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„Assessment of Essential Fatty Acids Status in Different Blood Components in Austrian Adults "

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AA	Arachidonic Acid
5-HETE	5- Hydroxyeicosatetraenoic Acid
ALA	A-Lionlenic Acid (α-linolenic)
AMDR	Acceptable Macronutrient Distribution Range
BF3	Boron Trifluride
BMI	Body Mass Index
CAD	Coronary Artery Disease
CE	Plasma Cholesterol Ester
CHD	Coronary Heart Disease
Chol	Cholesterol
CNS	Central Nervous System
CoA	Acetyl Coenzyme A
COX	Cyclooxygenases
COX-1	Cyclooxygenases 1
COX-2	Cyclooxygenases 2
CV	Coefficient Of Variation
CVD	Cardiovascular Disease
DGLA	Dihomo- γ -Linolenic Acid
DHA	Docosahexanoic Acid
DPA	Docosapentaenoic Acid
DPAn-3	Docosapentaenoic Acid
E%	Energy
EDTA	Ethylenediaminetetraacetic Acid
EFA	Essential Fatty Acids
EFAD	Essential Fatty Acids Deficiency
EPA	Eicosapentaenoic Acid
ESA	Eicostrienoic Acid
FA	Fatty Acid
FAME	Fatty Acid Methyl Esters
FAO	The Food And Agriculture Organization
FDA	Food And Drug Administration
FFQ	Food Frequency Questionnaire
FID	Flame Ionization Detector
GC	Gas Chromatography
GI	Gastrointestinal
GLA	γ -Linolenic Acid

HCV	Hepatitis C Virus
HDL	High Density Lipoprotein
HIV	Human Immunodeficiency Virus
HLCPUFA	High Long Chain Poly Unsaturated Fatty Acid
HUFA	Highly Unsaturated Fatty Acid
IBD	Inflammatory Bowel Disease
IL-1	Interleukin 1
IUPAC	International Union of Pure and Applied Chemistry
LA	Linoleic Acid
LC PUFA	Long Chain Polyunsaturated Fatty Acid
LCPn-3	Long Chain n-3 Polyunsaturated Fatty Acid
LCPn-6	Long Chain n-6 Polyunsaturated Fatty Acid
LCSFA	Long Chain Saturated Fatty Acid
LDL	Low Density Lipoprotein
LTB4	Leukotriene B4
LTs	Leukotrienes
MUFA	Mono Unsaturated Fatty Acids
NEFA	Non- Essential Fatty Acids
OA	Oleic Acid
PGE_2	Prostaglandin E2
PGE_2	Prostaglandin E2
PGs	Prostaglandins
PHVO	Partially Hydrogenated Vegetable Oil
PL	Plasma Phospholipids
PUFA	Poly Unsaturated Fatty Acid
RBC	Red Blood Cells
SCD	Sudden Cardiac Death
SD	Standard Deviation
SFA	Saturated Fatty Acid
TFA	Trans Fatty Acid
TG	Triglycerides
TLC	Thin Layer Chromatography
TNF	Tumour Necrosis Factor
TXs	Thromboxanes
USFA	Unsaturated Fatty Acids
WB	Whole Blood
WHO	World Health Organization

1. Introduction and objectives

The nutritional needs and habits have been changed widely during the last decades especially the western diet. Food variety and availability make the individuals' choices become more complicated. This would reflect the gradient in nutritional habits. Populations that have better access to processed and market foods differ in their fat and fatty acids intakes from other populations that rely on their traditional food types.

The eating patterns have been changed widely as a result of the increased in the living standards, industrialization, urbanization and the market globalization. However, those patterns have changed to be unhealthier dietary habits combined with less physical activities which make the energy balance goes to the overweight side (i.e. obesity) and nutrition related diseases (Ogura et al., 2010).

That was first noticed in 1972 in Greenland Eskimos population when compared with Danish population, they had lower levels of serum cholesterol (Chol), low density lipoprotein (LDL) and triglycerides (TG) with a low myocardial infarction rate (Amiano et al., 2001). Cree and Inuit nations still eat large quantities of fish and marine products (i.e. sea food) from the traditional food type which makes their n-3 fatty acids concentration one of the highest grade among other populations (Dewaillya et al., 2003).

Establishing the nutritional requirement values to support health and well-being of individuals and populations have and still are topics that require regular scientific updates. Constructing taxonomies and scientific categories of such requirements is considered to be very complex as they are adjusted with age and physiological status. In 2008, the third meeting of the Food and Agriculture Organization / World Health Organization (FAO/WHO) experts was held on fat in human nutrition (Burlingame et al., 2009).

Recent publications have addressed the impact of fat and fatty acids on health, their role in the body are somehow clear. Fat and fatty acids are now believed to have an

important role on the early life development, and later life nutrition related chronic disease. Fat and fatty acids requirements through life stages have been studied. Fat contributes with increasing the food palatability and soft mixture, plus they have critical role in early growth development (embryonic progress to childhood). Certain fatty acids have specific roles in vivo. While long chain n-3 polyunsaturated fatty acids (LCPn-3) enhance central nervous system (CNS) and brain development, saturated fatty acids (SFA) and *trans*-fatty acids (TFA) contribute with the cardiovascular disease (CVD) (Burlingame et al., 2009).

In 2004, omega-3 index expression had been defined as the red blood cells (RBC) percentage of Eicosapentaenoic Acid + Docosahexanoic Acid (EPA +DHA). RBC is preferable for assessing omega-3 with respect to the half-life of RBC being 4-6 times longer than plasma fractions (von Schacky, 2010). Omega-3 index is considered to be used as a risk biomarker for the coronary heart disease (CHD) especially sudden cardiac death (Harris, 2007a).

Many studies have analzyed plasma phospholipids (PL) and cholesterol ester (CE) to estimate the fatty acids (FA) composition to estimate the short term of dietary fat intake (King et al., 2006; Tvrzicka et al., 2002). Fatty acid compositions of RBC reflect the longer dietary intake (Harris and Thomas, 2009). However, assessing EFA and fatty acids composition from whole blood is not widely practiced. Nevertheless, the ideal method to assess dietary fat intake still does not exist especially for very long chain n-3 fatty acids (Amiano et al., 2001).

The objective of the study is to compare the fatty acid pattern in different blood components to find the simplest and most appropriate method for the rapid validation of the *essential fatty acids* in humans. Comparing the different blood components can lead to a specifying the preferred component for easy and rapid analysis of fatty acids while giving valid information about the status of essential fatty acids.

2. Literature Review

2.1. Fatty acid-structure and nomenclature

2.1.1. Structure

Fatty acids exemplified by a repeating series of aliphatic tail of methylene group with hydrocarbon (CH_3) in one hand and the carboxyl group (-COOH) on the other (Arab, 2003). The length of carbon chain varies from 2 to 30 carbons or more. Fatty acids are considered as the major components of the dietary fats that derived from acylglycerols, free fatty acids, phospholipids and sterol esters. Nevertheless, Triglycerides are considered the main source. 100g of TG yield approximately 95g fatty acids. However, fatty acids in the body are incorporated with blood lipids, in fats deposits and in structural lipids in biological membranes (Ratnayake and Galli, 2009).

Regarding the bonds between carbon-carbon in the fatty acids; fatty acids can potentially be classified as saturated fatty acids if no double bonds occur, unsaturated fatty acids if they contain 1 or more double bonds in the chain. Fatty acid is called monounsaturated when it contains 1 double bond, and polyunsaturated (PUFA) when more than 1 double bonds exist. The positions of these double bonds within the hydrocarbon chain have many possibilities which might formulate the fatty acid as *cis* or *trans* configured (Ratnayake and Galli, 2009).

2.1.2. Nomenclature

A chemical name should clearly describe the chemical structure. This has been a good practice for the fatty acids by using the systematic nomenclature which is recommended by the International Union of Pure and Applied Chemistry [IUPAC-IUB Commission on Biochemical Nomenclature, 1978]. Fatty acid is named on the basis of the carbon number, the number and the position of the double bond is relative to the carboxyl group (Arab, 2003). The carboxyl carbon is regarded as number 1 and the fatty acid chain's carbons are numbered accordingly from the carboxylic carbon. While the IUPAC system is accurate but fatty acid names are too long thus biochemists and nutritionists

use the ‘n-minus’ system for naturally occurring *cis* unsaturated fatty acids that categorize the fatty acids into different families which share same biosynthetic pathways. In regard to the bonds between carbon-carbon in the fatty acids; fatty acids may classified as saturated fatty acids if no double bonds occurred, unsaturated fatty acids if they contain one or more double bonds in the chain (Ratnayake and Galli 2009).

Omega system is referred to the ‘n-minus’ system. It was Holman RT who established the numbering system for the unsaturation of fatty acids, the "omega nomenclature" (Holman 1964; Holman 1998).

Delta (Δ) system is another widely used system to identify fatty acids in which it is based on the carbon atoms number between the carboxyl carbon and the nearest double bonds to the carboxylic group. In Δ system; all double bonds position are specified and also their *cis/trans* configuration (Ratnayake and Galli 2009).

2.2. Fatty Acid Classification

Fatty acids can be classified according to (1) total number of C atoms (even and odd chains), or (2) to the length of hydrocarbon chain (short, medium and long chains), or (3) according to the nature of the hydrocarbon chain nature (saturated, unsaturated).

2.2.1. According to the fatty acid synthesis

2.2.1.1. Non-essential fatty acids

Saturated fatty acids and monounsaturated fatty acids are non-essential fatty acids because humans can derive them from Carbohydrate. SFA can be biosynthesized in humans by the addition of 2-carbon units to the acyl chain. MUFA are biosynthesized by the insertion of a cis double bond between C9 and C10 counting from the carboxyl end of the acyl chain. For example; oleic acid (18:1n-9) can be derived from stearic acid (18:0) and palmitoleic acid (16:1n-7) to palmitic acid (16:0) (Paganelli et al., 2001).

2.2.1.2. Essential fatty acids

In 1929, Burr discovered the Long Chain Polyunsaturated Fatty Acid (LC PUFA) linoleic and linolenic acids (Holman 1998; Burr GO et al., 1930). However, Burr and Burr are considered to be the first who invented the term essential fatty acid (EFA) in 1929 (Burr and Holman 1988). Arachidonic Acid (AA), EPA, and DHA might be considered as conditionally essential as their production would be insufficient (Strijbosch et al., 2008). These publications have resulted towards identifying signs of fat free diet seen in rat which both lionleic and linolenic acids implement that effect (Holman 1998), nowadays essential had a different definition in which they are the fatty acids that have to be obtained from the food (Crawford et al., 2009).

Mammals lack the enzymes to introduce double bonds at carbon atoms beyond C9 thus all fatty acids containing a double bond at positions beyond C9 have to be supplied in the diet. The two lacking enzymes are named Δ12 and Δ15 desaturases. While in plant, Linoleic acid can be desaturated to α-linolenic acid by the insertion of a double bond between carbon 3 and 4 (from the methyl carbon). In mammalian cells; three important families of fatty acids occurred: Omega-3, Omega-6, and Omega 9. The first two families are essential in mammals and must be supplemented from the diet. Consequently, cell membrane composition of the EFAs is determined by the dietary intake (Strijbosch et al., 2008).

The parental essential fatty acids are the two basic precursors of poly unsaturated fatty acids (PUFA) (lionleic acid 18:2n-6) for the omega 6 family, and (α-linolenic acid 18:3n3) for the omega-3 family (Tvrzická et al., 2002). It should be clear that all EFA are PUFAs but not all PUFAs are EFA (Undurti 2008).

2.2.2. According to the fatty acid structure

2.2.2.1. Saturated fatty acids (SFA)

Fatty acid contains only single carbon to carbon bonds, in nature this occurs in unbranched structure with even carbon atoms. Chemical structure in general is R-COOH wherein R group is straight hydrocarbon chain of the form $CH_3(CH_2)_X$. According to the chain length; FAO/WHO Experts Consultation recommended the following subclasses:

- Short chain fatty acids: between 3-7 carbon atoms.
- Medium chain fatty acids: between 8-13 carbon atoms.
- Long chain fatty acids: between 14 and 20 carbon atoms.
- Very long chain fatty acids: within 21 or more carbon atoms.

