ADDIS ABABA UNIVERSITY COLLEGE OF NATURAL SCIENCES CENTER FOR FOOD SCIENCE AND NUTRITION

Lipid Profiles and Dietary Intake of Long-Chain Omega-3 Polyunsaturated Fatty Acids of Food Science and Nutrition Students of Addis Ababa University, Ethiopia

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List of Abbreviations and Acronyms

SFA	saturated fatty acid
MUFA	monounsaturated fatty acid
PUFA	polyunsaturated fatty acid
CVD	cardiovascular diseases
LA	linoleic acid, 18:2n-6
ALA	alpha linolenic acid, 18:3n-3
EPA	eicosapentaenoic acid, 20:5n -3
DHA	docosahexaenoic acid, 22:6n -3
LDL	low-density lipoprotein
HDL	high-density lipoprotein
FA	fatty acids
MG	monoacylglycerols
DG	diacylglycerols
TG	triacylglycerols
PL	phospholipids
DPA	docosapentanoic acid, 22:5n-3
CHD	coronary heart disease
EPHI	Ethiopian Public Health Institute

mg	milligram
g	gram
DW	dry weight
n-3	omega 3
n-6	omega 6
LC	long chain
oC	degree centigrade
AAU	Addis Ababa University
REC	Research Ethics Committee
EFA	essential fatty acids
HUFA	highly unsaturated fatty acids
WHO	world health organization
FFQ	food frequency questioner
AI	adequate intake
wk	week
d	day
cm	centimeter
kg	kilogram
BMI	body mass index
ISSFAL	International Society for the Study of Fatty Acids and Lipids

Abstract

Lipid profiles and Dietary intake of Long Chain Omega-3 Polyunsaturated Fatty Acids of Food Science and Nutrition Students at Addis Ababa University, Ethiopia

Bethlehem Taye, Hinsta Mateos and Ashagrie Zewdu Woldegiorgis

Background

Intake of foods which are rich in long chain polyunsaturated fatty acid reduced the risk of Cardio Vascular Disease.

Objectives

The objective of this study was to evaluate the relationship between blood plasma lipid profile and dietary intake of LC n-3 PUFA of Food Science and Nutrition students at Addis Ababa University (AAU).

Methodology

The Study design was a cross-sectional study which evaluated the effect of fish and fish product consumption over blood plasma Total Cholesterol, Triglyceride, LDL, of a total of 40 young and educated men and women who attend at Center for Food Science and Nutrition Addis Ababa University. A pretested, structured, interviewer administered Food Frequency Questionnaire were used.

Results

From the food items listed on the food frequency questionnaire the majority of their intake was 100% low except for canned tuna (7.5%) and canned sardine (2.5%). Among the total participant n= 40 the distribution of cholesterol was determined to be: 90% was at desirable level, 7.5% at border line and 2.5% at high cholesterol level. Distribution of LDL was 57.5% at normal level, 30% at low border line, 7.5% at high borderline and 5% at high LDL level. Distribution of HDL 2.5% desirable, 70% satisfactory and 27.5% too low, and the distribution of Triglycerides 90% normal, 7.5% borderline high and 2.5% high .

Conclusion and Recommendations

The dietary intake of food rich in n-3 by 40 students was found to be lower than the recommended dietary intake. This was supported by the 97.5 % of the HDL level that was below the desirable level. Moreover, LDL cholesterol at normal level was only 57.5%.The population groups of this research deliberately targeted postgraduate students of Addis Ababa University Centre for Food Science and Nutrition because they were considered to know the effects of saturated and unsaturated fatty acid in health. The low intake LCPUFA that were observed in this educated student group can be extrapolated to the high probability of other groups of the society being at higher risk also for chronic diseases such as CVD.

Key Words: *Fats; n-3 fatty acids; Post Graduate Students; Low Density Lipoprotein; High Density Lipoprotein; Triglycerides; Chronic Diseases*

CHAPTER ONE

1. Introduction

1.1. Background

Dietary fat is the dens macronutrient, and includes all the lipids in plant and animal tissues that are eaten as food. Dietary fatty acids (FA) are long hydrocarbon chains, with a methyl group at one end (the omega or *n*-end) and an acid group at the other. The most common dietary fatty acids have been subdivided into three broad classes according to the degree of unsaturation; (i) saturated fatty acids (SFA) have no double bonds; (ii) monounsaturated fatty acids (MUFA) have one double bond: and, (iii) polyunsaturated fatty acids (PUFA) have two or more double bonds (FAO 2010; Lunn and Theobald 2006).

Dietary fats and oils provide calories and essential fatty acids (EFA), and are sources of fat-soluble vitamins A, D, E, and K. Certain types of fat, however, can increase the risk of chronic cardiovascular diseases (CVD) that affect the heart, blood vessels, and brain. The type of fat that is consumed can have either positive or negative effects on the risk of CVD. Trans fatty acids and SFA are generally considered unhealthy (USDA, 2011).

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Biologically significant polyunsaturated fatty acids (PUFA) include two with 18-carbon chains, linoleic acid (LA, C18:2n-6) and a-linolenic acid (ALA, C18:3n-3), which are considered EFA. The longer chain members of this biochemical class have been termed long chain polyunsaturated fatty acids (LC PUFA) or, alternatively, highly unsaturated fatty acids (HUFA) (Stanely *et al.*, 1998).

The short chain n-3 PUFA, 18-carbon alpha linolenic acid (ALA, 18:3n-3) must be acquired nutritionally but its metabolites, 20-carbon eicosapentaenoic acid (EPA, 20:5n-3) and 22-carbon docosahexaenoic acid (DHA, 22:6n-3), can be synthesized by cells. The rate of conversion of ALA into longer n-3 PUFA is, however, rather slow and nutritional intake plays a large part in determining the abundance of EPA and DHA in the membrane (Brian, 2008).

The relationship between n-3 FA and CVD was proven following the observation that the Greenland Inuit had low mortality from coronary heart disease (CHD) despite a diet that is rich in fat. A clinical study by the University of Edinburgh which is based on literature searches from PubMed, showed there is an inverse relationship between fish consumption and CHD and the risk of death from coronary heart attack (Din *et al.*, 2004).

Omega-3(n-3) FAs, however, have anti- inflammatory, antithrombotic, antiarrhythmic, hypolipidemic, and vasodilatory properties. These beneficial effects of n-3 FA have been shown in the secondary prevention of CHD, hypertension, type-2 diabetes, and, in some patients with renal disease, rheumatoid arthritis, ulcerative colitis, Crohns disease, and chronic obstructive pulmonary disease (Simopoulos, 1999). Fish and fish oil are the main sources of LC n-3 PUFA EPA and DHA. Cold water fish oils contain high quantities of EPA and DHA acids. These FA have multiple general health benefits when consumed in the daily dietary as a nutritional supplement (Jeff *et al.*, 2008; Welch *et al.*, 2010; Din *et al.*, 2004).

1.2. Statement of the problem

Chronic diseases such as CVD, cancer, and diabetes mellitus are mostly caused by lifestyle behaviors like unhealthy diet, physical inactivity, alcohol and tobacco use. These risk factors are expressed through raised blood pressure, raised glucose levels, abnormal blood lipids, particularly LDL and becoming overweight. According to WHO, it was estimated that death due to chronic diseases was 35 million in 2005 and if no action was taken, it would increase by 17% between 2005 and 2015 which includes young and middle aged people (WHO, 2005). By 2020 their contribution is expected to rise to 73% of all deaths and 60% of the global burden of disease. Moreover, 79% of the deaths attributed to these diseases occur in the developing countries. Four of the most prominent chronic diseases – cardiovascular diseases (CVD), cancer, chronic obstructive pulmonary disease and type-2 diabetes are linked by common and preventable biological risk factors, notably high blood pressure, high blood cholesterol and overweight, and by related major behavioural risk factors: unhealthy diet, physical inactivity and tobacco use. (WHO, 2018)

In sub-Saharan Africa the prevalence of non-communicable disease increases rapidly, particularly in urban areas. This is particularly so in the case of Ethiopia, where major non-communicable disease are causing higher morbidity and mortality both in rural and urban populations (Awoke *et al.*, 2014). In Ethiopia, even if there is no reliable data on the cause of the death, according to research which is done by verbal autopsy in the capital city Addis Ababa, the rate of death by non-communicable disease was 42% from 2006 to 2009 (Awoke *et al.*, 2012).

Diet which mainly includes saturated and *trans* fat increases bad cholesterol LDL and causes chronic non-communicable diseases such as diabetics, CVD, cancer whereas diets which include PUFA(which is mainly sourced from sea foods) has a significant effect on reducing non-communicable disease, especially CVD. The population groups of this research are students of Addis Ababa University Food Science and Nutrition Students who are considered to know the effects of saturated and unsaturated fatty acids in health., By evaluating the dietary selection and the lipid profiles of this group if may be possible to determine who is at risk, and as a consequence, determine if there other groups who might be at risk.

PUFAs such as Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are mainly found in oily fish. Several studies in adults have used these biomarkers to evaluate the dietary intake of fish and dairy products or specific FA estimated with FFQ (McNaughton *et al.*, 2007). However, among young educated adults, there is a lack of research comparing dietary assessment methods with biomarkers of fatty acids in general and, to our knowledge, no studies that compare plasma phospholipids EPA, and DHA, and the frequency of fish and fish product intake estimated with a food frequency questioner (FFQ) in Ethiopia.

1.3. Significance of the study

The prevalence of non-communicable diseases is increasing worldwide and is becoming a significant cause of mortality even in developing countries like Ethiopia. It is mainly caused by lifestyle behaviors like unhealthy diets, lack of activity, tobacco smoking, and alcohol intake. Chronic diseases and poverty are interconnected in a vicious cycle. In many countries, it is the poorest people who are most at risk of developing chronic diseases and dying prematurely from them, as they are least able to cope with the resulting financial burden. Chronic diseases can cause poverty in individuals and families, and draw them into a downward spiral of worsening disease and poverty (Tesfaye 2008), (Marc *et al.*, 2006).

Currently, Ethiopia has set no guideline values for dietary recommendations for levels of plasma phospholipid LC n-3 PUFA concentrations in men and women, therefore an effort to produce baseline data on the nutritional benefit associated with fish consumption in the study could contribute to determining such values. Also the findings of some proximate profiles will be necessary to ensure that they meet the dietary requirements and commercial specifications.

Further, the information is useful to help consumers choosing their prefer red fish species for their nutritional wellbeing. For those LC n-3 PUFA that are below these recommended levels in their blood plasma, higher LDL, lower HDL cholesterol concentrations (in the form of medications or n-3 PUFA supplements) will be suggested to reach the recommended levels.

1.4. OBJECTIVES

1.4.1. General objective

- To evaluate blood plasma lipid profiles and dietary intakes of long chain omega- 3 fatty acids of Centre for Food Science and Nutrition students at Addis Ababa University

1.4.2. Specific objectives

- To determine food frequency of foods rich in omega-3 fatty acids of Centre for Food Science and Nutrition students at Addis Ababa University

- To analyze lipid profiles (HDL, LDL, Cholesterol and Triacylglycerides) of Centre for Food Science and Nutrition students at Addis Ababa University

- To evaluate the association between dietary intake and lipid profiles of Centre for Food Science and Nutrition students at Addis Ababa University

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Dietary Fats and Fatty Acids

Fats, oils and lipids consist of a large number of organic compounds, including fatty acids (FA), monoacylglycerols (MG), diacylglycerols (DG), triacylglycerols (TG), phospholipids (PL), eicosanoids, docosanoids, sterols, sterol esters, fatty alcohols, hydrocarbons and wax esters. Dietary fat comprises all the lipids in plant and animal tissues. The most common fats are glycerolipids, which are essentially composed of TG. Fatty acids constitute the main components of these lipid units and are required in human nutrition as a source of energy which produces 9kcal per gram, and for metabolic and structural activities. The most common dietary fatty acids have been subdivided into three broad classes according to the degree of unsaturation; (i) saturated fatty acids (SFA) have no double bonds; (ii) monounsaturated fatty acids (MUFA) have one double bond: and (iii) polyunsaturated fatty acids (PUFA) that have two or more double bonds. In general, these fatty acids have an even number of carbon atoms and have un-branched structures (FAO 2010; Lunn and Theobald, 2006).

