	-		ns	-	

IQR= Interquantile Range

3.5 Correlation between different parameters in coffee drinkers and non-drinkers

In non-coffee drinkers, post-loading glucose correlated significantly and positively with fasting glucose ($p<0.01$)); triglyceride correlated significantly and positively with fasting insulin ($p<0.05$); and cholesterol correlated significantly and positively with LDL-C ($p<0.01$) **(Figure 3.6)**. In coffee drinkers, fasting glucose correlated significantly and positively with post-loading glucose ($p<0.01$); cholesterol correlated significantly and positively with LDL-C ($p<0.01$); and HDL-C correlated

significantly and negatively with LDL-C (p<0.05) but positively with grades of coffee consumption (p<0.05) **(Figure 3.6).**

Figure 3.6 Correlation between difference parameters in non-coffee drinkers

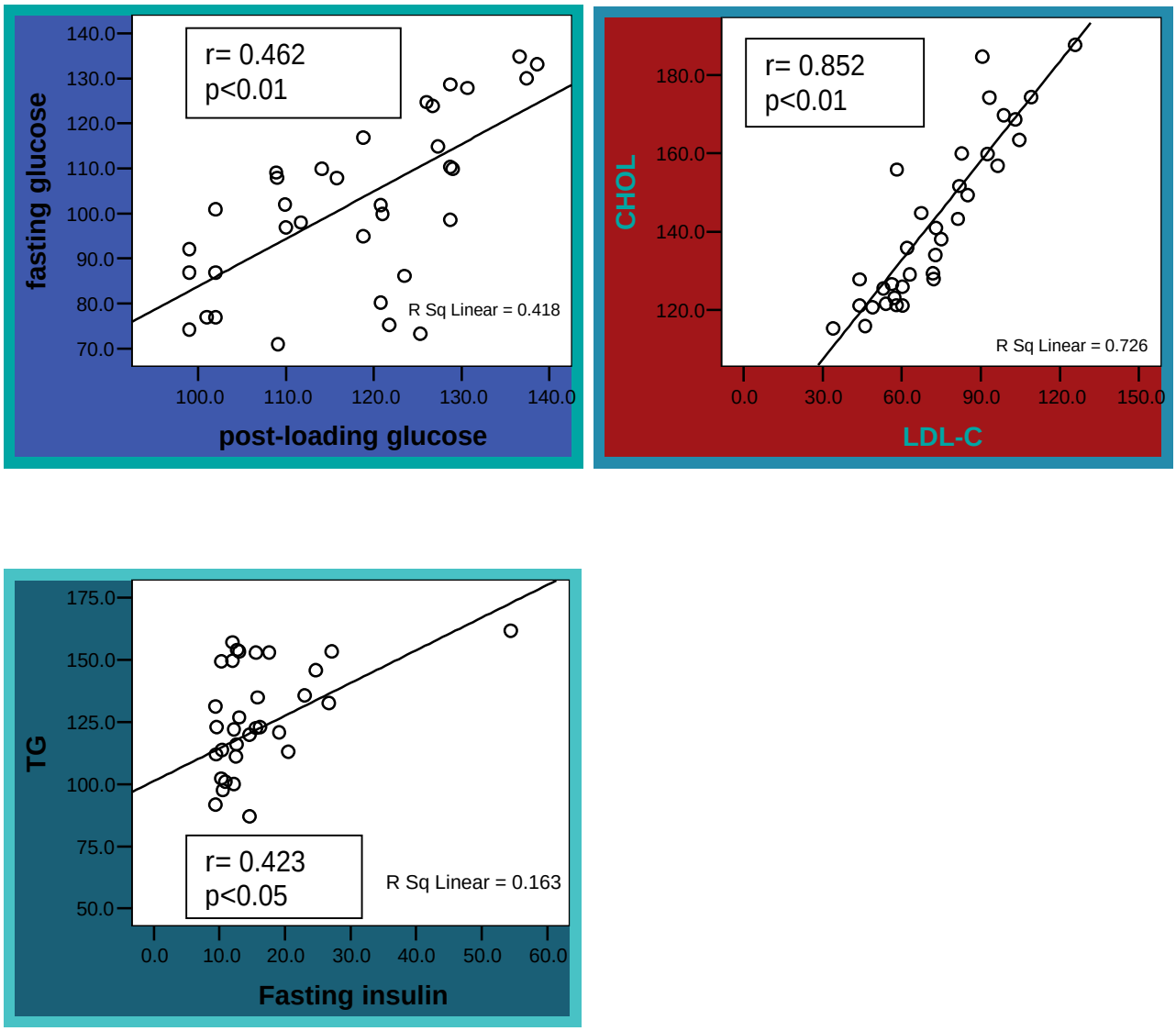
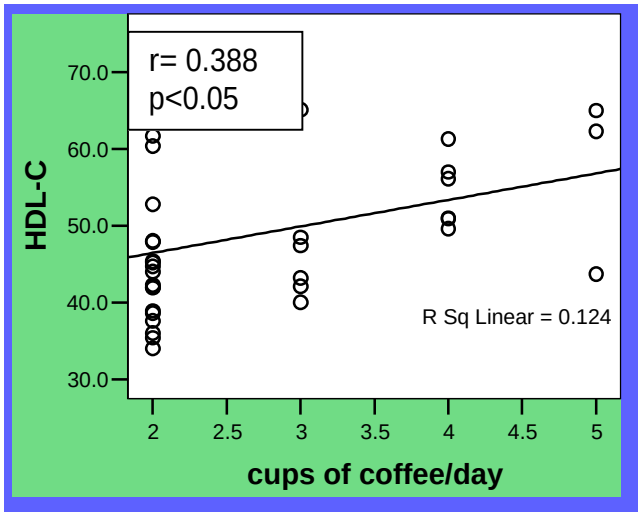
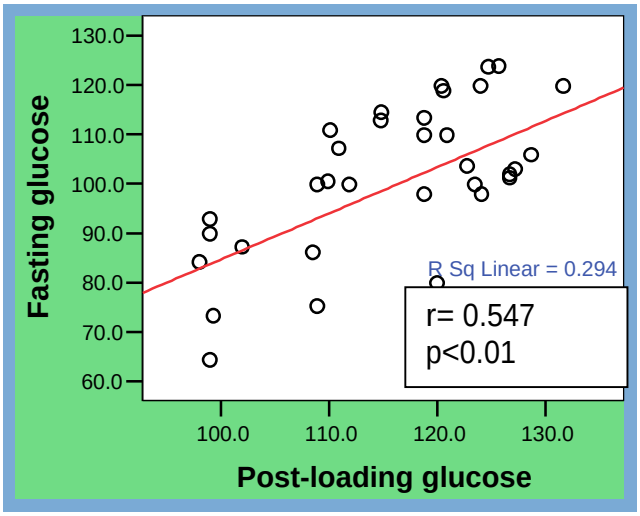
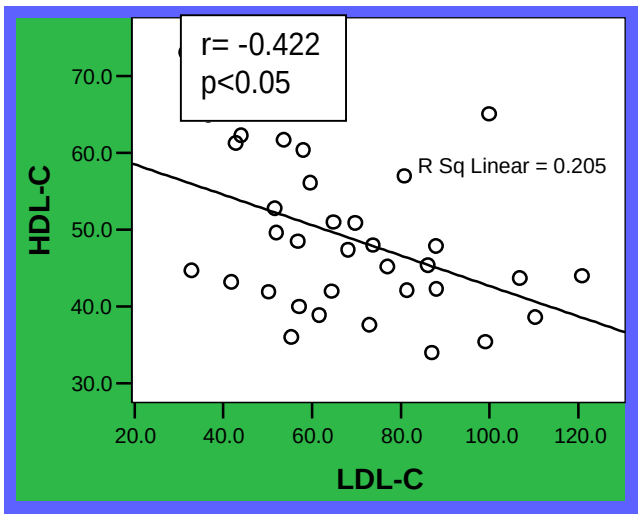
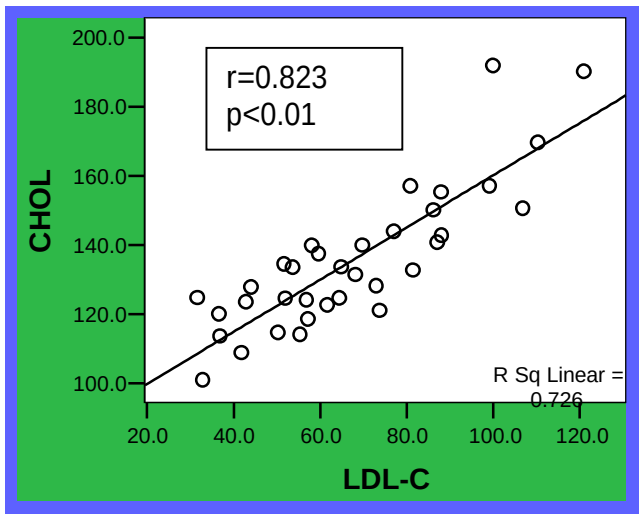