2.2.2.2. Unsaturated fatty acids (USFA)

Fatty acid contains double bonds and hence more chemically reactive than SFAs, the more double bonds the more reactivity found. According to the double bond number; Unsaturated Fatty Acids (USFA) are categorized into two subcategories: Monounsaturated Fatty Acids [MUFA], and Polyunsaturated Fatty Acids [PUFA]. However, according to the chain length; FAO/WHO Experts Consultation recommended the following subclasses:

- Short chain unsaturated fatty acids: with 19 or fewer carbon atoms.
- Long chain unsaturated fatty acids: with 20-24 carbon atoms.
- Very long unsaturated fatty acids: with 25 or more carbon atoms. (Ratnayake and Galli, 2009).

2.2.2.3. Cis-Monounsaturated fatty acids (*cis*-MUFA)

Naturally the largest parts of the double bonds of the unsaturated fatty acids in food fats are found in the *cis* configuration (Ratnayake and Galli 2009). There is a suggestion

that MUFA reflect SFA dietary intake but doesn't reflect MUFA intake which might be explained by MUFA being endogenously biosynthesized from PUFA (MA et al., 1995).

2.2.2.4. Trans-fatty acids (TFA)

USFA that contain at least one double bond in the *trans* configuration is called trans fat. The disadvantages of such recent FA are now known to lead to increase the risk of non communicable diseases such CHD and metabolic syndrome. Such FAs have affections on blood lipoproteins, while they increase Low Density Lipoprotein (LDL) (the same effect of SFA) and TG, they decrease HDL (Asgary et al., 2008).

Small amount (2-6%) of *trans* configuration of the USFA double bonds are presented in ruminant deposition and milk fats; although the most naturally USFAs double bonds of the food fats are found in *cis* configuration. It was in 1929 when *trans* isomers of fatty acids were demonstrated (Bertram 1928). The major intake of the *trans* fatty acids in human diets arise from food technological treatment such as hydrogenation of oils (Ratnayake and Galli 2009).

2.2.2.5. Polyunsaturated fatty acids (PUFA)

According to the double bonds location; natural PUFAs from the methyl terminus of the acyl chain with all *cis* configuration can be alienated into 12 different families from n-1 to n-12. However, regarding the presence and human health and nutrition, the most important two families are n-3 and n-6. Both are essential for humans because they cannot be synthesized from the organism, and have hence to be provided via dietary intake (Ratnayake and Galli 2009). High long chain poly unsaturated fatty acids (HLCPUFA) are found in retina membranes, brain synapses and sperm (Arab, 2003).

2.2.2.5.1. n-6 PUFA

Linoleic acid structure was elucidated by (Erdmann et al., 1909), and synthesis description was, at a later time, presented by (Raphael et al., 1950). n-6 PUFA is an

important family with respect to human health. It is considered essential. In nature, there are many important n-6 PUFAs such as lionleic acid (LA), γ linolenic acid (GLA), docosapentaenoic acid (DPA), aracidonic acid (AA), etc. Nevertheless, LA is the parent of this family. It is widely distributed in almost every dietary fat; therefore some population might over consume LA. In vivo, LA can produce AA and Dihomo- γ -Linolenic Acid (DGLA) and those are substrates for eicosanoids. AA is mainly present in animal products especially free-range animals and rarely in the plant kingdom (Ratnayake and Galli, 2009).

2.2.2.5.2. n-3 PUFA

n-3 PUFAs family is present in both the animal and plant kingdoms and it is considered very important with respect to human health and nutrition. There are many important n-3 PUFAs such as eicosapentaenoic acid EPA, docosapentaenoic acid DPA, and docosahexanoic acid DHA. Nevertheless, ALA α-lionlenic acid is the parent of this family. However, each fatty acid has a main role in vivo. For example; EPA is a precursor for the n-3 derived prostaglandins (PGs) and thromboxanes (TXs), DHA is the precursor of docosanoids and it is highly presented in some highly specialist tissues like brain and retinal cells, sperm and cardiomyocytes (Ratnayake and Galli, 2009).

Several studies have demonstrated that n-3 FA lower levels of triacylglycerol and the increase level of HDL (Dewaillya et al., 2003), n-3 FA can also decrease the atherogenic eicosanoids production and modulate plasma lipids (Sun et al., 2008). It has been suggested that plasma n-3 PUFA (especially 22:6n3) form good biomarkers for dietary intake (MA et al., 1995).

Fish is the richest source for major n-3 PUFAs (EPA and DHA). However, there is a high correlation between serum EPA and DHA in cholesteryl ester and phospholipids fractions and fish intake which makes very long chain n-3 PUFA considered as a valuable biomarker fish dietary intake (Amiano et al., 2001).

2.2.2.6. Furan Fatty acids

Spiteller has reviewed that there are a large group of unusual fatty acids distinguished by the furan ring occurred in a low level in many natural products and also in human blood. Human beings obtain furan fatty acids from food (fish, soft corals, vegetable oils) and incorporate them into PLs and CEs (Spiteller, 2005; Ratnayake and Galli, 2009). Furan fatty acids can be found in different amounts amongst all the blood samples, in serum they were found only in PL but not in CE or TG (Wahl et al., 1995).

Furan FA (F-acids) are identified by the fatty acids characterized by side chain of a propyl or pentyl in one of the alpha-positions; and the other side chain with SFA with COOH end (Spiteller, 2005). Yet, some features of this family need to be clinically tested. For instance, furan fatty acids have the ability to be radical scavenging which might contribute to the cardioprotective functions of the fish and fish oil (Ratnayake and Galli, 2009).

2.2.3. Membrane Lipids

2.2.3.1. Phospholipids

Phospholipids are considered to be the main class of membrane lipids. They consist of four substances: the platform where the fatty acids attached with, one or more fatty acids, phosphate and an alcohol attached to the phosphate. They are also known as glycerophospholipids. As shown in (Fig 2.2.3.1.) X group natures in the phospholipids produce many classes such as phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine in biological membrane. However, R1 in the sn1 is usually esterified to SFA while R2 in the sn2 is esterified to PUFA while at sn3 the PL molecule is provided by a hydrophilic region (phosphorus with nitrogen base or sugar molecule).

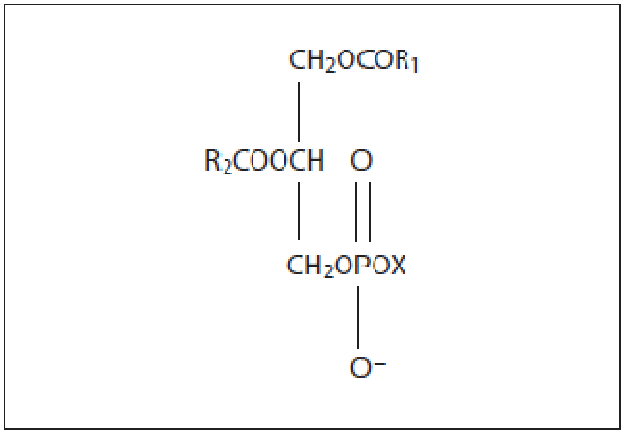

Fig 2.2.3.1. Chemical structure of phospholipids, P= phosphate, R1, R2= fatty acids, X= Choline, ethanolamine, serine, inositol or glycerol {Adapted from (Ratnayake and Galli 2009)}.

2.2.3.2. Cholesterol

Cholesterol is a member of the sterol lipids, although it is an important constituent of the membrane lipids. Sterol lipids consist of 3 main components: the steroid nucleus of the 4-ring structure, the hydrocarbon side chain and the alcohol group. Cholesterol is

regarded as the animal fat sterol as it rarely occurs in vegetable oils in trace amounts (Ratnayake and Galli 2009)

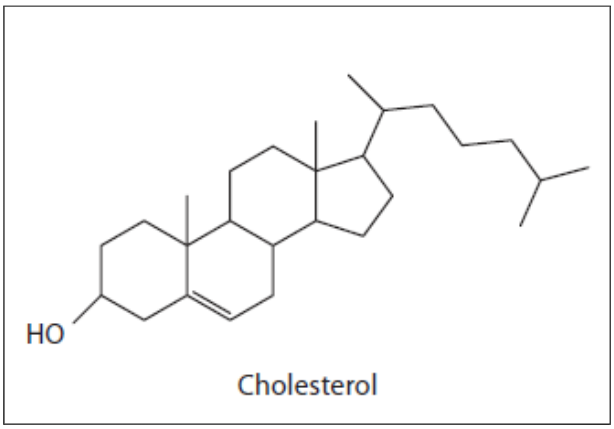

Fig 2.2.3.2. Chemical structure of a cholesterol {adapted from (Ratnayake and Galli 2009)}.

2.3. Fat digestion, absorption and delivery to tissue

Digestion breaks down the large components of food into substances the gastrointestinal tract lining can absorb. This process requires secretion of enzymes from different part of the Gastrointestinal (GI) tract. Triglycerides are the main component in the natural fat intake with other few types of fatty acids such as SFA and USFA. The main site for fat digestion is the small intestine (Frances Sizer and Ellie Whitney, 2007).

However, as dietary fat intake does not exist for pure fat and other components thus when fat enters the stomach it floats on the top of the fluid components in the stomach. Bile is the main factor to emulsify fat in the small intestine while digestive enzymes complete most fat digestion in the small intestine. Those digestive enzymes cleave triglycerides into free fatty acids, glycerol, and monoglycerides (Frances Sizer and Ellie Whitney, 2007)

Free fatty acids are absorbed by the intestinal villi. The small volume of short chain fatty acids and glycerol allow them to be absorbed into the bloodstream immediately to be used by cells or stored in adipose tissue. However, short chain fatty acids is oxidized in the liver, while long chain fatty acids combines with protein (as they are not soluble in the blood aqueous median) to form chylomicrons and pass to bloodstream via the lymph vessels (Ratnayake and Galli 2009).

Dietary fatty acids absorption would reach > 95%. On the other hand, absorption of dietary TG depends on the TGs physical natures that are found in the food (Ratnayake and Galli, 2009). To increase the absorption of the long chain PUFAs (especially DHA and EPA), Garaiova suggested to pre-emulsification the oil mixture before eating while that had no effect on shorter chain SFAs absorption (Garaiova, 2007).

TGs are mainly structured of fatty acids which are also found in PL and CE. Fatty acids in vivo are usually found combined and not as free fatty acids (Arab, 2003).

Similar amount of the n-3 FAs intake either in form of capsules or salmon fish have different plasma EPA and DHA concentrations, from the later were significantly higher according to many studies (Ratnayake and Galli, 2009).

2.4. Fatty acids synthesis

Many fatty acids can be synthesized, elongation and desaturated in vivo which affect the fatty acids measuring as indicator for the dietary intake (Arab, 2003). Nonessential fatty acids NEFA (SFA and MUFA) are totally identified to be supplied from dietary intake and also from in vivo biosynthesis from acetate precursors which might be PUFA or non-fatty components such as carbohydrates and glucogenic amino acids (Brenna and Lapillonne 2009). TFA, LA, ALA, Long Chain Saturated Fatty Acid LCSFA (22:0 and 24:0) are obtained from dietary intake, while 16:0, 18:0, 16:1n7, 18:1n7 and OA are synthesized endogenously from carbohydrates (King et al., 2006).

Synthesizing fatty acids in vivo swing between three processes; (1) synthesize SFA from acetyl Coenzyme A (CoA) units that derive from carbohydrate intake, (2) elongate the FA by entering 2 carbon atoms at once which create new fatty acid chain (usually occurs in endoplasmic reticulum), and (3) desaturate fatty acid by entering double bond and remove the hydrogen (converting the saturated bond to an unsaturated one). The last process requires desaturase enzymes, and mammalian are deficient in Δ12 that convert Oleic Acid (OA) to LA (n-9 to n-6), and Δ15 that convert LA to ALA (Arab 2003).

LA and ALA as mentioned before are considered the EFA parent in which their metabolized produce the main two families of PUFA. This metabolize occurs by the same microsomal enzyme system (Ratnayake and Galli, 2009). In tissue structural lipids in both humans and animals the two biochemistry (desaturation and elongation) are altering to produce up to 22 carbons fatty acid chain or long (Holman 1998). Human can enter double bonds at the Δ9 position but they cannot enter it between Δ10 and the methyl terminal end. Those reactions use the same enzymes but they are independent and never cross their reactions series (Ratnayake and Galli, 2009).