2.1.1. Saturated Fatty Acids

Saturated fatty acids (SFA) are long-chain carboxylic acids that usually have between 12 and 24 carbon atoms and have no double bonds. Thus, SFA are saturated with hydrogen since double bonds reduce the number of hydrogens on each carbon. Because SFA have only single bonds, each carbon atom within the chain has 2 hydrogen atoms except for the omega carbon at the end that has 3 hydrogens. Saturated fats are primarily found in foods that come from animals, such as meat and dairy but can also be found from plant. Most common saturated fatty acid in animals, plants and microorganisms is palmitic acid (16:0). Stearic acid (18:0) is a major FA in animals and some fungi, and a minor component in most plants. Myristic acid (14:0) has a widespread occurrence, occasionally as a major component (Arild and Christiann, 2005).

2.1.2. Unsaturated fatty acids

Unsaturated fatty acid can be classified into monounsaturated fatty acids (MUFA) which have one carbon–carbon double bond, and polyunsaturated fatty acid (PUFA) which has more than one double bond. The most common MUFA are oleic acid (18:1n-9) and palmitoleic acid (16:1n-7) most of them have 16–22 carbon atom and a double bond with the *cis* configuration. This means that the hydrogen atoms on either side of the double bond are oriented in the same direction. *Trans* isomers may be produced during industrial processing (hydrogenation) of unsaturated oils. The presence of a double bond causes restriction in the mobility of the acyl chain at that point. The *cis* configuration gives a kink in the molecular shape and *cis* fatty acids are thermodynamically less stable than the *trans* forms. The *cis* fatty acids have lower melting points than the *trans* FAs or their saturated counterparts (Arild and Christiann, 2005).

2.1.3. Polyunsaturated fatty acids (PUFA)

Most fatty acids (FA) can be synthesized in the body, but humans lack the enzymes required to produce two FA. These essential fatty acids (EFA) are omega-3 PUFA a- linolenic (n-3) acid and the omega-6 PUFA linoleic acid (n-6).

Omega-6 fatty acids are PUFAs s that has the first double bond at the sixth carbon from the end of the FA chain, like linoleic acid, which has 18 carbons and two double bonds. These are the types of FA present in some of the most commonly used vegetable oils such as corn oil, safflower oil, and sunflower oil.

Omega-3 fatty acids are PUFA that has the first double bond located at the third carbon from the end of the FA chain. The long-chain n-3s from marine sources are EPA DPA and DHA, with 20 and 22 carbons and with five or six double bonds. There are also plant sources of n-3 fatty acids with 18 carbons and three double bonds. Humans may have the ability to convert some of the dietary alpha- linolenic acid (ALA)which is found in higher plants (soya bean oil, flaxseed and rape seed oils) to the LC n-3 PUFA eicosapentaenoic acid and docosahexaenoic acid, however conversions in adults have been found to be very low and more limited due to insufficient desaturase and elongase enzymes. The long chain n-3 PUFA, in particular EPA and DHA and the short chain n-3 PUFA, ALA are the focus of this research (Arild and Christiann 2005; Lunn and Theobald, 2006; Dickinson, 2012).

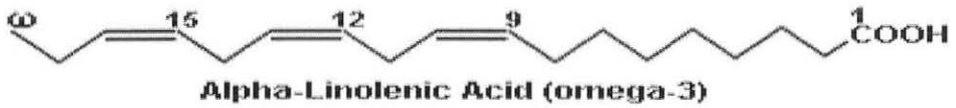


Figure 1. Chemical structure of alpha-linolenic acid (ALA, 18:3n-3)

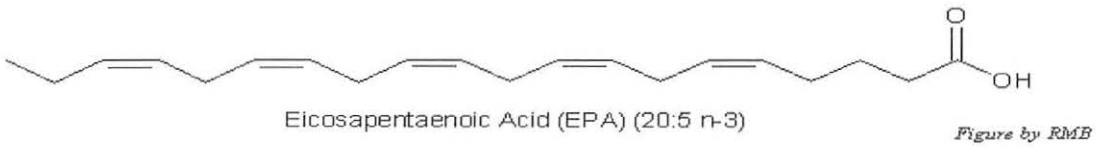


Figure 2. Chemical structure of Eicos ape ntae noic Acid (EPA, 20:5n-3)

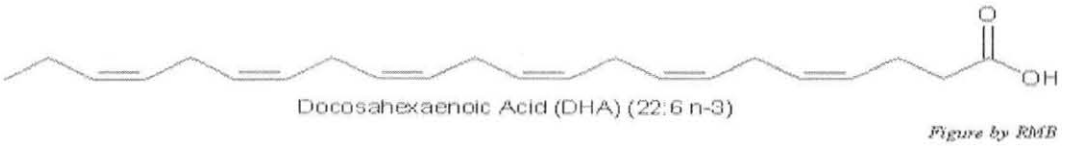


Figure 3. Chemical structure of Docos ahe xae noic Acid (DHA, 22:6 n -3)

2.1.3.1. Docosahexaenoic Acid and Eicosapentaenoic Acid

Eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) are the most important n-3 fatty acids in human nutrition. EPA and DHA are components of marine lipids. Marine fish such as mackerel, salmon, sardine, and tuna are excellent sources of EPA and DHA (Ackman, 2008).

Docosahexaenoic acid is the predominant n-3 fatty acid found in the brain and is linked to many aspects of neural function, including neurotransmission, ion channel regulation and gene expression. It is also associated with an impairment of PUFA metabolism has also been hypothesized to occur in children suffering from autistic spectrum disorders.

2.1.3.2. Alpha Linolenic acid

Alpha Linolenic acid (18:3n-3, αLNA) is an essential fatty acid in the diet of humans and is the principal n-3 polyunsaturated fatty acid (PUFA) in the western diet.
The major dietary sources of αLNA are green leaves, and oils used on cooking such as rapeseed oil and soybean oil where it accounts for up to 10% of total fatty acids. Some seeds (e.g., flaxseed (also known as linseed)) and nuts (e.g., walnut) are particularly rich in αLNA, as are the oils extracted from those seeds and nuts. ALA can be converted to longer-chain n-3 PUFA such as eicosapentaenoic acid (EPA;
20:5n-5) and docosahexaenoic acid (DHA; 22:6n-3) by the pathway shown in Figure 4. (Grham and Philip 2005)

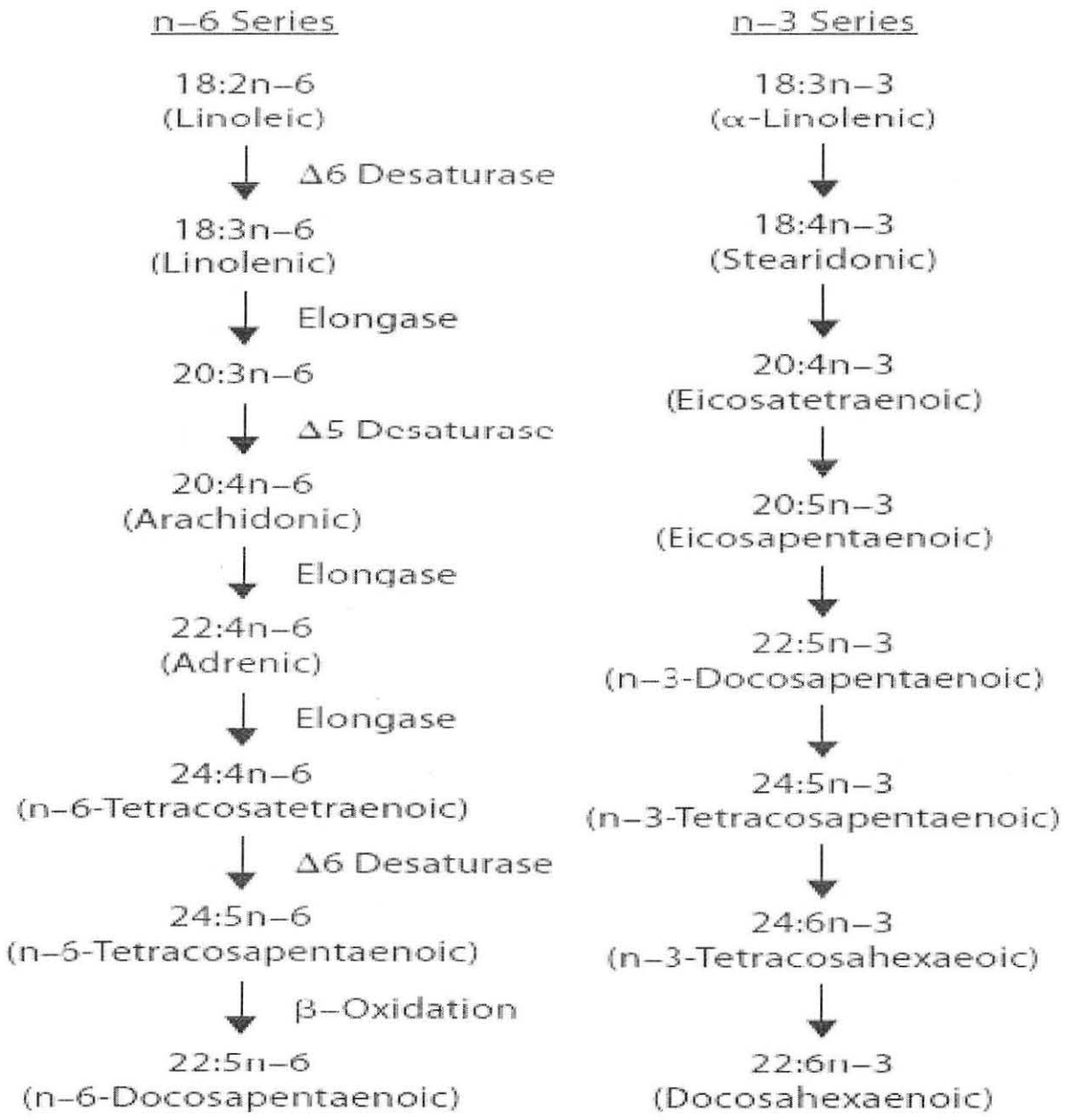


Figure 4. n–6 and n–3 fatty acid metabolis m (Ratenayake and Galli, 2009)

2.2. Recommended intake for EPA and DHA

According to global recommendation for EPA & DHA, omega-3 PUFA should be 1-2% of total energy taken per day (WHO, 2002). DHA: 0.1-.018%E for 0-6 months, DHA: 10-12 mg/kg bw for 6-24 month, EPA + DHA: 100-150 mg 2-4 years, EPA + DHA: 150-200 mg4-6, EPA + DHA: 200-250 mg, EPA + DHA: 0.3 g/d of which at least should be 0.2 g/d (FAO, Fats and fatty acids in human nutrition 2010). For the general adult population for cardiovascular health at least 500 mg/day of EPA+DHA is needed and for pregnant/lactating women DHA: 200 mg/day (ISSFAL, 2004). Infants, when breastfeeding is not possible0.2-0.5% wt total fat (Koletzko, *et al.* 2008). For the general population 3-5 servings/wk of fish, 300-400 mg EPA+DHA/day (Table 1) (2008; Simopolous 1989).