Figure 3.7 Correlation between difference parameters in coffee drinkers









4. Discussion

The present study conducted in Addis Ababa has shown the association between coffee consumption and insulin sensitivity status of apparently health individuals. Insulin sensitivity status of coffee drinkers and non-coffee drinkers for this study was assessed by their serum level of fasting and post-loading glucose and insulin, and serum lipid profile with adjusted confiding factors, such as age and sex. Increase in blood concentration of free fatty acid in coffee drinkers of this study may suggest that coffee consumption decreases insulin sensitivity. However, lowering of LDL-C, triglyceride, cholesterol and post-loading glucose according to grades of coffee consumption, and lower in the ratio of blood concentration of TG to HDL-C and LDL-C to HDL-C for coffee drinkers may oppose the positive effect of coffee consumption on insulin resistance. Studies carried out in USA **(5, 8, 12)** reported that non-caffeine parts of coffee, such as chlorogenic acids, potassium, niacin, and magnesium may improve insulin sensitivity. These mechanisms may explain the recent findings that habitual coffee intake is associated with a lower risk of developing type 2 diabetes mellitus.

The current study suggested that serum concentration of both fasting and post-loading glucose was nearly similar for coffee drinkers and non-coffee drinkers of men, women, young and older groups (Table 3.8 and Table 3.11). However, the median of both fasting (P>0.05) and post load serum glucose level (P>0.0.05) was non-significantly lowered according to grades of coffee consumption (figure 3.2). The consumption was more strongly associated with decreased concentration of post-load plasma glucose than fasting plasma glucose. Similar results were obtained from studies carries out in Japan **(25)** and USA **(67)**.These two studies reported that total caffeine estimated from coffee was associated with a statistically significant lower fasting glucose concentration. On other hand, epidemiologic studies in humans appeared to show a higher blood glucose level shortly after taking caffeine or caffeinated coffee suggested that coffee consumption might decrease insulin sensitivity **(68)**.

The other results of the current study showed that there was no significant difference in serum concentration of fasting and post-loading insulin between coffee drinkers and non-drinkers of men and women, young and older groups (Table 3.9 and Table 3.12). However, moderate-coffee drinkers showed non-significance higher serum fasting insulin level, where as heavy coffee drinkers appeared to have low post-loading insulin than other groups (Table 3.7). Short-term metabolic studies by American Diabetes Association showed that high coffee consumption for 4 weeks

Significantly increased fasting insulin concentrations compared with coffee abstinence due to lipolsis effects of caffeine. Consumption of some what weaker coffee and caffeine intake were non-significantly associated with higher fasting insulin concentrations. The increased fasting insulin concentration after high coffee consumption in these studies probably reflects decreased insulin sensitivity. In short-term metabolic studies, caffeine intake acutely lowered insulin sensitivity over 100–180 min **(69)**. Studies in Finland showed that 5 weeks of caffeine intake, complete tolerances to the effects of caffeine on fasting insulin concentrations developed, but effects on norepinephrine and free fatty acid concentrations partly remained for the high-dose caffeine treatment. Increase in free fatty acid in our study corroborates these findings that coffee consumption decreased this lipid uptake by adiposities. However, it is difficult to exclude the possibility that the elevated insulin concentrations after coffee consumption were due to higher insulin secretion or to reduce hepatic insulin clearance as a result of increased free fatty acid concentrations **(33)**. As the same time, a long-term study on consumption of regular coffee in the Netherlands suggested that an inverse relationship between regular coffee intake and serum level of fasting insulin both men and women. This relationship was stronger in women than in men but the researchers did not find a relationship between decaffeinated coffee intakes and serum level of fasting and post-loading insulin **(70)**.