The pioneers to describe the in vivo conversion of stearic acid into oleic acid were (Schoenheimer et al., 1936). Synthesis of linoleic acid and linolenic acid were first described by (Raphael et al., 1950). Wider and Holman reported that LA is the precursor

of AA, while ALA is the precursor of the pentaene and hexaene acids. In rat fed fat free diet; oleic acid was the precursor of mead acid (which was known as trienoic acid) (Holman, 1998). However, it is well known that LA and ALA are the precursors of the LCPn-3 and LCPn-6 (Sprecher et al., 1995). LA can be converted to LCPUFAn-3 in vivo (Sun et al., 2008)

Since 1963, there were many experimental studies investigating the effect of linoleate and linolenate intake on the concentration of AA and ESA (eicostrienoic acid). It was shown that intake of linolenate and linoleate reduce the ESA level in rat liver. When supplied the rat (through fat free diet course) with linolenate, a slight reduction on the AA and DPA levels appeared (Mohrhauer and Holman, 1963).

Fatty acids' intake has a noticeable effect on the de novo fatty acids biosynthesized. Both PUFA and SFA suppress FA synthesizing. High fat intake leads to a reduction in the de novo FA synthesis (Arab 2003), while high carbohydrates intake elevates the liver conversion of carbohydrates to fatty acids especially SFA (14:0 and 16:0), n-7 and n-9 (King et al., 2006). However, habitual dietary intake, physical activity, genetic characters and hormonal status have also an influence on the individuals' fatty acids profile (Hodson et al., 2008; Ratnayake and Galli, 2009).

Alcohol would be metabolized to SFA in vivo which increase the SFA concentration. Alcohol also decreases the 18:2n6 in plasma PL and CE (MA et al., 1995), inhibit Δ6 and Δ5 desaturases which decrease the conversion of LA to AA, ALA to EPA and DHA, and also decrease the LCPUFA concentration (Ratnayake and Galli 2009). However, alcohol drinkers showed to have higher 16:0 in RBC PL than non-drinkers (Heude et al., 2002).

Undurti in the Lipids in the Health and Diseases journal summarized the inhibitor and activator factors of Δ6 and Δ5 desaturases that interference the EFA metabolism. It was mentioned that SFA, TFA, cholesterol, alcohol intakes, adrenaline level, insulin deficiency, diabetes, hypertension, oncogenic viruses (such hepatitis C), glucose rich diet and protein deficient inhibit Δ6 and Δ5 desaturases which reduce the formation of GLA, DGLA, AA, EPA, and DHA. Whilse fat free diet, caloric restriction, insulin and co-factors

(e.g. zinc, magnesium, pyrixodine, and nicotinic acid) would enhance their regulation (Undurti, 2008); lipid lowering drugs have an effect on the LA conversion (Ratnayake and Galli, 2009)

Smoking has an effect on the LA conversion, the serum LCPUFAs, and lower serum AA and DHA. Negative smokers might face changes in n-3 biosynthetic pathways in epithelial cells; in addition women who smoke during their pregnancy would cause a reduction in their infants LCPUFA levels (Pawlosky, 2007; Ratnayake and Galli, 2009).

Oral hormonal replacement therapy in postmenopausal women would enhance the elongation and the desaturation activity that transform LA to AA in plasma CE. Increasing AA level enhance the eicosanoids synthesizing which has an important role in CVD (Lewis-Barned et al., 2000).

LA metabolic cascade was described by (Marcel et al., 1968) as follows:
18:2ω6 –› 18:3ω6 –› 20:3ω6 –› 20:4ω6 –› 22:4ω6 –› 22:5ω6

ALA metabolic was described by (Klenk and Mohrhauer, 1960) as follows:
18:3ω3 –› 18:4ω3 –› 20:4ω3 –› 20:5ω3 –› 22:5ω3 –› 22:6ω3

AA and EPA biosynthesizing depends on the LA and ALA desaturation at the position 6. The assumption of having a large amount of DHA and low levels of 22:5n6 in the membrane lipids would be justified by the faster conversion of EPA to 24:6n3 than AA to 24:5n6 (Sprecher et al., 1995). The conversion of n-6 PUFA is accelerated by the LA high intake and concentration with low ALA in lipids tissue (Ratnayake and Galli 2009). On the other hand, the n-3 and n-6 reactions rates of desaturation and elongation were found to be similar (Sprecher et al., 1995).

The conversion of ALA to n-3 LCPUFA is not sufficiently provided and thus the adequate intake of EPA and DHA are recommended (Ratnayake and Galli, 2009).

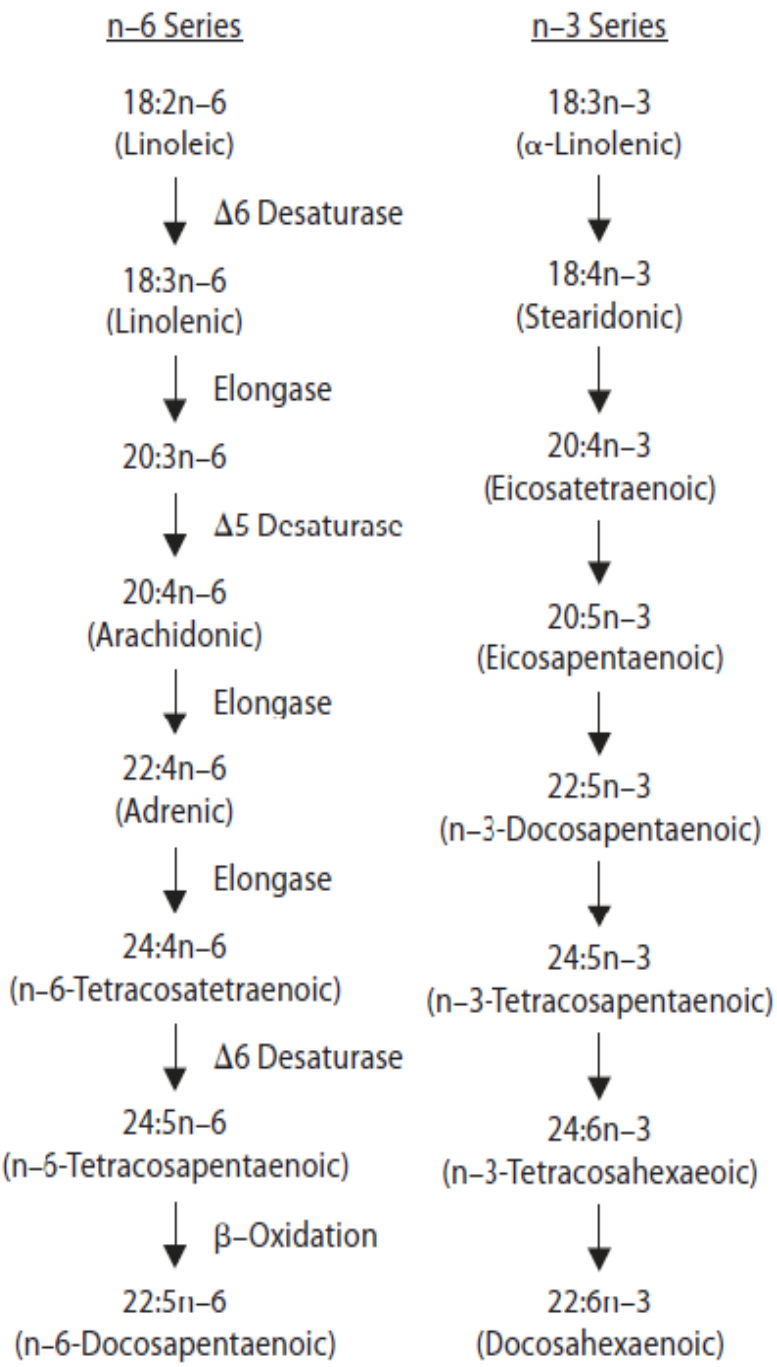

Fig 2.4. Representation of the pathways of n–6 and n–3 fatty acid metabolism (Ratnayake and Galli, 2009)

2.5. Essential fatty acids in human health and disease prevention

2.5.1. Heart diseases

The attention paid for the blood lipid bprofile with respect to cardiovascular disease is ample. Meta-analyse-studies demonstrated a positive correlation between total fat intake and total cholesterol, LDL and HDL cholesterol levels; however in other studies these results could not be confirmed. These conflicting results can be caused by the fact that the intake of fat vary widely in their fatty acids composition pattern. It is also well known the reduction of body weight through changing the energy balance has an impact on the blood lipid profile. For each 1 kg body weight loss, TGs were lessened by 0.011 mmol/l, and HDL increased by 0.011 mmol/l (Elamdfa and Kornsteiner, 2009).

High intake of SFA and cholesterol along with low intake of PUFA stimulate serum cholesterol level and CHD risk (Skeaff and Miller, 2009). Nevertheless, TFA consumption plays a role on the CHD as it affects the serum lipoproteins. TFA are considered as pro-inflammatory (Galli and Calder 2009).

The role of EPA in preventing thrombosis and atherosclerosis was studied by (Dyerberg et al., 1978). In recent days, WHO is demonstrated with the protective effect of linolenic acid, EPA, and DHA and the inverse effect of the SFA myristic and palmitic with regards to the increasing the risk of cardiovascular disease and reducing the mortality (Anderson et al., 2009). Especially n-3 LCPUFA (Skeaff and Miller, 2009) from fish and fish oil supplements were found to be protective agents against cardiovascular diseases (Welch et al., 2006). The FAO/WHO was reported that replacing SFAs with PUFAs in dietary intake lower LDL-cholesterol, total cholesterol/ HDL-cholesterol ratio and reduce the risk of CHD (Elmadfa and Kornsteiner, 2009).

Harris has reported that the intake of n-3 (EPA and DHA) fatty acids has a beneficial effect on the heart disease. 850 mg/day would decrease 25% of death from CHD and 45% of sudden cardiac death. In case of primary prevention two meals of fatty fish correspond to (500mg) EPA+DHA per week would be recommended, while for

secondary prevention 1 g EPA+DHA /day would decrease the risk of CHD death (Harris and von Schacky, 2004). EPA and DHA supplementation reduce the CVD risk by stabilizing the arterial plaque which is considered as a potential mechanism to secondary reduce inflammation (Harris, 2010a).

In Japan, 7.8 out of 100.000 persons would suffer from sudden cardiac death (SCD) (von Schacky and Harris, 2007) with an omega-3 index in RBC equal to 10%, while in Western countries the incidence is much higher and amounts to 150 out of 100.000 with an omega-3 index in RBC equal to 4.5%. In Seattle, a case-control study showed that when omega-3 index is 3.3% the sudden cardiac death was 1.0 while when the index increased to 6.5% the risk decreased to 0.1 person per 100,000 per year (von Schacky, 2009).

2.5.2. Overweight and Obesity

Recently, obesity is considered as an epidemic since the prevalence in most countries has increased significantly. FAO/WHO reported in 1994 that high fat intake increases the risk of obesity, CHD and certain cancer types (Smit et al., 2009; Ogura et al., 2010; Kobayashi et al., 2001).

While some prospective studies have demonstrated the hypothesis of food with high in fat and energy promote weight gain, other prospective studies debated this hypothesis and found some conflicting results. However, in short term intervention studies, it was clear that reducing body weight could be achieved by lowering the energy percentage from fat but not on an ad libitum basis (Elmadfa and Kornsteinerm, 2009).

The effect of diet rich in medium-chain fatty acids on controlling weight was demonstrated by (Kaunitz et al., 1958). n-3 PUFA might positively contribute in controlling some metabolic mechanism of obesity (Nobili et al., 2011). In rat-based studies fish oil supplements were proven to decrease obesity but not in human. There is a positive relation between EPA and body mass index (BMI) in human (Ogura et al., 2010). Adiposity and weight increase are enhanced with diet poor in PUFA (Lweis-Barned et al., 2000).