According to the Australian Heart Foundation, to lower the risk of CHD, consumption of about 500 mg per day of combined DHA and EPA through a combination of the two or three serves (150 g/serve) of oily fish per week, fish oil capsules or liquid food and drinks enriched with marine n-3 PUFA and also to consume at least 2g per day of ALA.

Table 1.Global Recommendations for EPA and DHA Intake (GOED, 2014)

Organization	Target Population	Recommendation
World Health Organization (WHO)	General adult population	n-3 PUFAs: 1-2% of energy/day
Food and Agriculture Organization of the United Nations (FAO)	0-6 month	DHA: 0.1-.018%E
	6-24 month	DHA: 10-12 mg/kg bw
	2-4 years	EPA + DHA: 100-150 mg
	4-6 years	EPA + DHA: 150-200 mg
	6-10 years	EPA + DHA: 200-250 mg
	Pregnant/Lactating Women	EPA + DHA: 0.3 g/d of which at least should be 0.2 g/d
International Society for the Study of Fatty Acids and Lipids (ISSFAL)	General adult population for cardiovascular health	atleast500mg/day of EPA+DHA
	Pregnant/Lactating Women	DHA: 200 mg/day
World Association of Prenatal Medicine	Pregnant/Lactating Women	200 mg DHA/day
	Infants, when breastfeeding is not possible	0.2-0.5% wt total fat
World Gastroenterology Organization	General adult population	3-5 servings/wk of fish
NATO Workshop on n-3 and n-6 Fatty Acids	General adult population	300-400 mg EPA+DHA/day

According to the Australian NHMRC Nutrient Reference Values for Australia and New Zealand, which included recommendations on ALA, DHA, EPA and DPA adequate intake (AI) for ALA: 1.3 g/day for men and 0.8 g/day for women and DHA + EPA + DPA: 160 mg/day for men and 90 mg/day for women. The upper limit for children, adolescents and adults was set at 3000 mg/day for combined DHA, EPA and DPA. This upper limit is unlikely to be met by the consumption of seafood alone.

No upper limit was set for ALA because there is no known level at which adverse effects occur. To prevent chronic disease, dietary intakes for DHA, EPA and DPA have been set at 610 mg/day for men and 430 mg/day for women. The NHMRC report suggests achieving this by replacing energy-dense low- nutrient foods with marine n-3 PUFA-rich foods, such as oily fish.

The acceptable distribution range of ALA intake to reduce chronic disease risk equates to 0.4-0.5% of total dietary energy at the lower end, and 1% of total dietary energy at the upper end, as relevant for the age- and sex-specific AIs. These values were based on intakes to optimize the reduction in chronic disease risk, notably CHD (David *et al.*, 2008).

Heat-treatment (cooking and frying) did not in general significantly decrease the contents of EPA and DHA compared to raw fish species. Boiled trout appeared to be a more valuable fish dish for obtaining the officially recommended appropriate daily intake of EPA + DHA for humans (Michail *et al.*, 2006). The total lipid concentration, Eicosapentaenoic acid concentration and Docosahexaenoic concentration of some fish items are listed below (Table 2).

Table 2.Total fat, EPA & DHA concentration of some sea foods (USDA 2017)

Description	Total lipid (fat)(g)/ 100 g	EPA(g)/ 100 g	DHA(g)/ 100 g
Fish trout, rainbow, farmed raw	01.507	0.217	0.516
Fish, mackerel, Atlantic, cooked, dry heat	17.81	0.504	0.699
Fish, mackerel, Atlantic, raw	13.89	0.898	1.401
Fish, salmon, Atlantic, farmed, raw	13.42	0.862	1.104
Mollusks, oyster, eastern, cooked, breaded and fried	12.58	0.202	0.218
Fish, salmon, Atlantic, farmed, cooked, dry heat	12.35	0.69	1.457
Crustaceans, shrimp, mixed species, cooked, breaded and fried	12.28	0.109	0.124
Fish, sardine, Atlantic, canned in oil, drained solids with bone	11.45	0.473	0.509
Fish, sardine, Pacific, canned in tomato sauce, drained solids with bone	10.45	0.532	0.864
Fish, tuna salad	9.26	0.014	0.055
Fish, tuna, light, canned in oil, without salt, drained solids	8.21	0.027	0.101
Fish, tuna, light, canned in oil, drained solids	8.21	0.027	0.101
Crustaceans, crab, blue, crab cakes, home recipe	7.52	0.227	0.216
Fish, catfish, channel, farmed, cooked, dry heat	7.19	0.02	0.069
Fish, mackerel, salted	25.1	1.619	2.965
Mollusks, mussel, blue, raw	2.24	0.188	0.253
Mollusks, oyster, eastern, farmed, cooked, dry heat	2.12	0.229	0.211
Mollusks, octopus, common, cooked, moist heat	2.08	0.152	0.162
Fish, tilapia, raw	1.7	0.005	0.086
Crustaceans, shrimp, mixed species, cooked, moist heat (may have been previously frozen)	1.7	0.135	0.141
Fish, perch, mixed species, cooked, dry heat	1.18	0.101	0.223
Fish, grouper, mixed species, raw	1.02	0.027	0.22
Crustaceans, lobster, northern, raw	0.75	0.102	0.068

2.3. Long Chain Polyunsaturated Fatty Acids (LC-PUFA) in Ethiopian Fishes

There are 180 different species of fish in Ethiopia and 30 of those are native to the country. The total area of the lakes and reservoirs stands at about 7000 to 8000 square km and the important rivers stretch over 7000 km in the country. The fishing contribution to the country's GDP is very low. Fish production potential of the country is estimated at 51,000 tonnes. Fresh fish are consumed in the vicinity of the Great Rift Valley lakes. Outside these areas, the do mestic market for fish is small (Janko, 2015).

Among the five popular lakes in Ethiopia such as Lakes Ziway, Langeno, Awassa, Chamo and Haiq there is a remarkably large variation in the lipid and FA contents of the herbivorous fish, *0. niloticus,* It is because of the prevalence of specific phytoplankton flora in certain lakes; Lakes Ziway, Langeno and Awassa, the phytoplankton were dominated by blue-greens and green algae containing none of or only traces of the important long-chain EPA and DHA whereas, in Lakes Chamo and Haiq, the phytoplankton community commonly included diatoms, a group of algae containing high levels of EPA and lake Haiq is superior in nutritional fat quality because it contained phytoplankton having very high levels of both EPA and DHA.

The concentration of FA content and quality can be affected by genetic variation, size of fish, season, and water temperature and the abundance and composition of the diet (Table 3) (Henderson and Tocher 1987; Zenebe *et al.*, 1998).

Table 3. Fatty acid contents (*mg/g DW*, mean) of muscle of various fish species from different Ethiopian Lakes (Zenebe *et. al.* 1998)

Fish Species	Fish Source	ALA	EPA	DHA	n-3	Total PUFA
O. niloticus	Lake Ziway	1.81	0.49	3.08	7.56	12.34
	Lake Langeno	0.22	0.79	3.25	6.38	8.47
	Lake Chamo	3.05	0.66	3.55	11.16	18.1
	Lake Awassa	0.3	0.48	2.69	4.86	7.88
	Lake Haiq	1.12	1.92	11.55	20.61	24.12
C. gariepinus	Lake Ziway	1.2	0.59	3.5	6.5	10.63
	Lake Langeno	0.48	1.81	3.43	6.91	8.53
	Lake Chamo	2.02	1.6	5.8	11.51	15.01
	Lake Awassa	0.32	0.53	4.78	6.61	9.38
	Lake Haiq	1.52	2.6	4.72	10.81	14.83
Barbus sp.	Lake Langeno	2.98	4.74	3.41	14.26	21.03
	Lake Chamo	0.57	0.61	4.66	7.39	9.98
	Lake Awassa	0.39	1.11	5.36	8.58	13.65
L. niloticus	Lake Chamo	0.82	0.64	3.62	7.05	10.31
T. zilli	Lake Ziway	1.4	0.51	3.44	7.22	12.4

2.4. Health implication of lipid profiles

Changes in lipid metabolism can result in modification of membrane composition and subsequently in changes in its permeability. It may also lead to disruption of signaling networks and could be associated with some pathological states, such as cancer, cardiovascular, neurodegenerative, and metabolic diseases, and similarly with inflammatory complications (Brenna, 2002; Brenna *et al.*, 2009).

Lipid profiles such as total cholesterol, LDL, HDL and triglycerides are biomarkers of diseases like CVD. Analysis of fatty acid (FA) composition in different blood and lipid fractions seems to be a valuable biomarker to assess the FA status in humans (Claudia *et al.*, 2010; Risé *et al.*, 2007; Hodson *et al.*, 2008; Fekete *et al.*, 2009).

In the body, cholesterol comes from two main sources: foods in the diet, or made by the liver in the body. Cholesterol is essential to proper body function such as synthesis of hormones, Vitamin D and bile. However, there is a link between blood cholesterol levels and the risk of heart disease. Cholesterol cannot travel alone in the blood and is transported by lipoprote ins (Whitney and Rolfes, 2008).

Triglyceride is the first and most important energy provider to the body either from the body or the diet. Triglyceride is transported to different parts of the body by lipoproteins called chylomicrons. Chylomicrons causes hyperglyceridemia postprandial but raised triglyceride is mainly caused by high very low density lipoprotein (VLDL) which is associated with excessive intake of carbohydrates. Cells all over the body remove triglycerides from the chylomicrons as they pass by so the chylomicrons get smaller and smaller. Within 14 hours after absorption, most of the triglycerides have been depleted (Whitney and Rolfes, 2008).

Low Density Lipoprotein (LDL) which is known as the "bad cholesterol" travels through the blood stream and delivers cholesterol to the cells in need. If the body has too much LDL cholesterol it can buildup in the walls of arteries and it forms fatty deposits called plaque. Over the time the plaque can narrow arteries and reduce blood flow. Coronary arteries which supply the heart are the common place where such plaque forms and this causes coronary heart disease possibly leading to a heart attack.

High Density Lipoprotein (HDL) it is made in liver and helps to remove excess cholesterol from cells, tissues and from plaque in blood vessels by returning it to liver to remove it from the body (Whitney and Rolfes 2008). According to the National Cholesterol Education Program (NCEP) the distribution of cholesterol in plasma are listed in (Table 4).

Table 4. Health implication of lipid profiles (NCEP May 2001)

Type of Cholesterol	Concentration mg/dL	Implication
LDL cholesterol	<100	Optimal
	100-129	Near optimal/ above optimal
	130-159	Borderline high
	160-189	High
	≥190	Very high
HDL cholesterol	<40	Low
	≥ 60	High
Triglyceride	<150	Normal
	150-199	Borderline high
	200-499	High
	≥ 500	Very high
Total Cholesterol	<200	Desirable
	200-239	Borderline high
	≥240	High

2.5. Chronic diseases in Ethiopia

Chronic diseases such as cardiovascular diseases (CVD), cancer, and diabetes mellitus are mostly caused by lifestyle behaviors like unhealthy diet, physical inactivity, alcohol and tobacco use. These risk factors are expressed through raised blood pressure, raised glucose level, abnormal blood lipid, particularly LDL and becoming overweight. CVD is one of the leading causes of morbidity and mortality worldwide, accounting for approximately 30% of all deaths (Emilie *et al.*, 2014; WHO, 2010; CDC, 2014).

According to WHO, it was estimated that death due to chronic disease was 35 million in 2005 and if no action was taken it would increase by 17% between 2005 and 2015 which includes both young and middle age people (WHO, 2005). It was also estimated by WHO in 2011 that 34% of Ethiopian population was dying from non-communicable diseases, with a national CVD prevalence of 15% (Awoke *et al.*, 2014).