Homeostasis model assessment for insulin resistance (HOMA-IR) is better for analysis of insulin sensitivity status of coffee drinkers and non-drinkers than using single glucose and insulin markers. This analysis suggested that insulin sensitivity status of coffee drinkers was nearly similar with that of non-drinkers. However, non-significantly lower of HOMA analysis (Figure 3.1) results in coffee drinkers may support the evidence that coffee drinkers showed more insulin sensitivity that of non-drinkers. Homeostasis model assessment (HOMA) analysis results reported by epidemiological studies in Sweden showed coffee consumption improves insulin sensitivity **(71)** but similar study in Finland reported HOMA value was significantly increased in coffee consumers as compared to coffee abstinence **(72)**.

Increasing blood concentration of free fatty acid in coffee drinking men and women, young and older groups of the present study may support the evidence that coffee consumption decreases insulin sensitivity in fat cells. So, insulin resistance in fat cells reduces the normal effects of insulin on lipids and results in reduced uptake of circulating lipids and increased hydrolysis of stored triglycerides. Increased mobilization of stored lipids in these cells elevates free fatty acids in the

blood plasma. Elevated blood fatty-acid concentrations (associated with insulin resistance and diabetes mellitus Type 2), reduced muscle glucose uptake, and increased liver glucose production all contribute to elevated blood glucose levels. Increasing in blood free fatty acid in coffee drinkers similar to the present study was also reported by short-term metabolic studies of American Diabetes Association and studies in Finland. However, results from study on Scottish **(73)** population for 8 years suggested that higher coffee consumption was associated with lower serum level of free fatty acid among men but not among women. On other hand, lowering of LDL-C, triglyceride and cholesterol but increase of HDL-C in coffee drinkers of not so-young and young groups of the present study may oppose the positive effect of coffee consumption on insulin resistance. Reduction of concentration of LDL-C with improvement of HDL-C in the blood of coffee drinkers than that of non-drinkers in men, women, young and older groups may provide supportive evidence for antioxidant activities of coffee. Similarly, study in England **(74)** reported that coffee ingestion resulted in a significant decrease in serum levels of cholesterol and LDL cholesterol, and a significant decrease in susceptibility of LDL to oxidation. This study indicated that coffee consumption might protect against atherosclerosis due to lowering serum lipid levels and improving LDL susceptibility. However, study in Finland reported that daily consumption of 7 to 9 cups of coffee per day of filtered coffee was associated with an increasing and decreasing serum concentration LDL-C and HDL-C respectively of men compared with a daily consumption of 3 cups/day or less; however, this association was not observed for women **(75)**. According to some reports in Germane, no significant associations were observed between coffee consumption and blood lipids **(76)**.

The ratio of TG to LDL-C and LDL-C to HDL-C serve as easily available laboratory markers for analyzing of insulin sensitivity and antioxidant activities of coffee. Low serum HDL-C combined with increased serum TG concentrations may predict the development of type 2 diabetes mellitus **(77)**. Non-significantly lower of the ratio of LDL-C to HDL-C , significantly lower of the ratio of TG to HDL-C in coffee drinkers may provide better evidence for positive effect of coffee on insulin sensitivity and antioxidant activities.

Significantly and negatively correlated between HDL-C and LDL-C in coffee drinker (Figure 3.7) but not in non-coffee drinkers of the present study may provide other evidence for antioxidant

activities of coffee. In addition, HDL-C was significantly and positively correlated with grades of coffee consumption in coffee drinkers can strength the above evidence.

Significantly higher blood concentration of free fatty acids and non-significantly increased concentration of insulin for coffee drinkers than that of non-coffee drinkers in men, female, young and older groups may support the evidence that coffee consumption decreases insulin sensitivity. However, significantly (older groups) and non-significantly (young groups) lower of LDL-C, triglyceride and cholesterol but non-significantly higher of HDL-C in the blood of coffee drinkers of older and young groups may indicate the positive effect of coffee consumption on insulin sensitivity. Reduction in concentration of LDL-C with improvement of HDL-C in the blood of coffee drinkers than that of non-coffee drinkers in men, women, young and older groups and significantly lower of the ratio of LDL-C to HDL-C may provide supportive evidence for insulin sensitivity and antioxidant activities of coffee. Reduction in the ratio of triglyceride to HDL-C in the blood of coffee drinkers may add other evidence for positive effects of coffee on insulin sensitivity.