2.5.3. Type 2 Diabetes Mellitus

EFAs/PUFAs ratio has an important role in diabetes mellitus (Undurti 2008). According to the strong association between type 2 diabetes and overweight and obesity, many studies were designed to establish the relation between type 2 diabetes and fat intake (Melanson et al., 2009, Elmadfa and Kornsteiner, 2009). Cohort studies found some conflicting results. Reduction in body weight and Insulin sensitivity was shown to be improved by regular physical activity (Elmadfa and Kornsteiner, 2009).

Insulin activates Δ6 desaturase while diet rich in glucose suppress Δ6 and Δ5 desaturases (Undurti, 2008). However, there are evidences that insulin resistance increases when diet is rich in SFA and poor in PUFA (Lweis-Barned et al., 2000). In animal studies, SFA rich food reduces insulin sensitivity as opposed to n-3 LCPUFA which increase it. Although there has been no guarantee evidence that replacing fat type or change in fat amount have a constant effect on insulin sensitivity, limited evidence showed an improvement in insulin sensitivity and glycaemic index in type 2 diabetes when swapping the SFA (animal source) with MUFA (vegetable source) (Sanders, 2009).

2.5.4. Essential fatty acids and depression

Abnormalities in fatty acids patterns were noticed in patients with psychiatric disorders such as high EPA/DHA ratio and low level of EPA and n-3 PUFA in CE and PL. Second to adipose tissue, cells of central nervous system are richest in fat (Bourre 2004) especially PUFAs (Heude et al., 2003). Thus, any abnormalities in amount and composition of ingested PUFA's will change the regulation of those cells. In bipolar patients, RBC concentrations of AA and DHA are significantly depleted as compared to healthy individuals affecting mood stabilizers. However, among schizophrenia patients, EPA was noticed to improve the disease symptoms (Chiu et al., 2003).

2.5.5. Cognitive function

AA and DHA are well established to be major structural components of the brain. A trail investigation showed that when school aged children were supplemented with LCPUFA (DHA and EPA) their learning and memory abilities were improved. Children with low level of n-3 LCPUFA appear to have learning problems. However, DHA long term supplementation has a positive effect on the children health and their school performance (Dalton et al., 2009).

2.5.6. Eicosanoid precursors and inflammation

As PUFA are responsible for the production of eicosanoids in vivo, Eicosanoids are derived by the oxidation of n-3 and n-6 essential fatty acids mainly from AA (Arab, 2003) which is the predominant PUFA in the body tissue is the product of desaturation and elongation of LA in the liver (Ratnayake and Galli, 2009). Eicosanoids are high potency hormones (Arab, 2003) and they are the connection between inflammation and immunity from one side and the PUFA from the other (Simopoulos, 2002). Eicosanoids and docosanoids have diverse physiological functions in blood pressure, platelet clotting, blood lipid profiles, the immune response and the inflammation response to injury infection (Ratnayake and Galli, 2009). Both n-3 and n-6 have anti-inflammatory properties as they produce different eicosanoids. Although the eicosanoids from n-3 and n-6 have reversed properties, EPA has higher affinity to cyclooxygenases and 5-lipoxygenase than AA (Simopoulos 2002). ALA, EPA and DHA play an important role in the immune function. While high intake of ALA (15g/d) suppress the IL-1 and TNF (tumor necrosis factor), high intake of n-6 (imbalance of n6:n3 PUFAs) will over produce the pro-inflammatory prostaglandins of the n-6 series and cytokines. Thus it can be concluded that LA increases while n-3 reduces the pro-inflammatory cytokine secretion (Simopoulos, 2002). Prostaglandin E2 (PGE_2) has pro-inflammatory effect which can cause pain, fever and edema. When supplemented with 2g EPA and DHA, those n-3 will work as anti-inflammatory and decrease the bacterial infection (Arab, 2003).

The pivotal of n-6 PUFA AA is a precursor of several lipid mediators that have biological actions (LTs, PGs, TX and HETEs) (Gomolka et al., 2011). The metabolism of the AA cascade through the cyclooxygenase enzymes (which has two isoforms Cyclooxygenases 1 (COX-1) and Cyclooxygenases 2 (COX-2)) increase the PG and Thromboxanes (TX) series. COX-1 and -2 are found in many normal human body's tissues. While COX-1 plays role in basal production of eicosanoids, COX-2 is enhanced by certain inflammation factors and also plays a role in the PG production (Ratnayake and Galli, 2009)

EFAs play a special role in regulating the prostaglandins (PGs) and the leukotrienes (LTs) which are released by almost all the body tissues. PGs and LTs play an important role in the inflammatory and immune system. DGLA, AA and EPA are the precursors of the 1-, 2- and, 3- LTs series, respectively (Belch and Hill, 2000). In addition, TFAs work as pro-inflammatory and *trans* LA and *trans* OA are stronger than *trans* 16:0 in pro-inflammatory effect (Galli and Calder, 2009).

Furthermore, EPA and DHA compete with AA on the production of PGs, TXs, LTs and 5-HETE in inflammatory cells. While AA produces them, EPA and DHA suppress their production (Ratnayake and Galli, 2009).

2.5.7. Inflammatory bowel disease IBD

The IBD diseases, Crohn's disease or ulcerative colitis are related to immunologic, environmental and genetic components. AA metabolites, prostaglandins, leukotrienes and cytokines are the most studied mediators. LTB_4 which is the end product of AA is shown to be high in ulcerative colitis patients. However, supplementation of Crohn's disease patients with 2.7g of n-3 decreased the relapse rate, which in the company of other studies concluded that n-3 showed significant improvement for the IBD patients (Simopoulos, 2002).

A cross sectional study done in Berlin, Vienna, and Bari to compare FAs profile between IBD patients and healthy individuals showed that SFA, MUFA were abnormal. Plasma 15:0 and 24:0 decreased while 16:0 and 18:0 series increased. PUFAs profiles were

similar between the 2 groups – except for 22:4n-6 which was higher in IBD patients- although most studies showed different levels in IBD patients (Hengstermanna et al., 2008).

2.5.8. Bacterial, viral, fungal and parasitic diseases

PUFA is suggested to inactivate HIV virus. It was found that plasma PL in AIDS patients is low in DGLA, AA, and DHA levels icnreasing the onset and the development of AIDS. LA, ALA, and AA can inactivate both gram-positive and gram-negative bacteria. There is a hypothesis that EFA/PUFA ratio has similar aspirin-like action and that subjects with lower PUFA levels are more able to develop HCV (hepatitis C virus), HIV (human immunodeficiency virus), malaria and bacterial infections. Throughout many studies it was established that PUFAs have anti-bacterial, anti-viral, anti-fungal and anti-parasitic actions (Undurti, 2008).

2.6. Essential fatty acids deficiency (EFAD)

LA and ALA are the essential fatty acids because they must be obtained from diet as the body does not have the ability to synthesize them in vivo. LA and ALA constitute the cell membranes and accordingly EFA deficiency might alter the membrane structure and fluidity, also can ffect the membrane-bound enzymes and receptors performances (Undurti, 2008; Bourre, 2004).

EFAD is the combination of the low intake with increased demand of physical requirements and sever malabsorption (Holman, 1998). Chronic malnutrition which includes low intake of EFA and protein (which is important for enzyme and lipoproteins synthesizing) causes low LA and its products (Johnson et al., 1985). Clinically, EFAD would be distinguished by many symptoms such as growth retard, renal toxicity, pulmonary abnormalities, and increased metabolic rate. However on the other hand in laboratarial experimentations, EFDA is distinguished by low level of omega-3, omega-6, AA, and high level of Mead Acid (Holman, 1998).

20:3n9 (mead acid) level in general is low in the plasma and tissue, while in the case of fatty acids deficiency the conversion of OA to 20:3n9 is increased according to the reduction in AA level. The ratio of 20:3n9/ 20:4n6 is therefore increased which is considered as an EFAD biomarker (Ratnayake and Galli, 2009). The high level of Mead acid is a result of the conversion of the metabolic pathway. Usually, LA is converted to AA, while the deficiency and low intake of LA lead to a low level of AA which enhances Oleic acid to be converted to Mead Acid (20:3n9) (Ratnayake and Galli 2009; Mohrhauer and Holman, 1963). Increased level of all n-7 fatty acids, and n-9 (especially OA) with low level of LA (and most n-6 FA) would be signs of EFAD (Jeppesen et al., 1998). It was shown in a study feeding rat fat free diet has increased dietary linoleate intake suppressed 20:3n9 level in liver and heart lipids (Mohrhauer and Holman, 1963).

The index of triene/tetraene to indicate the EFAD status was established by Holman in 1960, and this index is now known by Holman index. However, triene/tetraene ratio is being used to indicate the EFAD but not omega-3 status (Strijbosch et al., 2008; Mohrhauer and Holman 1963). Holman has stated triene/tetraene ratio > 0.2 to be abnormal and EFAD ratio is > 0.4 in PL fraction (Holman 1960; Holman et al., 1979).

Previously, EFAD was noticed clinically from the eczema symptoms in an infant who was on skimmed milk and sucrose diet instead of mother's milk, and the dermatitis that noticed in a man who also was on low fat diet for 6 months. EFAD occurs rapidly and severely in both young and adult subjects (Holman, 1998).

However, EFAD affect all body tissues (RBC, heart tissue, liver, kidney, muscle, skin and depot fat of rats (Holman, 1998). Since 1960s the correlation between CAD and EFAD were established by (Paganelli et al., 2001). It was found that the onset of certain types of diseases such as collagen vascular diseases, hypertension, diabetes mellitus, metabolic syndrome X, Alzheimer's disease, CHD, atherosclerosis and cancer are significantly triggered by the severly low plasma and tissue levels of GLA, DGLA, AA, EPA and DHA (EFA/PUFA deficient) (Undurti, 2008). ALA deficiency affects the performance of the certain cerebral structure and altering sensor organs receptors which decrease

the taste sensitivity. Subjects with ALA deficiency will need large amount of sugar to actually taste sweetness (Bourre, 2004).

For n-3 deficiency but not for DHA, 22:5n6/ 22:6n3 ratio is established in animal studies because 22:5n6 concentration increased in case of n-3 deficiency (Ratnayake and Galli, 2009). However, Holman was the first to report n-3 deficiency in 1982 (Holman, 1998). Excessive SFA leads to n-3 deficiency due to their effect on transport mechanism (Paganelli et al., 2001).

Increased intake of LA with low ALA intake decreases the EPA and DHA synthesis. In addition, western diet is extremely high in LA (10 folds higher than ALA intake) especially with people who do not consume LCPUFAn-3 (EPA/DHA). Thus, it is recommended to increase the intake of ALA (whether from plant or fatty fish) to prevent the EFAD and to ensure an adequate conversion of ALA to EPA and DHA (Ratnayake and Galli, 2009).

2.7. An essential fatty acid index necessity

In 2004, the definition of omega-3 index was established in RBC to be the sum of EPA and DHA. Using RBC rather than serum to assess the EPA+DHA decreases the biological variability as RBC half-life is 4-6 times longer and less influenced by day-to-day dietary intake. Thus, RBC omega-3 index is not influenced by fasting or feeding state. In case of short term dietary intake scenario, plasma fatty acid would be considered. Many factors affect omega-3 index values such as age, gender, BMI, alcohol drinking, physical activity and diabetes (von Schacky, 2010). Omega-3 index doesn't only differ according to those factors but also it varies within (2.6% to 14.9%) and between populations (3.4% to 9.5%) as well. However, the upper limit of the omega-3 index presents in Japanese population is 10% (von Schacky, 2009).

Omega-3 index is favoured as a marker for the CHD especially sudden death for many reasons. Most importantly is the inverse relationship between omega-3 index tissue levels and the risk of cardiac events and sudden death (Harris, 2007a; Albert, 2002;

Siscovick et al., 1995; Kuriki, 2006). Omega-3 FAs have a lowering effect on serum triglycerides level, blood pressure, and serum cholesterol (Anderson et al., 2009) which are considered as the main risk factors for cardiac diseases. Omega-3 index would reflect CHD more than other known risk factors plus that it is simple, safe, and inexpensive (Harris, 2007a). However, EPA and DHA were found to work as anti-atherosclerotic and enhanced plaque stability (Harris and von Schacky, 2004), they are considered as an indicator of the risk of sudden death according to the results of a prospective, nested case-control analysis study established the effect of WB EPA and DHA level (not the DPA and ALA) in lowering the risk of the sudden death (Albert et al., 2002).