In sub-Saharan Africa prevalence of non-communicable disease increases rapidly particularly in urban areas. This is particularly so in the case of Ethiopia, where major non-communicable disease are causing higher morbidity and mortality both in rural and urban populations (Awoke *et al.*, 2014).

In Ethiopia, even if there is no reliable data on the cause s of the death, according to a research which is done by verbal autopsy in the capital city Addis Ababa the rate of death by non-communicable disease was 42% from 2006 to 2009 (Awoke *et al.*, 2012).

2.6. Review of Researches on Serum Plasma Phospholipid n-3 Statuses

A research study was conducted on 200 French-Canadian men and women aged between 18 to 55 years. Dietary data were collected using a validated FFQ. Fasting blood samples were also collected and the plasma phospholipid (PL) FA profile was measured by gas chromatography. Low intakes of n-3 long-chain PUFA together with low percentages of n-3 long-chain PUFA in plasma PL were found in the studied French-Canadian population. Daily intakes of eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA)

were similar between men and women. Yet, alpha- linolenic acid (ALA) and total n-3 PUFA intakes were significantly higher in men compared to women. This research concluded that n-3 long-chain PUFA intake among this young and well-educated French-Canadian population is lower than the recommendations. Further, FFQ data was comparable to plasma PL results used to estimate DHA and total n-3 PUFA status in healthy individuals as well as to evaluate the EPA and DPA status in women (Véronique *et al.*, 2012).

The Department of Nutrition and Dietetics in Netherland systematically reviewed findings of scientific research studies and compared them with recommendation given by advisory committees by the USDA/USDHHS in a report, a report about dietary fats from the Institute of Medicine (IOM) in 2005, and a report about dietary fats from the European Food Safety Authority (EFSA) in 2010. These advisory committees recommend that; consumption of less than 10% of calories from saturated fatty acids (SFA) by replacing them with monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), keeping the intake of SFA as low as possible while consuming a nutritionally adequate diet and saturated fat intake should be as low as possible. The recommendations are based on the studies which included 3 types of studies: - (i) controlled trials that showed saturated fat consumption increases (LDL) cholesterol levels; (ii).Intervention studies that showed that the decrease of saturated fat and the simultaneous increase of polyunsaturated fat in the diet decrease CVD risk and, (iii). Prospective cohort studies that showed a positive association between saturated fat intake and coronary heart disease (CHD) risk. Reports from all three advisory committees mentioned that saturated fat intake increases LDL cholesterol and included the effect of LDL cholesterol on CVD as evidence for an association between saturated fat and CVD (Hoenselaar, 2012).

The relationship between n-3 FAs and CVD was proven following the observation that the Greenland Inuit had low mortality from CHD despite a diet that is rich in fat. A clinical study by the University of Edinburgh which is based on literature searches from PubMed showed there is an inverse relationship between fish consumption and CHD and the risk of death from coronary heart attack.

The diet and reinfarction trial (DART) randomized 2033 men with a recent myocardial infarction to three dietary interventions; patients who received advice on fish had a relative reduction in total

mortality of 29% year follow up ($p \leq 0.05$), mainly because of a reduction in deaths from CHD . Another study reviewed trial randomised 11324 patients after myocardial infarction to either a daily capsule of about 850 mg n-3 FA, 300 mg Vitamin E, both, or neither. After 3.5 years participants randomised to fish oil capsules had a reduction in relative risk of 15% in the composite primary end point of total mortality, non- fatal myocardial infarction, and stroke ($p = 0.023$). The relative risk of cardiovascular death was also reduced, by 30% ($p = 0.024$), and of sudden death by 45% ($p = 0.01$). These benefits were apparent within just four months of randomisation. Two smaller secondary prevention trials have also assessed the effects of n-3 FA. In an Asian population, patients with a suspected myocardial infarction randomised to fish oil capsules experienced a significant reduction in mortality from coronary heart disease after one year compared with placebo (Din *et al.*, 2004).

A study which was done in 2053 men and women in North America and Canada who were consuming a wide diversity of food including fish and, supplements of EPA & DHA. The results showes significant inverse relation between the levels of n-3 (EPA & DHA) and total FA in serum phospholipid and levels of this FA correlated with CHD (Bruce *et al.*, 2009).

A study in 14,422 men and women aged 39–78years from the EPICN (European Prospective Investigation into Cancer and Nutrition)-Norfolk cohort with 7-d diary data and included a sub-study in 4902 individuals with plasma phospholipid FA measures. Intakes and status of n-3 PUFA were measured, and the precursor product ratio of ALA to circulating n-3 PUFA was calculated. The results from this research determined, total n-3 PUFA intakes were 57–80% lower in non-fish eaters than in fish-eaters (Welch *et al.*, 2010).

A study designed to determine the effect of 2560 mg/day of mixed EPA/DHA n-3 PUFA supplements (650 mg EPA; 450 mg DHA; 180 mg other n-3 FAs in 36 professional NFL football players from the Pittsburgh Steelers Football Club, ages 23 to 41 years (average, 28.03 years), volunteered to be randomly assigned to either the treatment (n= 20) or the control (c = 16) arms of the study. There was an average increase of 106.67% for DHA and 365.82% for EPA in the treatment group. There was a 58.29% increase in the EPA and no change in the DHA of the control group. Treatment increased HDL (average percent change: +25.96, control +14.16),

decreased triglycerides treatment (–8.06, control +43.98), VLDL treatment (–13.98, control +23.18), intermediate density lipoprotein (–27.58, control +12.07), remnant lipoproteins (–23.86, control +8.33), and VLDL-3 (–17.10, control +7.77) (Yates *et al.*, 2009).

CHAPTER THREE

3. Materials and Methods

3.1. Study area and period

The real sample collection was conducted starting from February to April 2017 for three months in Addis Ababa University, Ethiopia. Addis Ababa has a population size of over 3 million (3,384,569) with annual growth rate of 2.1% (data obtained from central statistical agency of Ethiopia 2007). The city is divided into ten sub-cities and 100 Kebeles (lowest administrative units in Ethiopia). Addis Ababa is located at $9^{o}1'$ 48" North and 38^{o} 44' 24" East and the total land area is 54,000 hectares. Its average elevation is 2,405 m above sea level, and hence has a fairly favorable climate and moderate weather conditions (CSA, 2007).

3.2. Study design

The study design was a cross-sectional study which evaluated the effect of fish and fish product consumption over blood plasma total cholesterol, triglyceride, LDL, HDL and physical performance of young and healthy men and women who attend at Center for Food Science and Nutrition at Addis Ababa University.

Inclusion criteria

- ✓ ≥18 years of age
- ✓ Give verbal informed consent voluntarily
- ✓ Healthy

Exclusion Criteria

- ✓ A person who has diabetes or takes any cardiac medication will be ineligible to participate in the study
- ✓ Those who smoke cigarettes

3.3. Source of population

Source of population were young and healthy men and women who attend the Center for Food Science and Nutrition at Addis Ababa University.

3.4. Study population

The study population was young and healthy men and women who attend at Center for Food Science and Nutrition at Addis Ababa University who were ≥18 years of age and who gave verbal informed consent voluntarily.

3.5. Sample size

The total number of participants recruit from AAU Centre for Food Science and Nutrition were n=40. Because of the limited population group it was impossible to use sampling procedure (Yates, *et al.* 2009)

3.6. Study variables

- Lipid profile
  - ✓ Total Cholesterol
  - ✓ Triglyceride
  - ✓ LDL
  - ✓ HDL
- FFQ (Food Frequency Questionnaire)
- Height
- Weight
- BMI (Body Mass Index)

3.7. Data collection method

3.7.1. Dietary data collection

The questionnaire was prepared based on the nutritional status and commercial availability of foods which are rich in long chain omega-3 such as Canned Tuna, Cat Fish (Ambazza), Nile Tilapia (Korosso), Nile Perch (Nech Assa), Salmon, Lobster, Coral fish (e.g., hump head wrasse, potatogrouper, tiger grouper, and high fingrouper), Seafood sushi (other than fish sushi) (e.g., whelk sushi, octopus sushi, sea urchin sushi), Freshwater hairy crab, Seaweed, Tuna sushi, Mackerel, Canned Sardine and short chain omega-3 such as Omega-3 fortified food, Canola oil, Soybean oil, Omega-3 supplements, Flaxseed. We took their one month dietary intake and classified their intake, one day per month , 2-3 days per month, one day per week, 2-3 days per week, and their intake is calculated in serving, gram or any other amount.

Data on food consumption patterns were collected by using a pretested and interviewer administered questionnaire, the questionnaire was in English. Height/length of each participant was measured using a calibrated height- measuring board. Their heights were measured in duplicate and recorded to the nearest 0.1cm or in triplicate whenever the deviation between the first two measurements was > 0.5 cm. Weights were measured using a battery powered digital scale where participants wear light clothes, pockets emptied and no shoes. The weight scale was calibrated at least twice daily. The measurement were done twice and recorded to the nearest 0.1 kg.

3.7.2. Blood Sample Collection

The blood samples were collected by experienced nurses using sterile disposable needles from the veins of the upper arm after being cleaned by alcohol swaps to avoid contamination and all necessary safety measures was observed in the collection of blood samples. About 6 ml of blood was collected and placed in a trace-metal- free vacutainer tube. The collected blood was allowed to coagulate at room temperature and centrifuged in the field within an hour at 3000 rpm for 10 minutes. The plasma was separated and transferred into vials using a disposable plastic pipette (Eppendorf tubes 2 ml). The vials with plasma samples were labeled with identification codes and transported in ice and kept in -80^{o}C for further analysis at the Ethiopian Public Health Institute (EPHI).

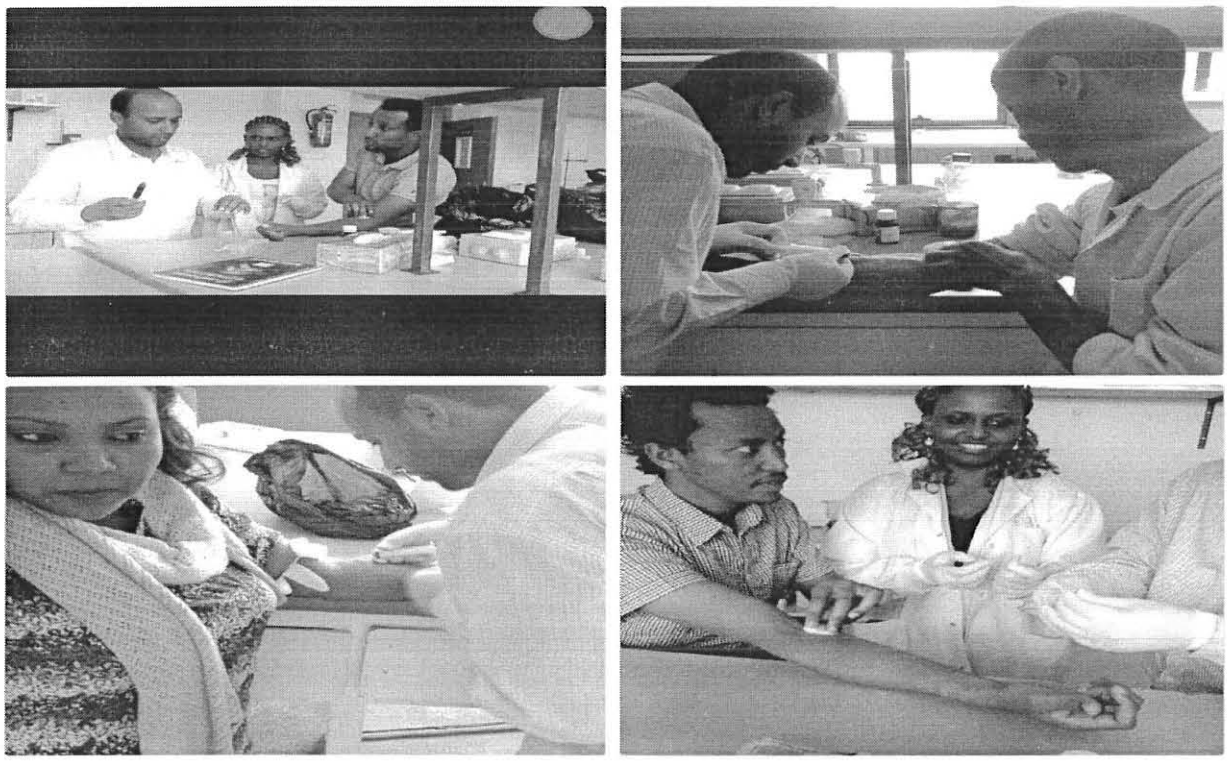


Figure 5. Blood Sample collection

3.8. Data quality assurance

To maintain data quality, data collectors were trained and they were selected based on educational level, work experience and familiarity (mostly nurses) with the work (most data collectors were nurses). Also, a 5% sample of pretest of the questionnaires was conducted on 10 individuals outside the study area to determine accuracy of responses, language clarity, appropriateness of data collection tool., Some modifications were made to the questionnaire on the basis of these findings. The collected data was reviewed and checked for omissions, readability of handwriting, completeness and consistency by the principal investigator and supervisor on a daily bases during the data collection.