5. Conclusions and Recommendation

WHO estimated the number of diabetic cases in Ethiopia to be 800,000 by the year 2000, and the number is expected to increase to 1.8 million by 2030. Most of Ethiopia adults consume coffee. Therefore, the potential impact that the humble coffee bean can have on diabetes incidence and progression needs to be carefully considered.

Although, results from many recent epidemiological studies were mixed, a number of them have found that consumption of caffeinated and decaffeinated coffee helps to improve insulin sensitivity. Therefore, the present study tried to investigate the effect of coffee consumption on human insulin sensitivity by assessing serum level of fasting and post-loading glucose and insulin, triglyceride, fatty acid, cholesterol, HDL-C and LDL-C in coffee drinkers compared to non-coffee drinkers.

The current study was revealed that free fatty acid was significantly but insulin was non-significantly higher in coffee drinkers than that of non-drinkers in men, women, young and older groups. These results may support the evidences that coffee consumption decrease insulin sensitivity. However, reduction in blood concentration of LDL-C and TG associated with increasing of HDL-C and significantly decreased of the ratio of TG to HDL-C in coffee drinkers of the study may provide the evidence that coffee has antioxidant activities and improves insulin sensitivity.

The results of this study reported that coffee has an effect on the lipid profile more than its effect on glucose metabolism. In addition, the effect of coffee consumption on free fatty acid, LDL-C and HDL-L was quite clear and stronger in men than the women and in older groups than the young groups.

This is the evidence suggesting the relationship between coffee consumption and insulin sensitivity of non-diabetic individuals in Addis Ababa. It is recommended that further study with larger sample size should be carried out. In addition, further metabolic studies are needed in order to investigate the long-term effects of coffee on glucose homeostasis and insulin resistance.

6. Limitation of the study

One of the limitations of the present study was cross-sectional nature. An association observed in this cross-sectional study did not indicate a causal relationship. The study was carried out in one kebele of Addis Ababa, which may not be representative of the Addis Ababa residents. The study population were mostly in the young students and may not consume coffee for longer periods. As our study was not based on the general population, selection bias might have affected the out come of the study. So, larger sample size in general population is required to confirm the result of the present study

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Appendix I Questionnaire

1. Personal identification
   - **a.** Full name of the subject_____
   - **b.** Subject identification number_____
2. Demographic details
   - **a)** Date of birth_____
   - **b)** Gender 1. Male ☐ 2. Female ☐
   - **c)** Address: City_____
     Woreda_____
     Kebele_____
     Phone number_____
3. Group: Study ☐ Control ☐
4. Life style

a) Daily coffee consumption (Cups per day)

1. <1 2. 1-2 ☐ 3. 3-4 ☐ 4. •5 ☐

b) Daily tea consumption (cups per day)

1. <1 ☐ 2. 1-2 ☐ 3. 3-4 ☐ 4. •5 ☐

c) Current alcohol use: Yes ☐ No ☐

d) Cigarette smoking: Yes ☐ No ☐

f) Parental history of diabetes Yes ☐ No ☐

5. Physical
   - Weight (kg): _____
   - Height (m): _____
   - BMI (kg/m^2): _____
   - Waist Circumference (cm): _____
   - Hip Circumference (cm): _____
   - WHR_____
6. Vital values
   - Pulse_____
   - Systolic/diastolic blood pressure (mmHg) _____

7. Chemistry

- Fasting plasma Glucose(mg/dl) _____
- Post load plasma glucose (mg/dl) _____
- Post load plasma insulin(• U/l)_____
- Fasting plasma insulin(• U/l) _____
- Free fatty acid(mg/dl) _____
- LDL-C(mg/dl)_____
- HDL-C(mg/dl)_____
- Triglyceride(mg/dl)_____

8. Any history of medical therapy

b) Current hypoglycemic therapy (oral and insulin)_____

c) Antihypertensive or hypolipidemic_____

d) Other medical therapies _____

9. Clinical history of major cardiovascular events

_____

Appendix II. Information sheet and constant form for participants of the study entitled Association of coffee consumption with insulin sensitivity and plasma lipid profile among non diabetic individuals in Addis Ababa.

A- Information sheet (English Version) Addis Ababa University, Department of Biochemistry, Addis Ababa, Ethiopia

Principal Investigator: Gizaw Mamo Gebeyehu

Advisors: - Dr. Rajinder Chawla, Dr. Melaku Umeta, Dr. Neena Chawla.