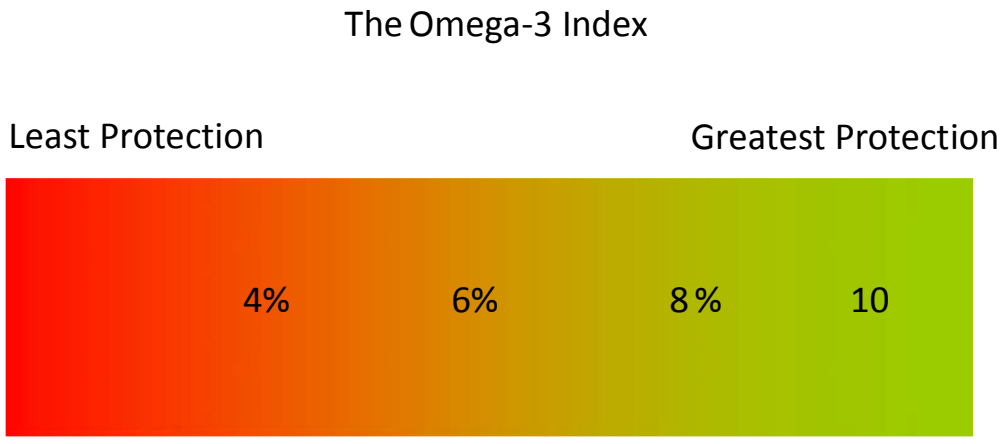

Fig 2.7.1. Summary of the proposed cut points for the Omega-3 Index (Harris and von Schacky, 2004)

The only two risk factors that have a statistically significant effect on CHD are: C-reactive protein and the blood omega-3 index. The omega-3 index is considered as a suitable biomarker independent from other risk factors for the sudden cardiac death risk. Recently Harris and Schacky established omega 3 index for cardiovascular patients (Harris and von Schacky, 2004). Harris found in one study that EPA+DHA content was 29% lower in patients with acute coronary syndrome than controls (1.7 ± 0.9% versus 2.4 ± 1.4%, $p<0.001$) (Harris, 2007b). However, omega-3 index is affected by the intake

of EPA + DHA, an increase of 0.24% will be noticed with each 4 g of EPA and DHA consumed monthly (von Schacky, 2010).

In another study Harris and von Schacky established an omega-3 index target for cardioprotective to be about 8% and when the index is lower than 4% the risk of CHD death increased (Harris and von Schacky, 2004; Ratnayake and Galli, 2009). However, 8% or greater is considered the optimal levels (Harris, 2010c). While Aarsetoey could not establish the above index, he however did not find a significant correlation between omega-3 index > 5.27% and all mortality risk reduction (Aarsetoey et al., 2008). In serum phospholipids, DHA ≥ 4.5% from total fatty acids was found to decrease 34% of CHD, while omega-3 index (EPA+DHA) ≥ 4.6 % was shown to lower 70% of the CHD risk (Halub, 2009).

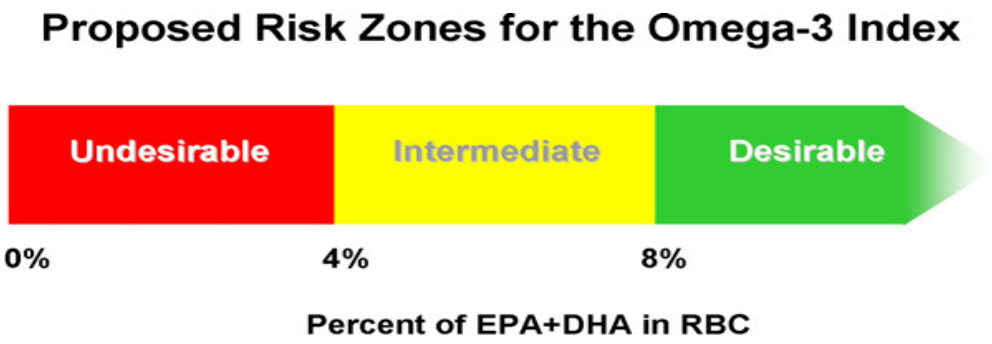

Fig 2.7.2. Proposed risk zone for the omega-3 index (RBC EPA+DHA) (Harris, 2007a)

Rupp distinguished between the two terms of omega-3 level and omega-3 index. The omega-3 level does not mean omega-3 index as omega-3 level includes other n-3 FA while the index includes only EPA and DHA (Rupp et al., 2004) the most two important n-3 LCPUFA in RBC membrane (Aarsetoey et al., 2008).

To our knowledge, there is no omega-3 index cut of point to compare between healthy subjects; this has lead to the urgent need for further studies to establish a valid omega-3 index. There is a rising requirement for a standardized method that assess EFA and the omega-3 index which is believed to be a CVD biomarker (Harris and Thomas, 2009). It has been suggested to consider the omega-3 index test as a part of routine blood test

(von Schacky, 2009). Similarity to the fact that HbA1c reflect glucose homeostasis (von Schacky and Harris, 2007), and LDL levels (LDL<100 mg/dl) (von Schacky, 2009) equal to (2.586 mmol/L) (Robins et al, 2001) omega-3 index reflects omega-3 fatty acids status (von Schacky and Harris, 2007). Only three laboratories in the world have installed the analytic methods to assess omega-3 index as it includes highly standardized analytic laboratories methodology (von Schacky, 2010).

Omega-3 index is considered as an accepted risk marker according to its consistency among the epidemiological data, between and within population in addition to the prospective cohort studies. In addition the strong associations between the omega-3 index and certain types of disease with low biological variability and being safe, quick, and cheap to be analyzed are all advantages for considering omega-3 index a biomarker (Harris and von Schacky, 2004).

2.8. Choices of tissue for measurement of fatty acids

Fatty acid composition in tissue and blood have been used as a biomarker for dietary intake because they are mainly derived from the intake (Hodson et al., 2008). Blood lipids a good biomarker to reflect dietary long-term intake and measure EFA status in animals (Holman, 1998; Ogura et al., 2010). Changing fat dietary intake will change fatty acid composition which would turn back entirely within days (Kuang 2009). Analyzing fatty acids from blood components (single blood draw) is considered minimally invasive.

Despite the huge publications regarding the fatty acids profile, it remains difficult to establish a general distribution level. This is because fat intake and its absorption and metabolism affect fatty acids profile in body tissues. Highly specialized cells contain high amount of AA and DHA in the structural lipids PLs. Higher intake of LA does not correlate with higher concentration of the AA in RBC. On the other hand, very high intake of LA enhances the storage of the LA in the adipose tissue (Ratnayake and Galli, 2009).

The status of FA is influenced by many factors, dietary intake, food preparation method (which would affect the bioavailability), individual age, BMI, and total energy intake

(Aarsetoey et al., 2008). Yet, blood and tissues' concentrations of PUFAs (n-3 and n-6) cannot reflect their accurate proportions in the dietary intake (MA et al., 1995; Sun et al., 2007). Assessing PUFAs' level in body is the best biomarker to determine the dietary intake especially the levels of EPA, DHA, and n-3 HUFA as they are not biosynthesized in humans (Kuriki et al., 2006). However, n-3, n-6, and TFA as they are synthesized exogenously can be measured in blood specimens (plasma, serum, RBC) (Sun et al., 2007).

2.8.1. Whole blood

The easiest in which it can be measured from a dried blood spot does not need pre-separation or lipid trans-esterification like in RBC and Plasma (Harris, 2007a). There is no need for the TLC procedure, and FFA can be easily obtained by FAME method (Bailey-Hall et al., 2008). Intakes of EPA and DHA were easily measured in whole blood, EPA increased from 0.6% to 1.4% within 10 days, while DHA increased from 2.9% to 4.3% (Rupp et al., 2004). WB lipids composition represents FA of all the circulating lipid classes (Agostoni et al., 2011).

2.8.2. Erythrocyte

Erythrocyte has been widely examined throughout many studies. Harris has compared between omega-3 index that is measured in RBC, plasma and plasma phospholipids. However, he preferred using RBC to measure EPA+DHA as this index is not influenced by feeding status (blood could be withdrawn from fed or fast individual), RBC is not influenced by day to day dietary intake according to its longer half life shelf (4 to 6 more time longer than plasma) (Sun et al., 2007), RBC can be stored in -80 C° for 4 years (Harris, 2007a). The half-life of EPA in RBC is 4 months thus RBC reflect the last months diet intake (Sun et al., 2007). RBC omega-3 status is believed to have the lowest biological variability because all the RBC's FAs are esterified in the membrane PL, thus it reflects longer status than plasma beside it doesn't alter by the fed state (Harris and Thomas, 2009; Ogura et al., 2010).

Plasma and RBC n-3 LCPUFA composition are higher than those of other lipid fraction such CE and TG. Therefore, RBC and plasma PL are considered as an n-3 LCPUFA biomarker (Kawabata et al., 2011)

The table below shows the advantages of using RBC as an omega-3 FA intake biomarker [modified from (Harris and von Schacky, 2004)].

Table 2.8.2. Advantages of RBCs as biomarkers for omega-3 FA intakes

1. Lipid bilayer—reflects tissue FA composition
2. The Omega-3 Index half-life is 4– 6 times longer than serum EPA + DHA , better reflecting long-term exposure
3. Not influenced by fasting or fed state
4. Responsive towards increasing intakes
5. Correlates well with other biomarkers of omega-3 FA intake (with WB in this study)
6. Less influenced by dyslipidemias than serum FA
7. Less variable than serum EPA + DHA composition
8. Laboratory assessment is simpler than lipoprotein or lipid fraction FA
9. Stable to variations in pre-analytical storage conditions

2.8.3. Plasma

Plasma and serum' PUFA are useful to assess particularly the short term dietary intake (Kuriki et al., 2006; Kobayashi et al., 2001). It is difficult to measure 22:5n6 in plasma because it presents in trace amount and the concentration go higher only in case of n-3 deficiency (Galli et al., 1971; Ratnayake and Galli, 2009). However, plasma FA composition is considered to be a biomarker for the marine origin n-3 PUFA (Kobayashi et al., 2001).

Plasma contains a mixture of lipoprotein associated FAs (cholesteryl ester, TG and PLs) plus the non esterified FAs which make plasma more variable in assessing FAs composition (Harris and Thomas, 2009). While chylomicrons reflect the last meal consumed, triacylglycerols reflect the previous few hours, and CE and PL reflect the previous few days (Arab, 2003).

Fatty acids' changing is faster in plasma than in RBC, and the later still faster than subcutaneous adipose tissue which has the slowest rate (Ogura et al., 2010).

2.8.4. Plasma Phospholipids

Thannhauser has illustrated that extracting phospholipids is efficient with the mixture of chloroform to methanol (2/1) (Thannhauser, 1936). Phospholipids' PUFA in erythrocytes and platelets are useful to assess the medium term dietary intake (Kuriki et al., 2006) while fatty acids of plasma PLs pool tend to reflect day to day intake more than RBC FA pool (Harris and Thomas, 2009; Kawabata et al., 2011). Fatty acid composition in PL has been considered as a good biomarker for omega-3 derived from fish intake. Studies showed an inverse correlation between omega-3 in serum PL and CHD risk (Holub, 2009). However, plasma phospholipids vary among countries, PLs from Caninio in Italy showed significant difference from Nurmijarv in Finland (Ching Kuang 2009).

2.8.5. Plasma Cholesterol ester

As for plasma phospholipids, plasma cholesterol ester content reflects weeks to months of the dietary intake (medium-term) (MA et al., 1995). Fatty acid composition of CE reflects FA pattern of tissue lipids. In 1963, Morris isolated acids from plasma cholesterol esters with 0, 1, 2, 3, 4, 5 and 6 double bonds (Nichols et al., 1966).

Cholesterol ester plasma lipoprotein fatty acid composition is affected by the rate of FA synthesis, FA dietary intake, organism requires and their non-enzymatic degradation (Tvrzická et al., 2002). Cholesterol ester was more sensitive to recent diet and EPA half-life was 4.8 days (Sun et al., 2007).