3.9. Ethical clearance

Ethical clearance was obtained from Research Ethics Committee (REC) of the College of Natural and Computational Sciences, AAU. A letter of support was also obtained from the Addis Ababa Regional Health Bureau.

3.10. Plasma Lipid Analysis

Total cholesterol (TC), HDL-cholesterol (HDL-c), LDL-cholesterol (LDL-c) and triacylglycerol (TAG) were quantified using enzymatic, colorimetric method using COBAS INTEGRA 400 plus and 501 systems, fully automated closed system available at EPHI. Analytical methods were as per the manufacturer's instructions.

Materials: COBAS INTEGRA 400 plus and 501 systems fully automated closed system

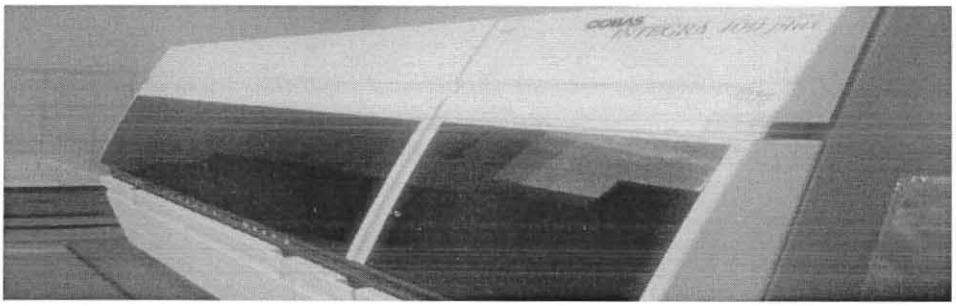


Figure 6. COBAS INTEGRA400 Plus fully automated cholesterol analyze

3.10.1. Total Cholesterol (TC)

Cholesterol is a steroid with a secondary hydroxyl group in the C3 position. It is synthesized in many types of tissue, but particularly in the liver and intestinal wall. Approximately three quarters of body cholesterol is newly synthesized and a quarter originates from dietary intake. Cholesterol assays are used for screening for atherosclerotic risk and in the diagnosis and treatment of disorders involving elevated cholesterol levels as well as lipid and lipoprotein metabolic disorders.

Reagents – working solutions

R1 PIPES buffer: 225 mmol/L, pH 6.8; Mg2+: 10 mmol/L; sodium cholate: 0.6 mmol/L; 4-aminophenazone: ≥ 0.45 mmol/L; phenol: ≥ 12.6 mmol/L; fatty alcohol polyglycol ether: 3 %; cholesterol esterase (Pseudomonas spec.): ≥ 25 µkat/L (≥ 1.5 U/mL); cholesterol oxidase (E. coli): ≥ 7.5 µkat/L (≥ 0.45 U/mL); peroxidase (horseradish): ≥ 12.5 µkat/L (≥ 0.75 U/mL); stabilizers; preservative.

Method: Testing TC was carried out through Enzymatic; Colorimetric method where cholesterol ester was cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids. Cholesterol oxidase then catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed effects the oxidative coupling of phenol and 4-aminoantipyrine to form a red quinine- imine dye. Absorbance was measured at 512/659 nm. COBAS INTEGRA analyzers automatically calculated the analyte concentration of each sample (Tietz 2001; Thomas 1998).

Cholesteryl ester + H_2O —(cholesterol esterase)→ cholesterol + fatty acid

Cholesterol+O_2 —(Cholesteroloxidase)→ cholest-4-en-3-one + H_2O_2

$2H_2O_2$+4-aminophenazone + phenol —(Peroxidase)→ 4-(p-benzoquinone- monoimino)-phenazone + 4 H_2O

3.10.2. High density lipoproteins (HDL)

This procedure provides instructions for performing in vitro test for the quantitative determination of the HDL cholesterol concentration in human serum and plasma on COBAS INTEGRA 400 plus and 501 systems.

High density lipoproteins (HDL) are responsible for the reverse transport of cholesterol from the peripheral cells to the liver. Here, cholesterol is transformed to bile acids which are excreted into the intestine via the biliary tract. Monitoring of HDL-cholesterol in serum is of clinical importance since an inverse correlation exists between serum HDL-cholesterol concentrations and the risk of atherosclerotic disease. Elevated HDL-cholesterol concentrations are protective against CHD, while reduced HDL-cholesterol concentrations, particularly in conjunction with elevated triglycerides, increase the cardiovascular risk.

Reagents: Working solution

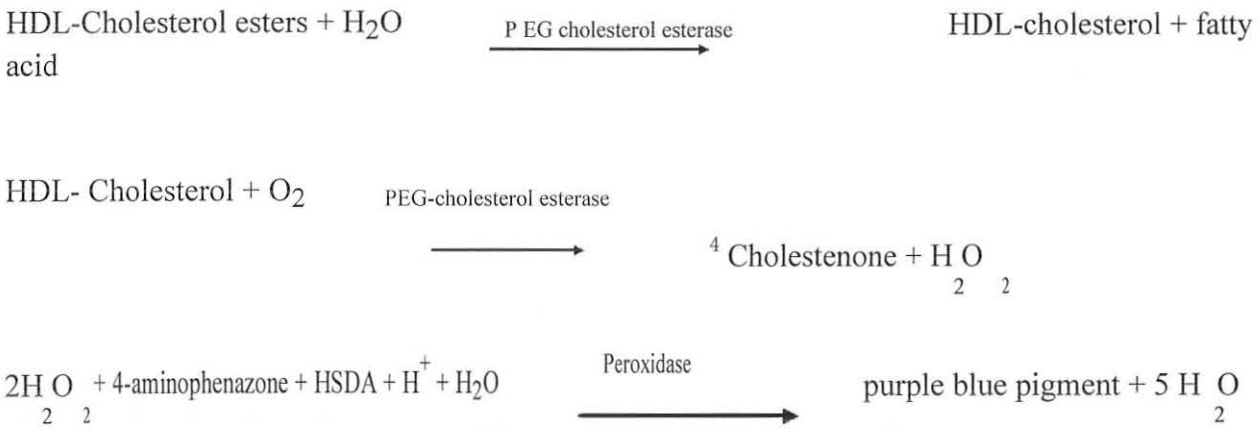
R1 HEPES buffer: 10.07 mmol/L; CHES;96.95mmol/L, pH 7.4; dextran Sulphate:1.5 g/L; magnesium nitrate hexahydrate:>11.7 mmol/L; HSDA:0.96mmol/L; ascorbate oxidase (Eupenicillium sp.,recombinant):>50µkat/L;peroxidase(horseradish):>16.7µkat/L; preservative

R 2 HEPES buffer: 10.07 mmol/L,pH 7.0; PEG-cholesterol esterase (Pseudomonas spec.): > 3.33µkat/L; PEG-cholesterol oxidase (Streptomyces sp.,recombinant:>127 µkat/L; peroxidase(horseradish):>333 µkat/L; 4-amino-antipyrine: 2.46 mmol/L;preservative.

Method: HDL-cholesterol was directly determined in the presence of VLDL, LDL AND chylomicronsbythe COBAS INTEGRA plus. No sample pretreatment step was required.

Homogenous enzymatic colorimetric assay: In the presence of magnesium ions and dextran sulphate, water soluble complexed with LDL, VLDL and chylomicrons were formed which were resistant to PEG- modified enzymes. The cholesterol concentration of HDL-cholesterol was determined by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups (approximately 40%). Cholesterol esters were broken down quantitatively into cholesterol and fatty acids by cholesterol esterase. In the presence of oxygen, cholesterol was oxidized by cholesterol oxidase to 4-cholestenone and hydrogen peroxide. The color intensity of the blue

quinoneimine dye formed is directly proportional to the HDL-cholesterol concentration. It is determined by measuring the increase in absorbance at 583 nm (Tietz 2001; Thomas 1998)



HSDA= sodium N-(2-hydrxy-3-sulfopropyl)-3,5-dimethoxyaniline

3.10.3. Low Density Lipoprotein LDL-Cholesterol

Low Density Lipoprotein (LDL) plays a key role in causing and influencing the progression of atherosclerosis and in particular, coronary sclerosis. LDLs are derived from VLDLs rich in triglycerides by the action of various lipolytic enzymes and are synthesized in the liver. The elimination of LDL from plasma takes place mainly by the liver parenchymal cells via a specific LDL receptor. Elevated LDL concentration in blood and an increase in their residence times, coupled with an increase in the biological modification rate, results in the destruction of the endothelial function and higher LDL-cholesterol uptake the monocyte/ macrophage system as well as by smooth muscle cells in vessel walls. The majority of cholesterol stored in atherosclerotic plaques originates from LDL. The LDL-cholesterol value is the most powerful clinical predictor among all of the single parameters with respect to coronary atherosclerosis. Therefore therapies focusing on lipid reduction primarily target the reduction of LDL-cholesterol which is then expressed in an improvement of the endothelial function, prevention of atherosclerosis and reducing its progression as well as prevention of plaque rupture.

This automated method for the direct determination of LDL-C took advantage of the selective micellarysolublization of LDL-C by a nonionic detergent and the interaction of suga r compound and lipoproteins (VLDL and chylomicrons). When a detergent was includedin the enzymatic method for cholesterol determinations (cholesterol esterase / cholesterol oxidase coupling reaction), the relative reactivities of cholesterol in the lipoprotein fractions increase in the order: HDL<chylomicrons<VLDL<LDL. In the presence of Mg^{2}, a sugar compound markedly reduces the enzymatic reaction of the cholesterol measurement in VLDL andchylomicrons. The combination of sugar compounds with detergent enables the selective determination of LDL-cholesterol in serum (Tietz 2001; Thomas 1998).

Reagents - working solutions

R1 MOPS (3-morpholinopropane sulfonic acid) buffer: 20.1 mmol/L, pH 6.5; HSDA: 0.96 mmol/L; ascorbate oxidase (Eupenicillium spec., recombinant): ≥ 50 µkat/L; peroxidase (horseradish): ≥ 167 µkat/L; preservative

R2 MOPS (3-morpholinopropane sulfonic acid) buffer: 20.1 mmol/L, pH 6.8; MgSO4·7H2O: 8.11 mmol/L; 4-aminoantipyrine: 2.46 mmol/L; cholesterol esterase (Pseudomonas spec.): ≥ 50 µkat/L; cholesterol oxidase (Brevibacterium spec., recombinant): ≥ 33.3 µkat/L; peroxidase (horseradish): ≥ 334 µkat/L; detergent; preservative

Method: Homogenous enzymatic colorimetric assay was used;

LDL-Cholesterol esters $+H_2O$ $\xrightarrow{\text{deteregent / cholesterol esterase}}$ cholesterol+ free fattyacid(selective micellarysolublization)

LDL- Cholesterol + O_2 $\xrightarrow{\text{Cholesterol oxidase}}$ ^{4} Cholestenone + H_2O_2

In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ4-cholestenone and hydrogen peroxide.