Name of the Sponsor: Addis Ababa University, Faculty of Medicine, Department of Biochemistry.

This information sheet is prepared by researcher at AAU for a project with the aim of assessing the association of coffee consumption with insulin sensitivity and plasma lipid profile among non-diabetic individuals in Addis Ababa.

1. **Aim of the Study**

Coffee is among the most widely consumed beverage in the world including Ethiopia. Most of Ethiopian adults are consuming coffee on daily basis, because the coffee ceremony is one of the most recognizable symbols of the Ethiopian culture. So knowledge of both the positive and negative health effect of coffee is important to allow individual to make informed choice regarding to coffee consumption. A prospective study performed in the Netherlands and Japan reported that coffee consumption was association with a decrease of type – 2 – diabetes. However, a population based study of Pima Indian and Finish failed to observe a protective effect of coffee. This study will reduce the gap between two studies and provide the first data in Ethiopia whether or not coffee consumption provides protective effective against the development of the type-2-diabetes.

2. Study design and procedure

If you agree to take part in the study, investigators will give you written information about the study and you will be given the consent form to sign.

Investigator will ask you some questions about your general health and will take a drop of blood from your finger and assess whether you qualify to participate in the study. If you are fit for the study 5ml of blood after an over night fasting and 2 hr after you are given with 75 g oral glucose will be collected.

3. Risk and discomfort

Participating in this project will not cause more discomfort and no need of extra sample other than samples taken for diagnostic purpose. But, there could be minor pain and change in color of your skin following the blood drawing. The amount of blood taken from each volunteer throughout the study period is 10mL which will not affect your health. There is no major risk in participating in this

research, as the whole procedure is carried out by physician and / or health professionals following the standard good clinical practice.

4. Benefits and Incentives

You will have the chance to know your blood sugar level from the laboratory result. And if your result reveals any incidental health problems (Hyperglycemia) that need immediate treatment, you will be referred to an appropriate health facility. The study could benefit many peoples who are consuming coffee on daily basis. You will not be provided with any direct incentives for your participation in the research. But the cost for blood collection will be covered by the project.

5. Confidentiality

All information about the patients will be kept confidential. Logbooks used in the laboratory will have no names but codes. The information sheet that links the coded number to participants name will be locked inside a box and it will not be revealed to any one except the principal investigator.

6. Right to refuse or withdraw

You have full right to refuse or with draw from participating in this study at any time before and after consent with out explaining the reason.

7. Whom to contact

This study protocol is reviewed and approved by ethical committee, Addis Ababa University Medical faculty Department of Biochemistry and the Ethiopian National Ethical Research Committee (NERC). The purpose of the review by these committees is to make sure that research participants are protected from harm. For more information you can contact the chairman of the committee _____, and _____) Chair person the NERC.

To know more information about the study you can contact any of the following individuals:

1. IRB address (tel.No. 0115538734),mailadress (aaumfi@ yahoo.com)
2. Ato Gizaw Mamo (mobile tel. No. 0911-80 43 67)
3. Dr. Rajinder Chawla (mobile tel. No. 9316919661)
4. Dr. Melaku Umeta (mobile tel. No. 0911-46-98-55)
5. Dr. Neena Chawla (mobile tel.No 9316919661)

Appendix III Information sheet (Amharic version)

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1. IRB address (tel.No. 0115538734),mailadress (aaumfi@ yahoo.com)
2. Ato Gizaw Mamo (mobile tel. No. 0911-80 43 67)
3. Dr. Rajinder Chawla (mobile tel. No. 9316919661)
4. Dr. Melaku Umeta (mobile tel. No. 0911-46-98-55)
5. Dr. Neena Chawla (mobile tel.No 9316919661)

Appendix IV Consent Form

For participation as a volunteer in the research undertaking

Code number_____

Name of study subject_____

I have been informed about a study that plans to correlate coffee consumption with insulin sensitivity and plasma lipids profile. For this purpose, blood needs to be taken from me. The aims of the study and the possible risks, including mild pain during blood collection were explained to me.

I am also informed that all the information contained with in 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.

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

Signature _____ Signature _____

(Participant) (Investigator)

Appendix V **Amharic Version**

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