2.8.6. Adipose tissue

Adipose tissue' PUFAs are useful to assess the long term dietary intake (Kuriki et al., 2006; MA et al., 1995) in which they are reflected in human over 2 and half years (Ogura et al., 2010; Kobayashi et al., 2001) to 3 years (Amiano et al., 2001). 680 days are estimated to be the half-life of LA in adipose tissue (MA et al., 2007).

For studying the EFA status, fatty acids' biomarkers in plasma and RBC PLs seems to be more favourable than adipose tissue. However, adipose tissue has the slowest rate in reflecting fatty acids changing when comparing with other lipids components (Amiano et al., 2001). In addition, participants prefer the blood withdrawal than the adipose tissue biopsy (as it is more invasive) (Amiano et al., 2001; MA et al., 1995) therefore, RBC and Plasma would be more appropriate especially when dealing with large-scale studies (Ogura et al., 2010).

MUFAs are the predominant FAs in Adipose tissue, followed by SFAs while PUFAs has the lowest level of presence. However, according to their liquid form room temperature; n-3 PUFAs do not accumulate in adipose tissue as it is not appropriate (Ogura et al., 2010). The correlation between n-6 FA, LA, and TFA were stronger in adipose tissue than in plasma or whole blood (Sun et al., 2007). The correlation between PUFA from subcutaneous adipose tissue and fat intake measured by FFQ were found to be positive (MA et al., 1995; Sun et al., 2007).

2.9. Recommendation of the quantity and quality of fat intake

It is known that a fat macronutrient yields double the value of energy (E) from an equal amount of grams weight of carbohydrate and protein. Studies approved that fat increases the palatability and reduce the satiety. However, high fat foods have many effects on the body in a way that they are more able to be stored than carbohydrates and proteins are, also they increase the total number and sizes of the body fat cells (Elmadfa and Kornsteiner, 2009)

Table 2.9. Dietary recommendations for fat and fatty acid intake for adults (Elmadfa and Kornsteiner, 2009)

Type of fat	Amount
Total fat E%	20-35
SFAs. E%	10
MUFAs, E%	By differences
Total n3,n6 PUFAs, E%	6- 11
n6 PUFA LA, E%	2.5-9
n3 PUFA : ALA, E%	≥ 0.5
n3 PUFA : EPA + DHA	0.250- 2 g/day
Trans fatty acids, E%	1

2.9.1. Recommendation of fat and fatty acid intake

2.9.1.1. Fats and fatty acid requirements for infant of 0-2 years

During the brain development, intake of LA and ALA is very important (Heude et al., 2002). Energy from fat lay between 40-60% of total energy during the first 6 months. At later stages, after 6 months till 3 years, the energy requirement from fat declined needs to be from 30-35% of total energy depending on the child physical activity. Roles of the lipid as structural components are very important especially for tissues of brain, retina and neural tissues. Those tissues are rich in LCPs especially (Uauy and Dangour, 2009). However, adequate supplements of LCPUFA should be provided to fetuses (Makrides, 2000).

Human milk is a good source for the DHA and AA and their precursors LA and ALA. DHA level in human milk depends on the maternal diet while AA is more constant. Therefore to supply the infant who depends on his mother's milk with DHA, mothers are therefore advised to be supplemented with DHA. While for the infant fed on formula, human milk considered the ideal to be matched in the concentration of LCPUFA, EPA, DHA and AA. DHA concentration is between 0.2 to 0.5% of total FAs. Added AA should be around the same concentration of DHA, also EPA should not exceed the DHA. EPA and DHA should be maximally account for 1.5% of the total energy in the formula (Uauy and Dangour 2009)

In order to prevent nutrition-related chronic diseases in infants aged 0-2 years, total fat intake should lay between 30-40 % according to the infant's physical activity. PUFAs in total are between 5-15% of total energy, including 4-13% from n-6 and 1-2% from n-3 of total energy. MUFA's requirement has no restriction within the total fat range while cholesterol should not exceed 300 mg/day (Uauy and Dangour, 2009). There is a hypothesis that n-3 intake during infancy reduce the risk of type 1 diabetes and both infection and non infection diseases (Undurti, 2008)

2.9.1.2. Fats and fatty acid requirements for children of 2-18 years

For children over 2 years, intake of SFA should not exceed 10% of total energy. PUFA's requirements lay between 5-15% by giving attention to the ratio between omega 6 and omega 3 to be between 5:1 and 10:1. No restriction to MUFA's within the total fat range with highly restricted to the hydrogenated fats (Uauy and Dangour, 2009).

2.9.1.3. Fats and fatty acid requirements for adults

Total fat intake requirements vary according to the individual's physical activity. The moderate physical activity individual would need 30% of energy while high physical activity individual requirements increase to 35% of energy. However, the acceptable macronutrient distribution range (AMDR) varies between 20 and 35% of total energy (minimum 15%, maximum 35%). A maximum level of SFAs should not exceed 10% of

total energy intake. However, MUFAs requirements depend on the total fat and fatty acid intake pattern. FAO/WHO calculated MUFAs intake by: Total fat intake minus SFAs minus PUFAs minus TFAs. The maximum PUFAs (n-3 and n-6) intake is 11%. ALA (n-3 FAs) requirements encouraged to achieve between 0.5 and 2% from total energy; 0.5% is the minimum in order to avoid deficiency symptoms. Therefore the lower range of LA (n-6 FAs) would be 2.5 while the average can reach 9% from total energy. TFAs intake adjusted for gender. The average acceptaned values for humen are approximately 1.5 g and 0.9 g for men and women respectively. Alterantively, near 0.5% E for both genders (Elmadfa and Kornsteiner, 2009).

However, for adults in the US, the Food And Drug Administration (FDA) in 1997 considered the safe dose is up to 3 g/day of EPA and DHA, although there were no significant clinical symptoms (bleeding) noticed with high dose of EPA and DHA (Harris and von Schacky, 2004). The American Society for Nutrition has published that taking high dose of EPA and DHA (3.4 g/d) lowers significantly serum triglycerides 27% comparing with 0.85 g/d (Skulas-Ray et al., 2011).

2.9.1.4. Fats and fatty acid requirements for pregnancy and lactation

FA n-3 and n-6 LCPUFA especially DHA requirement are high and very important during pregnancy (especially during the last trimester) for the fetus development especially that maternal DHA plasma phospholipids significantly decrease during pregnancy. It also contributes in the structure of brain cell membrane and prostaglandins and eicosanoids production (Makrides, 2000).

For the total fat intake requirements, pregnancy and lactating women needs do not differ from the non-pregnant and non-lactating ones of the same age. No additional requirements for SFAs and MUFAs while a warranty restriction for the food contain Partially Hydrogenated Vegetable Oil (PHVO). DHA recommendation in lactation and pregnancy is at least 200 mg/day, however, for pregnancy the highest demand will be in the third trimester. In case of pregnancy; the average for both DHA and EPA is 300

mg/day, while the AA upper limit is 800 mg/day (Brenna and Lapillonne, 2009). LCPUFA status is generally decreased in maternal and infant during the lactating period (Kuipers et al., 2011).

Smoking during pregnancy will affect infant's PUFAs level and reduce plasma concentration (20:3n6, AA, DHA, 20:3n6/LA, AA/20:3n6 and DHA/ALA) of the newborns (Ratnayake and Galli, 2009).

2.9.2. Recommendation of the n-6/n-3 ratio:

Holman found that when ALA intake is increased, n-6 family production is consequently suppressed. In contrast, when LA intake is increased, n-3 family production is consequently suppressed. That would explain the competition between n-3 and n-6 on the metabolism reaction enzymes. Increasing the intake of ALA to < 2% suppresses n-6 metabolism while LA intake should be increased 10 times to suppress n-3 metabolism to the same degree (Holman, 1998).

n-3 and n-6 hold important roles as precursors of bioactive lipid mediators, consequently the different ratios of n-6/n-3 might lead to different levels of those lipid mediators and metabolites (Gomolka et al., 2011).

Decreasing the intake of LA from 7 to 3% energy increases the EPA synthesis while increasing the intake of ALA from 0.3 to 1.1% energy decreases the DHA synthesis. In another way, increased intake of ALA and decreased intake of LA would be recommended to stimulate the synthesis of EPA and DHA. ALA and LA biological roles depend on the balance of their dietary intake, thus FAO/WHO in a 1994 meeting recommended a dietary intake balance to be between LA:ALA 5:2 and 10:1 (Ratnayake and Galli, 2009).

Over the past century, LA had been the area of medical interest and people were encouraged to increase their LA intake as the only source of PUFA while n-3 PUFA were ignored. The higher dietary intake of animal's meat rather than fish led to a fat intake

rich in LA and poor in ALA. All of the previous points led to n-3 EFAD especially in the American population (Holman, 1998).

The ratio of LCPUFA n-6:n-3 is used to present n-3 biostatus in addition to EPA+DHA (omega-3 index) (Harris and Thomas, 2009). n-6:n-3 ratio would be decreased with diet high in EPA and DHA which found in Japanese participants (increased n-3 and decreased n-6) (Ogura et al., 2010).

2.10. History of Fatty acids analysis

Through 1900s, fatty acids science was more sophisticated and had more achievement. Normann patented his discovery of the conversion of unsaturated fatty acids or their glycerides into saturated compounds by hydrogenation (Normann, 1903). Morrison was the first to prepare FAME with described boron trifluoride (Morrison et al., 1964).

It was the first time when Folch described the isolation of phospholipids, phosphatidylserine and phosphatidylethanolamine from brain (Folch, 1942). Folch established the lipids extraction method that is still used by most laboratories. He used the mixture of chloroform/methanol (2/1 Vol) (Folch et al., 1957)

Until mid 1940s, no method was established yet to evaluate individual fatty acid in mixtures (Holman, 1998). Burr and his students were the first to work on the method to distinguish PUFA's family. They measured the double bonds numbers by using alkaline isomerization and ultraviolet. Via this method they found the lionleic acid was the precursor of AA, that PUFA respond to fat intake, and finally that trienoic acid would be an index the EFA as its concentration increased (Burr and Holman, 1988).

2.11. Analysis of fatty acids by Gas Chromatography

Mikhail Tswett (a Russian botanist) can be considered the first who applied chromatography to separate various plant pigments by passing solution through glass column on a chalk column in 1903. Afterwards, scientists tried to minimize the column sizes to increase the separated efficiency, till 1960 columns sizes reach as small as 3 to 10 µm. then glass columns were replaced by stainless steel (Lin et al., 2009).

James and Martin are considered the first to use and publish gas chromatography (GC) in assessing the underivatized fatty acids, they described the method of free fatty acids separation (from 1 to 12 C) (James and Martin, 1952), in later years they used shorter FAME form to assess fatty acids by GC (James and Martin, 1956; James and Wheatley, 1956). However, Mohrhauer and Holman validated the EFA analyzing by GC method to

analyze EFA and their metabolic products in liver, with the alkaline isomerization method. Results were the same which established all the old results from studies used the later method (Holman, 1998; Mohrhauer and Holman, 1963).

The main three parts of the GC equipment are:

- The instrument-injector.
- The column.
- The detector.

Through those three parts, fatty acid –in the past- might lost while recently such techniques are improved and losing problems are solved in which all the process being fully automated and computerized (Tvrzická et al., 2002).

Analyzing fatty acids by GC involves main steps: lipid extraction from cells or tissue, methylation, then the injection into the GC (Ratnayake and Galli, 2009; Masoodet et al., 2005; Kurik et al., 2006) which is called the *conventional method*. It is applied in most lipid analysis laboratories (Kang and Wang, 2004).

However, there is an argument made about a simplified method in which the extraction (homogenate using organic solvents) is combined with the methylation. This has advantages such as saving time, materials and solvents, simple (easy to do), and can be used with dried or liquid small sample amount with large samples number. There is a study which has assessed the fatty acids profile in mouse heart tissue. Compareing the two methods (conventional method that combine extraction before methylation and simplified method without prior extraction) has concluded that the results from the simplified method is good. Also, It is more suitable for long chain PUFA ≥ 18 C. However, prior extraction is still needed for analyzing fatty acids from Phospholipids (Kang and Wang, 2004).