$2H_2O_2$ + 4-aminoantipyrine + HSDA + H^{+} + H_2O $\xrightarrow{\text{peroxidase}}$ Purple blue pigment + 5 H_2O

HSDA= sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline

In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-aminoantipyrine and HSDA to form a purple-blue dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically.

3.10.4. Triglycerides

This procedure provides instructions for performing the in vitro test for the quantitative determination of triglyceride concentration in human serum and plasma on COBAS INTEGRA 400 plus and 501 systems.

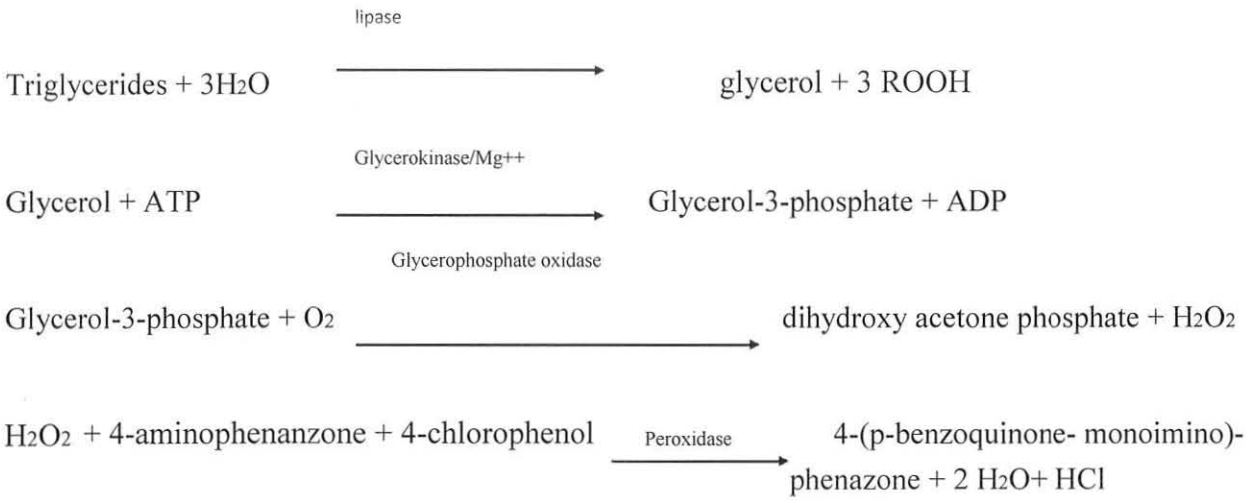
Triglycerides are esters of the trihydroxy alcohol, glycerol, with 3 long-chain FAs. They are partly synthesized in the liver and partly ingested in food. The determination of triglycerides is utilized in the diagnosis and treatment of patients having diabetes mellitus, nephrosis, liver obstruction, lipid metabolism disorders and numerous other endocrine diseases.

Method: This method was based on the work by Wahelfeld using a lipoprotein lipase from microorganisms for the rapid and complete hydrolysis of triglycerides to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced then reacted with a-aminophenanzone and 4-cholorophenol under the catalytic action of peroxidase to form a red dyestuff (Trinder endpoint reaction). The color intensity of the red dyestuff formed was directly proportional to the triglyceride concentration and measured photometricly (Tietz 2001; Thomas 1998).

Reagents - working solutions

R1 PIPES buffer: 50 mmol/L, pH 6.8; Mg2+: 40 mmol/L; sodium cholate: 0.20 mmol/L; ATP: ≥ 1.4 mmol/L; 4-aminophenazone: ≥ 0.13 mmol/L; 4-chlorophenol: 4.7 mmol/L; lipoprotein lipase (Pseudomonas spec.): ≥ 83 µkat/L; glycerokinase (Bacillus stearothermophilus): ≥ 3 µkat/L;

glycerol phosphate oxidase (E. coli): ≥ 41 µkat/L; peroxidase (horseradish): ≥ 1.6 µkat/L; preservative



3.11. Statistical analysis

Statistical analysis was undertaken using SPSS version 21. Within group differences were determined by independent sample t-test. Means and SD are included. *P* values <0.05 were considered significant. Frequency of dietary intake was examined by descriptive statistics.

CHAPTER FOUR

4. RESULT AND DISCUSSION

4.1. General characteristics of study population

A total of 40 volunteers, 20 male and 20 female, who were young and educated and who attended at the Center for Food Science and Nutrition at Addis Ababa University (graduating class of 2017) participated in this study. The mean age of the respondents was 29.28 years with minimum age of 22 and a maximum of 36. The mean age for females was 29.20 years and for males was 29.35 years. The average weight and height was 62.28 ± 1.55 kg and 1.67 ± 0.137 m respectively with average body mass index (BMI) of 21.75±0.64 kg/m ^{2} and 22.65±0.826 kg/m^2 (Table 5).

There was a significant difference between male and female participants in their height and weight *p-value* ≤ 0.05; this difference was expected since there is a physical difference between males and females but there was no significance difference in their BMI *p-value* ≥ 0.05. From N=40 participants 55% were at normal BMI level, 17% were overweight of which 15% male and 20% female. From the total N=40, 2.5% class 1 was classified as obese.

Table 5.General characteristics of study population

	Total	Males	Females	P-value
Sample size	N=40	N=20	N=20	
Age	29.28	29.35	29.20	0.87
Weight (kg)	62.28 ± 1.55	65.44±2.17	59.1±2.0	0.041*
Height (m)	1.67 ± 0.137	1.73±0.016	1.61±0.01	0.00*
BMI (kg/m^2)	22.2±0.52	21.75±0.64	22.65±0.826	0.39

- Data represented as mean ± standard deviation
- A *P-value* < 0.05, determined by independent sample t-test for differences between males and females, was considered statically significant

Obesity is one of the risk factor for CVD (Carl *et al.*, 2009). In our case only one participant (which is 2.5% of sample) was categorized at class-1obesity and 80% were categorized as normal and underweight BMI (Table 6). A research study conducted in Taiwan, showed that those with low HDL-C and normal/underweight had higher risk of mortality from coronary artery disease (Lin *et al.*, 2013).

Higher BMI is inversely associated with HDL and directly associated with TG. Body mass index (BMI) showed no significant association with LDL. Although the association between BMI and both HDL and TG may be explained by insulin resistance, the lack of a significant association between BMI and LDL remains an unexpected finding that requires further investigation (Shamai, *et al.*, 2011).

Table 6. Body status of the participants

• Body Status	BMI Cutoff pointkg/cm^2	Total N=40	Male N=20	Female N=20
Underweight	15-19.9	10(25%)	6 (30%)	4(20%)
Normal Weight	20-24.9	22(55%)	11 (55%)	11 (55%)
Overweight	25-29.9	7(17.5%)	3 (15%)	4(20%)
Class 1 Obesity	30-34.9	1(2.5%)	0	1(5%)
Class 1 Obesity	35-39.9	0	0	0
Class 1 Obesity	40	0	0	0

4.2. Intakes of Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA)

Fish and fish products like omega-3 (n-3) supplements are the healthiest diet because it is rich in long chain polyunsaturated fatty acid (LC PUFA) such as Eicosapentaenoic Acid (EPA, 20:5n-3) and docosahexanoic acid (DHA, 22:6n-3) (Kris-Etherton, et al. 2000). The study assumes the study groups have the potential to differentiate between healthy and unhealthy diet s.

We classified the intake of fourteen food items and the intake of n-3 supplement listed on food frequency questionnaire (FFQ) which are rich in LCPUFA such as EPA and DHA into high (those who have taken these food items more than or equal to two times a week) and to low (those who have taken the food items less than two times a week) (Kris-Etherton *et al.*, 2002; Kromhout, *et al.*,1985). According to the Australian Heart Foundation to lower the risk of coronary heart disease (CHD), consumption of about 500 mg per day of combined DHA and EPA is recommended through a combination of the two or three serves (150 g/serve) of oily fish per week, fish oil capsules or liquid food and drinks enriched with marine n-3 PUFA and also to consume at least 2 g per day of ALA (David *et al.*, 2008).

Table 7. Sources of Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA)

Food	High (>1/week)			Low (<1/week)		
	Total (N=40)	Male (N=20)	Female (N=20)	Total (N=40)	Male (N=20)	Female (N=20)
Canned Tuna	3	2	1	37	18	19
Lobster	0	0	0	40	20	20
Fresh Water Hairy Crab	0	0	0	40	20	20
Cat Fish (Ambaza)	0	0	0	40	20	20
Salmon	0	0	0	40	20	20
Nile Tilapia (Korosso)	0	0	0	40	20	20
Nile Perch (NechAssa)	0	0	0	40	20	20
Tuna Sushi	0	0	0	40	20	20
Sea Food Sushi	0	0	0	40	20	20
Seaweed	0	0	0	40	20	20
Coral Fish	0	0	0	40	20	20
Mackerel	0	0	0	40	20	20
Canned Sardine	1	0	1	39	20	19
Omega 3 Fortified milk, bread egg	0	0	0	40	20	20
Omega3Supplement	0	0	0	40	20	20

From the selected fourteen food items and one item from the n-3 supplement which are the source of LCPUFA we classified the intake into: low (those who have taken the source less than once per week) and high (those who have taken the sources more than or equal to two times a week). From the total participants(N=40) the frequency of intake of canned tuna was 7.5% high and 92.5% low and the frequency of canned sardine was 2.5% high and 97.5% low, and frequency of intake of the rest of the sources such as lobster, freshwater hairy crab, cat fish (Ambaza) Nile Tilapia, (Korosso), Nile Perch (NechAssa), tuna sushi, sea food sushi, seaweed, Coral Fish, mackerel, n-3 fortified milk bread egg and n-3 supplement was 100% low (Table 7).

Despite these findings it is necessary to recognize that, there are other factors like age, smoking, sex, and physical activity which commonly affect blood levels of EPA+DHA (Block *et al.*, 2008; Flock *et al.*, 2013).

Blood levels of EPA+DHA are variable across the globe, with most of the countries and regions of the world having levels that are considered low to very low (Renata, *et al.,* 2014). Based on the results from the FFQ it is possible to say that this study group showed low to very low EPA+DHA blood levels. Blood levels of EPA+DHA have long been known to correspond to dietary intakes of EPA+DHA (Kobayashi *et al.*, 2001; Bjerve *et al.*, 1993; Lands, 1995).

Japanese people are among the most important consumers of fish in the world (66.2 kg/cap in 2003, Source FAO (Mariojouls 2006). FA compositions of plasma EPA =110 μg/mL, DHA=191 μg/mL, EPA + DHA=301 μg/mL are considered as high (Konagai, *et al.,* 2013). The typical North American diet provides about 1–3 g of ALA per day but only 0.10–0.15 g of EPA plus DHA per day (Raper *et al.*, 1992; Kris-Etherton *et al.*, 2000). Very low blood levels were observed in North America, Central and South America, Europe, the Middle East, Southeast Asia, and Africa (Stark, et al. 2016) and EPA =11 μg/mL, DHA=39 μg/mL, EPA+DHA=49 μg/mL are considered as low (Van der Pols *et al.*, 2011).