In another study, GC technique was endeavoring to shorten the time consumed in analyzing human plasma FAME. While the standard method consume approximately >70 minutes per sample, the adapted fast GC method reduces the time to <12 minutes

which makes it more reasonable for large studies. Nevertheless 24:1n9 would be overlapped with DHA thus this method is not appropriate for analyzing samples that contain those two fatty acids (Masood et al., 2005).

TLC method is quite slow, time- and material-wise comsuption (Kang and Wang, 2004). It can oxidize PUFA if exposed to air, thus it is not appropriate for large number of samples, it can also generate organic waste and possible contamination which make cholesterol ester and phospholipid method inconvenient (Burdge et al., 2000).

3. Materials and methods

3.1. Study design

This study is a part of the Austrian Study on nutritional status and aimed to assess the essential fatty acids status at population level. The observational study included 140 participants (69 female, 71 male). The mean age (years) at enrollment was 38.8 ± 11.1 for the female, 37.8± 11.2 for the male. The mean body mass index (BMI) was 23.4 ± 3.01 kg/m^2 for the female and 24.53 ± 2.6 kg/m^2 for the male. Dietary intakes were assessed by a 24h recall and a food frequency questionnaire (FFQ). Fasting blood glucose (no caloric intake within last 8 hours) ranges between 4-6 mmol/l (60 -110 mg/dl), it reflects normal blood glucose. If the range goes higher than 110 mg/dl, it reflects hyperglycemia (Earl et al., 2002) ranged from 6 to 11 mmol/l (Preissig and Rigby, 2010), while beneath 60 mg/dl it reflects hypoglycemia (Miller et al., 2001) similar to blood glucose ≤2·2 mmol/l (Vlasselaers et al., 2009).

The American Heart Association had established guidelines for triglyceride levels in which Normal range, low risk < 150 mg/dl (<1.7 mmol/l), borderline high 150- 199 mg/dl (1.7- 2.2 mmol/l), and high level of TG when higher than 200 mg/dl (>2.2 mmol/L)(American Heart Association, 2010; Genest et al., 2009).

3.2. Subjects characteristics

Table 3.2.: Subject characteristics (age and BMI are shown as means ± SD)

	Total	**Female**	**Male**
n	140	69	71
Age (Years)	38.28 ± 11.13	38.8 ± 11.12	37.77 ±11.20
BMI (kg/m^2)	23.97 ± 2.86	23.4 ± 3.01	24.53 ± 2.6
Smoking (Y) **n (% of sample)**	79 (56%)	35 (51%)	44 (62%)
Normal blood glucose (4-6 mmol/l) **n (% of sample)**	109 (88%)	54 (78%)	55 (77%)
Hypoglycemic blood glucose (< 2.2 mmol/l) **n (% of sample)**	18 (13%)	11 (16%)	7 (10%)
Normal blood TG (<1.7 mmol/l) **n (% of sample)**	122 (87%)	65 (94%)	57 (80%)
Hyperlipidemia (> 2.2 mmol/l) **n (% of sample)**	9 (6%)	2 (3%)	7 (10%)

3.3. Analysis of fatty acids by Gas Chromatography

Fatty acids pattern was analysed with gas chromatography (*PERKIN Elmer, Vienna, Austria*). Carrier gas was Helium and equipped with a 30m X 0.25 mm ID fused silica column (RTX-2330) and a flame ionization detector (FID) which is a universal detector. Most nuitrition practisioners use capillary columns of 30m X 0.32 (or 0.25) mm length as it is adequate for analytic labour (Ratnayake and Galli, 2009). The separation of FAME appears on the GC baseline according to the FA chain length, number of double bonds and their position (Tvrzická et al., 2002).

The samples were dissolved in 50 µl hexane (*Vial: CHROMACOL C 821 UK; biolab Ges.m.b.H*) and 1.0 µl was injected in the gas chromatography with 30:1 split flow with helium as a carrier gas. The experiment time was 29.14 minutes, sampling rate was 6.25000 pts/s and FAMEs were identified by FID which was 0.38 mV. FID is still used because of its reasonable price in comparison to mass spectrometric (Tvrzická et al., 2002). The initial temperature was 90 deg, ramp 1 with 13/min to 155 C°, ramp 2 with 2.9/min to 185 C° and held for 5 min, ramp 3 with 5/min to 229 C°. It is worth mentioning that the temperature program affect the resolution of all interested components within suitable time (Tvrzická et al., 2002).

The order of the fatty acids in the baseline depends on the numbers of carbon atoms and then the degree of unsaturation. The four or five double bonds are appeared after the SFA FAME, in addition PUFA n-6 family appeared after PUFA n-3 even if they have same carbon atoms number and double number (Tvrzická et al., 2002).

The methylation was controlled by methylating Fatty acid supplement: *SAFC Biosciences F7050; 115K5410*. Free Fatty Acids FFAs were identified with reference to standard mixture of known composition Standard -*C37, Supelco analytic 18919-1AMP; LB63416*- is run on the same column under identical condition. The area % fatty acid compositions are calculated and all peak areas of the identified fatty acids were taken as 100%.

3.4. Overview of the parameters

Table 3.4. Overview of the analysed parameters

Parameters	**Matrix**	**Methods**
Fatty acids in whole blood	Whole blood	Methyl/ BF_3; GC
Fatty acids in Red Blood Cells	Red Blood Cells	Methyl/ BF_3; GC
Fatty acids in Plasma	Plasma	Methyl/ BF_3; GC
Fatty acids in Plasma Phospholipids	Plasma	TLC; Methyl/ BF_3; GC
Fatty acids in Plasma Cholesterol Ester	Plasma	TLC; Methyl/ BF_3; GC

BF_3= Boron trifluride methanol solution 14%, Methyl = Methanol for analysis, GC= Gas chromatography, TLC= Thin layer chromatography

3.5. List of fatty acids analysed

Table 3.5. List of fatty acids analysed (Adapted from Kornsteiner 2008)

Fatty Acid	**abbr.**	**common name**	**systematic name**
14:0		Myristic acid	Tetradecanoic acid
15:0		pentadecanoic acid	pentadecanoic acid
16:0		Palmitic acid	Hexadecaneoic acid
17:0		Margaric acid	Heptadecanoic acid
18:0		Stearic acid	Octadecanoic acid
20:0		Arachidic acid	Eicosanoic acid
16:1n7		Palmitoleic acid	cis9-Hexadecenoic acid
18:1n9 trans		Elaidic acid	trans9-Octadecenoic acid
18:1n7 trans		trans-Vaccenic acid	trans11-Octadecenoic acid
18:1n9 Cis	OA	Oleic acid	cis9-Octadecenoic acid
18:1n7 Cis		Vaccenic acid	cis11-Octadecenoic acid
20:1n9		Gadoleic acid	cis11-Eicosenoic acid
18:2n6 trans		Linolelaidic acid	
18:2n6 Cis	LA	Linoleic acid	all-cis9,cis12-Octadecadienoic acid
18:3n6	GLA	γ-Linolenic acid	all-cis6,9,12-Octadecatrienoic acid
20:2n6		Eicosadienoic acid	*all-cis*-11,14-eicosadienoic acid
20:3n6	GDGLA	Dihomo-γ-linolenic acid	all-cis8,11,14-Eicosatrienoic acid
20:4n6	AA	Arachidonic acid	all-cis5,8,11,14-Eicosatetraenoic acid
22:4n6	DTAn-6	Adrenic acid	all-cis7,10,13,16-Docosatetraenoic acid
18:3n3	ALA	α-Linolenic acid	all-cis9,12,15-Octadecatrienoic acid
20:5n3	EPA	Timnodonic acid	all-cis5,8,11,14,17-Eicosapentaenoic
22:5n3	DPAn-3	Docosapentaenoic acid	all-cis7,10,13,16,19-Docosapentaenoic acid
22:6n3	DHA	Cervonic acid	all-cis4,7,10,13,16,19-Docosahexaenoic acid
20:3n9	MA	Mead acid	all-cis11,14,17-Eicosatrienoic acid

3.6. Calculations of the fatty acid indexes

Table 3.6. Fatty acid indexes' calculations.

SFA	14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0
Atherogenic SFA	14:0 + 16:0
TFA	18:1n9t + 18:1n7t + 18:2n6t
Atherogenic FA	14:0 + 16:0 + TFA
Cis-MUFA	16:1n7 + 18:1n9c + 18:1n7c + 20:1n9
Total-MUFA	16:1n7 + 18:1n9c + 18:1n7c + 20:1n9 + 18:1n9t + 18:1n7t
Cis-PUFA	18:2n6c + 18:3n6 + 20:2n6 + 20:3n6 + 20:4n6 + 22:4n6 + 18:3n3 + 20:5n3 + 22:5n3 + 22:6n3 + 20:3n9
Total-PUFA	18:2n6c + 18:3n6 + 20:2n6 + 20:3n6 + 20:4n6 + 22:4n6 + 18:3n3 + 20:5n3 + 22:5n3 + 22:6n3 + 20:3n9 + 18:2n6t
USFA	Cis-MUFA + Cis-PUFA
LCP-n 6	20:2n6 + 20:3n6 + 20:4n6 + 22:4n6
LCP-n 3	20:5n3 + 22:5n3 + 22:6n3
n3 index	20:5n3 + 22:6n3
n-6	20:2n6 + 20:3n6 + 20:4n6 + 22:4n6 + 18:2n6c + 18:3n6
n-3	20:5n3 + 22:5n3 + 22:6n3 + 18:3n3
n-7	16:1n7 + 18:1n7t + 18:1n7c
n-9	18:1n9t + 18:1n9c + 20:1n9 + 20:3n9
LA/ ALA	18:2n6c / 18:3n3
Holman Index	20:3n9 / 20:4n6
n3 HUFA score	LCPn-3 / LCPn-3+LCPn-6

3.7. Reagents

- Methanol: Methanol for analysis, AMSURE. 1.06009.2500.
- Standard fatty acid supplement: SAFC Biosciences F7050; 115K5410.
- Sodium hydroxide: Riedel- de. Haën 06203.
- n-Hexane p.a. MERK KGaA 1.04367.2500.
- Chloroform p.a. MERCK KGaA 1.02445.2500.
- 2,6-Di-tert-butyl-p-cersol (BHT) Butylated Hydroxytoluene SIGMA B-1378 [128-37-0].
- BF_3 Boron trifluride methanol solution 14% SIGMA B-1252; Batch 078K5301.
- Diethyl ether: ANALAR NORMAPUR 23811.326.
- Heptane (Petroleum) Riedel- de. Haën 32287.

- Acetic acid 99.8% SIGMA- ALDRICH 33209.
- 11 mM tris-buffer pH 7.6: 4 g tris-(hydroxymethyl)-aminomethan (Riedel-de Haën 33742) are dissolved in 3000 ml demineralized water.
- Cholesterol analytic calibration standard for GC, Serva CAS. No. 57-88-5.
- MeOH extracting agent: 50 ml Mehtanol+ 3mg BHT+1g NaOH; for 45 minutes in ultrasonic bath.
- Folch extract : Chloroform p.a.: Methanol p.a. 2:1 v:v + 50mg BHT.

3.8. Equipments

- Drier: CAMAG TLS Plate Heater III CERAN SCHOTT ; cat: 022.3306 ; ser no: 121102.
- TLC: CAMAG ; cat: 022.7808; ser no: 121027 LINOMAT 5 .
- Freezer: SANYO model: MDF-U53V; serial no: 09080772.
- TLC Plates: Silica gel 60 F254 glass plates 20X20 cm, MERCK 1.05715.0001 HX 953371.
- Vial: CHROMACOL C 821 UK; biolab Ges.m.b.H.
- Needles: HAMILTON 1750 LTN; ga 22/51 mm/pst 2) 500 µl; P/N 8121 7/01; WO: 137422.
- Technospin Sorvall Centrifuges Instruments DU PONT.
- Champers.

3.9. Analyzing Fatty Acids from different tissues/systems

3.9.1. Preparation and methylation

In general, analyzing fatty acids from body tissues goes through 3 main steps: tissue preparation to extract the lipids, transformation the derivatives lipids to fatty acid methyl esters (FAMEs) (KANG and WANG, 2004), and then to detect fatty acids profile usually GC is used to analyze FAMEs (Ratnayake and Galli 2009). The method from the five systems resembles each other starting from the second step. However, extracting the lipids from the tissues and cells differ according to the system nature.