4.3. Intake of Alpha Linolenic Acid (ALA)

Food items such as soya bean oil, canola oil, flaxseed oil and rapeseed oil are rich sources of short chain n -3 fatty acids called alpha linolenic acid (ALA,18:3n-3). ALA is an EFA, meaning that human body can't make it, so must be supplied in the diet. In this research we classified the intake into low (those who have taken the sources of ALA less than ones per week) and high (those who have taken the sources of ALA more than or equal to two times a week). From the total participants (N=40) the frequency of intake of soya bean oil was 80% low and 20% high, flaxseed 92% low and 8% high and frequency of intake of canola oil was 100% low.(Table 8).It is not clear whether ALA prevents recurrent coronary events, although there are trends suggesting that this may be the case. Due to the paucity of high-quality conclusive data, the Heart Foundations of Australia recommend an intake of at least 2 g/day of ALA (NHFA 1999). Some epidemiological studies show that a high intake of ALA is associated with a low rate of CHD (de Lorgeril and Salen, 2004). It is not clear whether this reflects a specific protective effect of ALA or a surrogate effect of healthy eating patterns (Djousse, 2003). A 2004 meta-analysis of five prospective cohort studies and three clinical trials in patients with CHD found that high ALA intake is associated with a 21% reduction in fatal CHD, but this did not reach clinical significance (Brouwer *et al.*, 2004). A 2005 18-year follow-up from the Nurses Health Study 200 suggested that, after accounting for coronary risk factors and other FA, the intake of ALA was inversely associated with the risk of sudden cardiac death especially in women with high ALA intake. The inverse association between ALA and sudden cardiac death was linear and remained significant in women who also consumed high amounts of marine LC n-3 PUFA. This inverse association between ALA intake and CHD may not be as strong in men (Brouwer, Katan and Zock 2004), (Brouwer *et al.*, 2004). Human body can convert some ALA into EPA and then to DHA, but only in very small amounts. Therefore, getting EPA and DHA from foods (and dietary supplements is the only practical way to increase levels of these n-3 FA in human body.

Table 8. Sources of Alpha Linolenic Acid (ALA)

	High (>1/week)			Low (<1/week)		
	Total (N=40)	Male (N=20)	Female (N=20)	Total (N=40)	Male (N=20)	Female (N=20)
Soya bean oil	8	4	4	32	16	16
Canola oil	0	0	0	40	20	20
Flaxseed	3	1	2	37	19	18

4.4. Lipid Profile

The mean Cholesterol level was 157.9gm/dL±5.6, Low Density Lipoprotein (LDL) 98.3g/dL±4.71, Triglycerides 102.4g/dL±5.6 and High Density Lipoprotein (HDL) was 44.7g/dL±1.31. A P-value < 0.05, determined by independent sample t-test for differences between males and females, was P=0.045which indicates there is a significance difference between males and females in HDL cholesterol level (Table 9).

Table 9. Lipid Profile Results

Lipid Profile	Total subjects (N=40) g/dL	Male (N=20) g/dL	Female (N=20) g/dL	P-value
Cholesterol (CHO)	157.9±5.6	158.3±9.14	157.5±6.5	0.28
Low Density Lipoprotein (LDL) g/dL	98.3±4.71	98.5±8.3	94.4±7.8	0.46
High Density Lipoprotein (HDL) g/dL	44.7±1.31	38.4±1.65	48.0±1.9	0.045*
Triglycerides g/dL	102.4±5.6	171.3±8.3	103.7±7.8	0.93

- Data represented as mean ± standard deviation
- A *p-value* < *0.05*, determined by independent sample t-test for differences between males and females, was considered statically significant

Lipid profiles such as total cholesterol, Triglyceride, LDL & HDL cholesterol. From N=40, the average cholesterol level was 157.9g/dL which is < 200g/dL which indicates the desirable cholesterol level & among N=40, 36 of them were at desirable cholesterol level & 3 at border line and 1 with high cholesterol level (NCEP May 2001).

The average LDL level was 98.35g/dL which is also <100g/dL which indicates the normal LDL level & from N=40 individuals 57.5% at normal level, 30% at low border line, 7.5% at high borderline and 5% at high LDL level (NCEP, 2001).

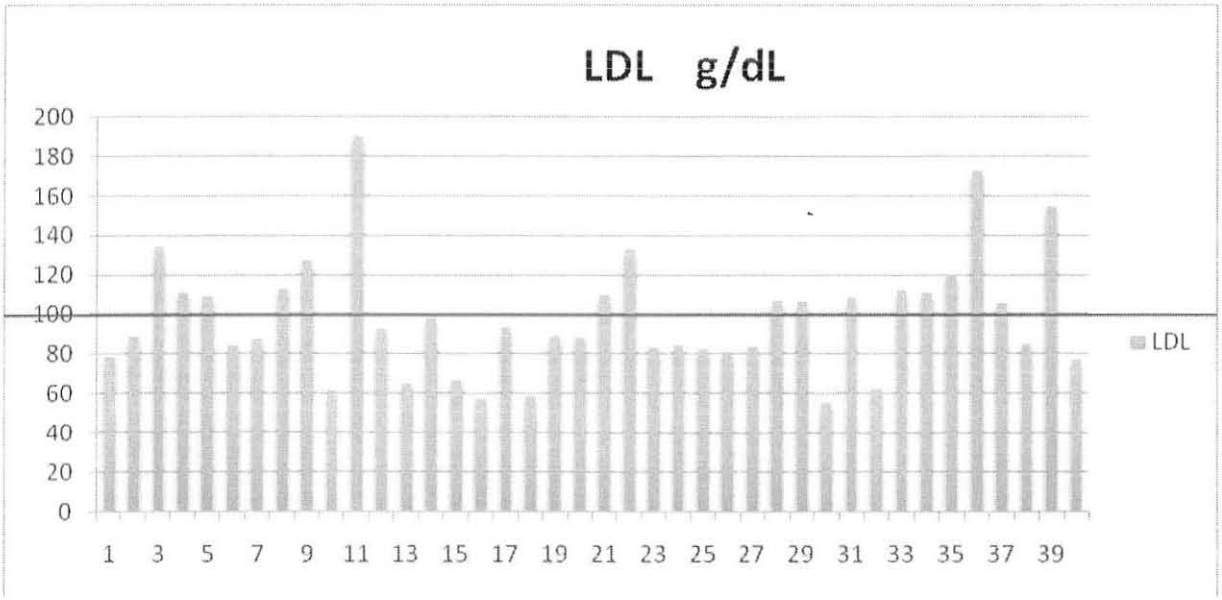


Figure 7. LDL cholesterol distribution, (N=40)

In LDL level from N=40 individuals 23 of them which is 57.5% were at normal level, but "normal" levels of circulating fatty acids are yet to be defined. The lack of established reference ranges for saturated, *trans*, monounsaturated and polyunsaturated FA has resulted in the poor interpretability of human research (Abdelmagid *et al.*, 2015; Baum, 2013).

The average HDL of N=40 individuals was 44.7g/dL which is at satisfactory level but the average HDL of males N=20 was 38.4g/dL is at a low level, and those of females was N=20 was 48g/dL which is at satisfactory level.

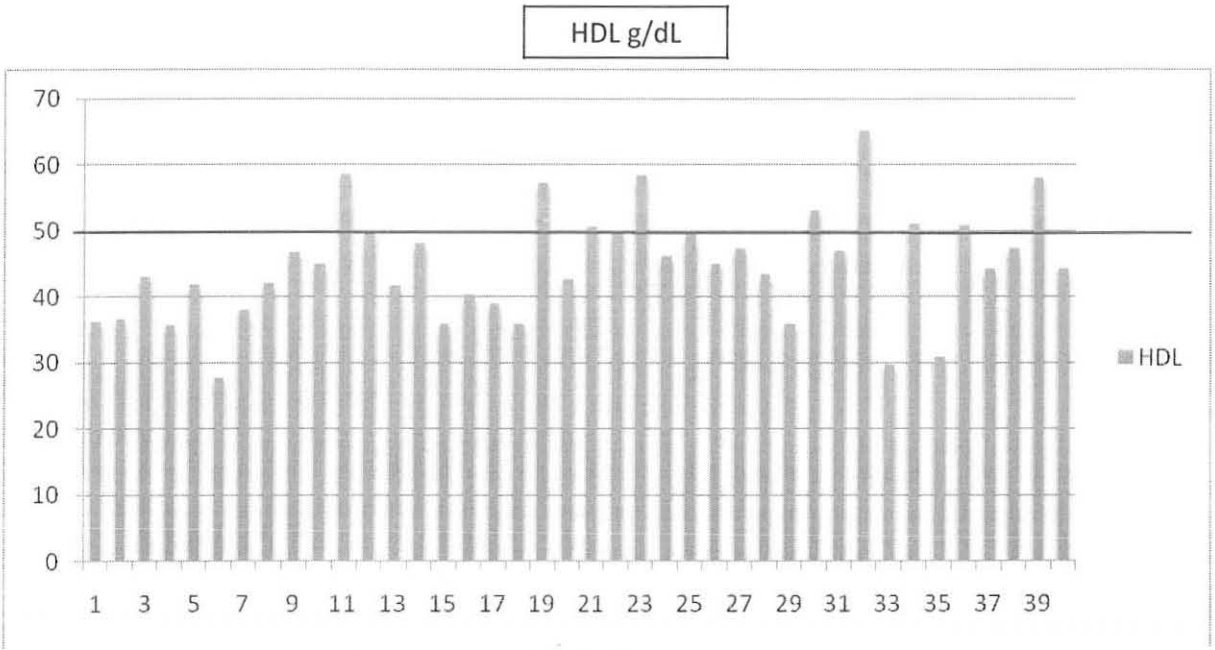


Figure 8. HDL cholesterol distribution

From the participants N=40, the intake of diets rich in n-3 fatty acids was low and so was the HDL level which at 97.5% was not considered to be a desirable level (Table 10). Daily fish oil supplement providing 2.1g docosahexaenoic acid (DHA, 22:6 n-3) and 0.8g eicosapentaenoic acid (DHA, 20:5n-3) for 6 weeks was taken. The proportion of EPA and DHA in plasma, erythrocytes, leucocytes and platelet phospholipids was increased by the supplement. Plasma concentrations of triacylglycerol and Very-Low-Density-Lipoprotein-cholesterol were lowered and those of HDL were increased (Hinds 1992).

Table 10. Distribution of Cholesterol among the participants (NCEP May 2001)

Health indicator	Mean g/dL	Status	Cut off g/dL	Subjects Total N=40	Male N=20	Female N=20
Cholesterol	157.92±5.6	Desirable	< 200	36	19	17
		Border line	200 < CHO < 240	3	0	3
		High	≥ 240	1	1	0
LDL	98.35±4.71	Normal	< 100	23	14	9
		Low border line	100 < LDL <129	12	4	8
		High borderline	130 < LDL <159	3	1	2
		High	160 < LDL <189	2	1	1
		Excess	≥ 190	0	0	0
HDL	44.75±1.31	Too low	<40	11	8	3
		Satisfactory	40<HDL<60	28	12	16
		Desirable	≥60	1	0	1
Triglycerides	102.46±5.6	Normal	< 150	36	17	19
		Borderline high	150 < TRG <199	3	3	0
		High	200 < TRG <499	1	0	1
		Excess	≥500	0	0	0

From the total participants N= 40 the average triglyceride level was 102.46 g/dL, according to National Education Cholesterol program, triglyceride level which is < 150 g/dL is considered as "normal" triglyceride level(Table 10). The average triglyceride level on male is 171.3 g/dL which lies on the borderline "high" triglyceride level. From the participants N=40, 36 had triglyceride levels at the "desirable" TG level; having a desirable TG does not keep them out of the risk zone since their HDL level was low, due to n-3 FA lower levels of triacylglycerol and increased levels of HD. (Dewailly, et al. 2003).Marine n-3 PUFA supplementation of 1,000–4,000 mg/day decreases TG levels by 25–30% and increases HDL-C levels by 1–3%. (NHFA, 2009; Harris, 1997; Buckley *et al.*, 2007; Balk *et al.*, 2006).

CHAPTER FIVE

5. Conclusion and Recommendations

5.1 Conclusion

Our population groups were young of the 40 individuals the average age was 29.3 and educated. These students were expected to know the health benefits of the food items that we had listed in the questionnaires because they are either PhD or MSc students at Addis Ababa University's Center for Food Science and Nutrition.

The significant difference between males and females in height and weight was to be expected since there are physical differences between the sexes, but there was no significant difference in their BMI; the average BMI was 22.2±0.52kg/m^2which is the desirable level.

As we have seen from this study from N=40 individuals, N=20 females and N=20 males, the intake of fish and fish products (except canned tuna and sardine) the intake of other fish items and n-3 supplement was 100% lower than the recommended levels to minimize CVD. Even if these groups are considered knowledgeable, their intake of a supposed "healthy" diet was poor, even from the commercially available fishes like Nile Tilapia, Nile perch and cat fish. These locally available fishes contain sufficient content of long chain fatty acid levels to meet the recommended dietary intake levels, for example Nile Tilapia (Korroso) contains 1.92mg/g EPA and 11.55 mg/g DHA; cat fish (Ambaza) contains 2.6mg/g EPA and 4.7mg/g DHA and Nile Perch (Nech Assa) contains 0.64mg/g EPA and 3.62mg/g DHA respectively.

Using as a reference the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) it was possible to draw these conclusions. From the total population, 90% of the population was at desirable total cholesterol and triglyceride level, which should be less than 200g/dL and 150g/dL respectively.

Low Density Lipoprotein (LDL), which is considered as "bad cholesterol"; from the total population 57.5% were at normal LDL cholesterol level but the remaining 42.5% are at high and borderline high LDL cholesterol level which is mainly caused by consumption of *trans* and saturated fat.

High Density Lipoprotein (HDL) which is made in liver and helps to remove excess cholesterol from cells, tissues and from plaque in blood vessels by returning it to liver to remove it from the body; in our population 97.5% were not at the desirable HDL cholesterol which was less than 60mg/d..

5.1. Recommendation

- Non-communicable diseases like cardiovascular disease (CVD) are a risk even for developing countries like Ethiopia so including a healthy diet like fish on a daily basis can minimize the risk of CVD.
- Raised levels of Low Density Lipoprotein (LDL) are mainly caused by increased intake of saturated acid and trans- fat; therefore by replacing saturated and *trans*-fat with Long chain polyunsaturated fatty acids (LCPUFA) can minimize these raised levels of LDL.
- A decreased level of High Density Lipoprotein (HDL) in this young and educated population indicates a relatively low frequency of having healthy dietary intakes.
- Currently, Ethiopia has set no guideline values of dietary recommended levels of plasma phospholipids LC n-3 PUFA concentrations for men and women, therefore an effort to produce baseline data on the nutritional benefits associated with fish consumption in the study could contribute to setting such recommended levels.
- The consumption of fish products in theses young and educated population was low it might be the low availability in the market, government and other stakeholders should be encouraged to work harder in this area to raise domestic market production.

- Even though several trials have been undertaken we couldn't analyze blood plasma LCPUFA therefore the determination of some findings will be necessary to ensure that these meet the dietary requirements and commercial specifications.
- There are some limitations in this study; one of the limitations of this study was the sampling time: some of the participants consume fish at the fasting season and some did not. Samples were collected in both seasons but we missed those who did not consume fish during fasting season because most of them prefer to consume fish when the fasting season is over; instead they rather prefer consuming meat, poultry and dairy products.

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Appendix

Title: Awareness of Intake of Diets Rich in Long Chain Omega–3 Fatty Acids to Decrease Risk Factor for Cardiovascular Disease in Addis Ababa Food Science and Nutrition Students

Name of Principal Investigator: Bethelhem Taye

Name of the Organization: Addis Ababa University

Name of the sponsor: Self

Information sheet and consent form prepared for students who are attending at center for food science and nutrition prior to the study to participate in this research project.

Introduction: - this information sheet and consent form is prepared with the aim of assessing the food frequency diversity of students who are attending at center for food science and nutrition.

The research group includes the principal investigator and professional nurses

Purpose of the study: -To evaluate the relationship between blood plasma lipid profile and long chain omega – 3 fatty acid of food science and nutrition students at Addis Ababa University

Procedure: - the study involves students who are attending at center for food science and nutrition. You are selected to be one of the study participants and if you are willing to participate. We are so happy finally you are kindly requested to give your genuine response in the questionnaires. Benefits, risk and/or discomfort: - by participating in this research project you may feel some discomfort in wasting your time (a maximum of 30 minutes). However, your participation is definitely important to assess the magnitude of non-communicable diseases. There is no risk or direct benefit in participation in this research project. Incentives/payments for participating: - you will not provide any incentives or payment to take part in this project.

Confidentiality: - we will keep the confidentiality by using codes instead of any personal identifiers and is meant only for the purpose of the study. Right to refusal or withdraw: - you will not be forced to participate; you have the full right to refuse and have the right to discontinue the process at any point in this research.

Person to contact: - this research project was reviewed and approved by the ethical committee of the Addis Ababa University. If you have any question you can contact any of the following individuals and you may ask at any time you want.

Name: Bethelhem Taye Tele: + 251-912010426

E- mail: adthelast@gmail.com

If you have read the document and you have been given the chance to ask any questions now or at a later time or if the document has been read and explained to you agree to be in this study, may I continue?

Yes ----------

No ----------

Demographic and socio-economic Characte ristics

Questions	Responses and coding category
ID No.	
Sex	
Age	
Weight	
Height	

Food Fre quency Questionnaire

Interviewer: ID: □□

Sample Person ID: □□□□
Date of Interview: □□-□□-20□□
dd m m y y

Time started □□:□□ am / pm Time ended □□:□□ am / pm

Inte rvie wer observation form

Did you or the respondent have difficulty with this intake interview?
(0) No (1) Yes
What was the reason for this difficulty?

Do you take any cardiac medication? Yes ___ No __

Do you smoke cigarette? Yes ___ No __

General Introduction:

I would like to ask you about eating patterns over the past one month, please tell me over the past one month did you eat each of the foods I ask you. If you didn't eat any of the food over the past one month, we will go to the next question; if you ate more than one time in the past one month, please tell me how often you ate it and how much you ate each time. There is no right/wrong answer for each question, if you want to make any changes be sure to let me know. If you don't have any question, could we start now?

Over the past 1month, how often did you eat (food)?

(00) Never

(1) 1 time per month

(2) 2-3 times per month

(3) 1 time per week

(4) 2 times per week

(5) 3-4 time per week

(6) 5-6 times per week

(7) 1 time per day

Over the past one month	Over the past one month
1. How often did you eat Canned Tuna? (00) NEVER (Go to question 2) 01 02 03 04 05 06 07	4. How often did you eat Cat Fish (Ambazza) (00) NEVER (Go to question 5) 01 02 03 04 05 06 07
Each time you ate Canned Tuna, how much did you usually eat? (1) _____ serving (2) _____ gram (3) _____ other amount	Each time you ate Cat Fish (Ambazza), how much did you usually eat? (1) _____ serving (2) _____ gram (3) _____ other amount
2. How often did you eat Nile Tilapia (Korosso) (00) NEVER (Go to question 3) 01 02 03 04 05 06 07	5. How often did you eat Salmon (00) NEVER (Go to question 6) 01 02 03 04 05 06 07
Each time you ate Nile Tilapia (Korosso), how much did you usually eat? (1) _____ serving (2) _____ gram (3) _____ other amount	Each time you ate Salmon, how much did you usually eat? (1) _____ serving (2) _____ gram (3) _____ other amount
3. How often did you eat Nile Perch (Nech Assa) (00) NEVER (Go to question 4) 01 02 03 04 05 06 07	6. How often did you eat Lobster (00) NEVER (Go to question 2) 01 02 03 04 05 06 07
Each time you ate Nile Perch (Nech Assa), how much did you usually eat? (1) _____ serving (2) _____ gram (3) _____ other amount	Each time you ate Lobster, how much did you usually eat? (1) _____ serving (2) _____ gram (3) _____ other amount

Over the past one month	Over the past one month
7. How often did you eat coral fish (e.g., hump head wrasse, potato grouper, tiger grouper, and high fingrouper)? (00) NEVER (Go to question 2) 01 02 03 04 05 06 07	10. How often did you eat other seafood sushi (other than fish sushi) (e.g., whelk sushi, octopus sushi, sea urchin sushi)? (00) NEVER (Go to question 11) 01 02 03 04 05 06 07
Each time you ate Coral Fish, how much did you usually eat? (1) _____ serving (2) _____ gram (3) _____ other amount	Each time you ate seafood sushi, how much did you usually eat? (1) _____ serving (2) _____ gram (3) _____ other amount
8. How often did you eat freshwater hairy crab (00) NEVER (Go to question 9) 01 02 03 04 05 06 07	11. How often did you eat seaweed (00) NEVER (Go to question 12) 01 02 03 04 05 06 07
Each time you ate freshwater hairy crab, how much did you usually eat? (1) _____ serving (2) _____ gram (3) _____ other amount	Each time you ate seaweed, how much did you usually eat? (1) _____ serving (2) _____ gram (3) _____ other amount
9. How often did you eat tuna sushi? 00) NEVER (Go to question 2) 01 02 03 04 05 06 07	12. How often did you eat Mackerel 00) NEVER (Go to question 13) 01 02 03 04 05 06 07
Each time you ate tuna sushi, how much did you usually eat? (1) _____ serving (2) _____ gram (3) _____ other amount	Each time you ate Mackerel, how much did you usually eat? (1) _____ serving (2) _____ gram (3) _____ other amount

Over the past one month	Over the past one month
13. How often did you eat Canned Sardine? (00) NEVER (Go to question 14) 01 02 03 04 05 06 07	16. How often did you take Canola oil? (00) NEVER (Go to question 17) 01 02 03 04 05 06 07
Each time you ate Canned Sardine, how much did You usually eat? (1) _____ serving (2) _____ gram (3) _____ other amount	Each time you took Canola oil, how much did You usually take? (1) _____ serving (2) _____ gram (3) _____ other amount
14. How often did you eat omega-3 fortified food (00) NEVER (Go to question 15) 01 02 03 04 05 06 07	17. How often did you take Soybean oil? (00) NEVER (Go to question 18) 01 02 03 04 05 06 07
Each time you ate omega-3 fortified food, how much did you usually eat? (1) _____ serving (2) _____ gram (3) _____ other amount	Each time you took Soybean oil, how much did you usually take? (1) _____ serving (2) _____ gram (3) _____ other amount
15. How often did you take omega-3 supplements (00) NEVER (Go to question 2) 01 02 03 04 05 06 07	18. How often did you take Flaxseed? (00) NEVER (Go to question 19) 01 02 03 04 05 06 07
Each time you tookomega-3 supplements, how much did you usually take? (1) _____ serving (2) _____ gram (3) _____ other amount	Each time you took Flaxseed, how much did you usually take? (1) _____ serving (2) _____ gram (3) _____ other amount

Thank you!!!

Declaration

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

Name: Bethelhem Taye

Signature _____

Date _____

The thesis has been submitted with approval as supervisors

Dr. Ashagrie Zewdu (Advisor)

Signature _____

Date _____

Dr. Hintsa Mateos (Co. Advisor)

Signature _____

Date _____