Venous blood was drawn from the participants after an overnight fast into a tube with EDTA (as anticoagulant) and transferred to the institute laboratory in a locked dry ice box to avoid any enzymatic lipolysis. From each participant; 100 µl of whole blood,

400 µl of plasma (300 µl in separated cup for CE and PL and 100 µl for total plasma), and 200 µl of erythrocyte packed cells were filled in cups labelled with the participant's ID number and stored in a -80 C^0 freezer. During the analysis phase, cups were taken out from the freezer and liquefied by hand temperature. Samples were saponified with 1 ml NaOh in methanol for 5 minutes at 100 C^0 boiled water bath then fatty acids were methylated using 1 ml BF_3 at 100 C^0 boiled water bath for 5 minutes then extracted with Hexane. A new bottle of BF3-MeOH reagent; in about weekly basis; was used to avoid any artifact production to prevent the loss of HUFA as the BF3-MeOH has a limit shelf life even when refrigerated (Ratnayake and Galli, 2009).

MeOH extract agent (50ml Methanol/1gNaOH/3mg BHT) was used for the analysis of fatty acids by the method of (Folch et al., 1957). BHT is believed to minimize fatty acids oxidation (Bailey-Hall, 2008). Fatty acids were transmethylated by alkaline methanolysis (1 ml) using the BF3 (1 ml) reagent kit. However NaOH salt alters the lipids distribution and eradicates them from the upper phase while in the absence of it; lipids might be lost during washing (Folch et al., 1957). The antioxidant 2,6-Di-tert-butyl-p-cersol (60mg/l) was added to the MeOH to prevent the USFAs oxidation.

Fatty acid methyl esters (FAMEs) were washed and extracted with 500 µl Hexane (enhancing the efficiency of fatty acid methylation) (Araujo et al., 2008). The resulting assortment separated into two phases. The lower phase is the total pure lipids extract (Folch et al., 1957). The hexane upper layer was transferred to a new glass tube and evaporated with nitrogen (N2) then FAMEs were ready to be resolved by gas chromatography. The extracts were dissolved with 50 µl hexane and flushed with nitrogen then stored in the labelled GC Vials (CHROMACOL C 821 UK; biolab Ges.m.b.H) at -80 C^0 for up to 3 months prior to GC analysis for the fatty acid composition.

3.9.2. Extracting Fatty Acids Methyl Ester from venous whole blood

A 35 µl of whole blood were transferred to glass tube and transmethylated using the method of Jordi Folch directly from the venous blood tubes to detect the fatty acids profile (Folch et al., 1957). However, using BF_3/MeOH FAME method transesterified TG thus it is appropriate for analyzing whole blood fatty acids (Bailey-Hall et al., 2009). The CVs for the major fatty acids in whole blood were as follows: 2.09% for 16:0, 3.71% for 18:0, and 5.48 % for cis 18:1n–9, 3.82% for 18:2n–6, 5.06% for 20:4n–6, 9.94% for 18:3n–3, 4.81% for 20:5n–3, and 5.73% for 22:6n–3.

3.9.3. Extracting Fatty Acids Methyl Ester from plasma

Plasma was immediately separated from erythrocytes by centrifugation at 4000 rpm at room temperature for 10 minutes. A 35 µl of the sample were transferred to glass tube and transmethylated by the method of Jordi Folch (Folch et al., 1957). The CVs for the major fatty acids were as follows: 2.13% for 16:0, 1.86% for 18:0, 6.85% for cis 18:1n–9, 3.37% for 18:2n–6, 5.10% for 18:3n–3, 7.08% for 20:5n–3, and 4.04% for 22:6n–3.

3.9.4. Extracting Fatty Acids Methyl Ester from erythrocytes

As mentioned above; plasma was immediately separated from erythrocytes by centrifugation at 4000 rpm at room temperature for 10 minutes. Then erythrocytes-packed cells (1.5 g) were treated with 11mM tris-buffer. Erythrocytes packed cells were washed 3 times with an EDTA -containing saline solution to remove white cells and plasma contamination (Bailey-Hall et al., 2008). A 35 µl of the sample were transferred to glass tube and transmethylated by the method of Jordi Folch (Folch et al., 1957).

The CVs for the major fatty acids were as follows: 2.62% for 16:0, 2.41% for 18:0, 2.01 % for cis 18:1n–9, 3.75 % for *trans* 18:1n–9, 2.22 % for 18:2n–6, 2.76% for 20:4n–6, 4.11% for 18:3n–3, and 4.94% for 22:6n–3.

3.9.5. Extracting Fatty Acids Methyl Ester from plasma phospholipids and plasma cholesterol ester

Plasma phospholipids and plasma cholesterol ester were extracted from blood plasma. The Isolation of the lipids classes were completed by TLC (thin layer chromatography). However, TLC considered as the most convenient technique for small amount of lipids components isolation and to achieve excellent separation (Ratnayake and Galli, 2009). Separation can be achieved by 1 dimensional TLC in single run using mobile phases consisting of a mixture of Petroleum and diethyl ether with acetic acid.

The lipids were extracted from 200µl Plasma by mixing the sample with 5ml Folch extract. The mixture was shacked in cool dark room for 1 hour. After centrifuged on 4000 rpm for 15 minutes; upper layer was abated while the waste shifts to the base of the tube. The extract was evaporated with nitrogen (N2) gas in warm bath (40 C^0) and the lipids were re-dissolved in 100µl chloroform.

However; 500µl of plasma were trailed with 5ml of extract; the shake mixtures outcome was not clear, harder to be abated with very high FFA concentration in the GC baseline. Therefore 200µl Plasma was used in this method.

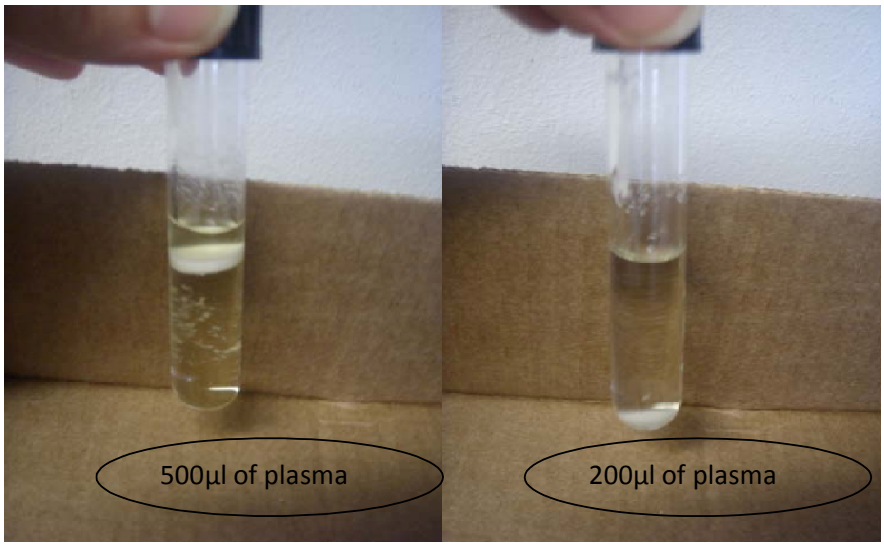

Fig 3.9.5. Lipids extracted from plasma by Folch extract.

TLC was performed by using Silica gel *60 F254* glass plates 20X20 cm from *MERCK*. First the plates were activated with 70 ml methanol in the chamber for 1hr and 15minutes. Standards and aliquots of the lipid extract were applied as narrow bands (0.5 cm in length) on the plate. During the sample injection and application; a flow of nitrogen was blown across the injection point to facilitate the spotting of a tight lipid band. Six samples were spotted per plate (1.5 cm space); though, 12 and 9 samples could be trailed per plate; but encountered CE separations materialized.

Meanwhile the extraction chamber is activated by a modified solvent system of (85ml Petroleum+ 15ml diethyl ether + 2ml acetic acid) for 35 minutes. Solvent system separated the sum of sphingo- and phospholipid fraction from the cholesterol fraction (Kornsteiner 2008). The plate with the injected samples was placed in the extraction chamber for 35 minutes in order to apply the phospholipids and cholesterol ester extraction from the lipids. The PL and CE fractions were made visible under iodine vapor. Lipids bands were identified by co-migration with standard phospholipids and standard cholesterol ester and quantified under identical conditions.

The phospholipids and cholesterol ester fractions were scraped into separate labelled test tubes for the preparation of fatty acid methyl esters process. Each phospholipid and cholesterol ester sample was then esterified in the presence of methanolic NaCl/ BF_3 and analyzed by gas -liquid chromatography.

The CVs for the major fatty acids were as follows for *phospholipids*: 6.97% for 16:0, 5.32% for 18:0, 9.63% for 18:2n–6 *cis*, 7.95% for 18:2n-6 *trans*, 9.20% for 20:4n–6, 9.13% for 18:3n–3, 7.8% for 20:5n3.

Additionally for *cholesterol ester*: 7.67% for 16:0, 4.94% for 18:0, 10.85% for 18:2n–6 *cis*, 7.98% for 18:2n-6 *trans*, 9.7% for 20:4n6, 10.25% for 18:3n–3, 11.68% for 20:5n–3.

Table 3.9. Summary for the main steps in analyzing:

Sample/tissue	**Sample µl**	**Me/NAOH ml**	**BF_3/ml**	**Extraction (ml)/N2**	**Dissolved µl Hexane**
Whole Blood	35	1	1	2 (4x0,5)	50
Erythrocytes	35	1	1	2 (4x0,5)	50
Plasma	35	1	1	2 (4x0,5)	50
Fatty acid supplement	15	1	1	2 (4x0,5)	50
Phospholipids/ Cholesterol ester	200 µl of the sample in 5 ml Folch extraction	1	1	2 (4x0,5)	50

3.10. Statistical analysis

All statistical analyses were implemented by using version 17.0 of the SPSS statistical package. GC computer program for fatty acids integration was performed using version 6.3.0.0445 TotalChrom Workstation; PerkinElmer. Fatty acids' Area [µV.s] from the Chromatogram were completed and calculated by using Microsoft ®Office Excel 2007 (12,0,4518,1014) MSO (12,0,4518,1014).

Student's T-Test was used to calculate the correlation between normal distribution values while Mann-Whitney-U-Test to calculate correlation of the un-normal distribution values. P-values were considered as significant for $p < 0.05$.

Partial Spearman's rank-correlation coefficients were calculated for the influence of BMI, age, gender, and smoking status on the fatty acids profile. Normally this Partial correlation can only be carried out with normal FA distribution. Explanatory variables were either continuous (age (y), body mass index (BMI kg/m^2) or categorical (Gender (Male\Female), smoking (Y\N), blood glucose (normal, hyperglycemia and hypoglycemia), triglyceride (normal/ hyperlipidemia). To compare the continuous covariates between levels of the categorical factors, two sample t-tests were performed, whilst associations between the categorical factors were assessed using Pearson's correlations for the normal distribution and Spearman nonparametric coefficient correlation.

For each of the whole blood, RBC, plasma, plasma phospholipids and plasma cholesterol ester fatty acids, the mean and two standard deviations (2 SD) were tabulated after checking that the distribution of each fatty acid was approximately normal. Pearson's correlation was used to assess the relation between the FAs index in the 5 different methods. Spearman rank correlation coefficient was of similar degree and was used to determine the strength of the association.

The aim of the study is two-fold: first, to find the best blood component for essential fatty acids analys; and second, to assess the essential fatty acid status in the tested population.

4. Results

Data on the range of the fatty acids contents of whole blood, RBC, plasma, plasma phospholipids and plasma cholesterol ester of 140 Austrian healthy adults. This present study attempts to find the most appropriate method for a large number of samples that:

- Gives an accurate fatty acid profile (especially essential fatty acids in concern),
- Is easy-to-do (may not require professional workers),
- And consumes less time, less material, less agents and less sample size.

4.1. Fatty acids in whole blood, red blood cells, plasma, plasma cholesterol ester, plasma phospholipids

Of the 140 blood samples; 120 WB samples, 131 RBC samples, 131 plasma samples, 128 CE samples and 117 PL samples were analysed. Same ID of the blood samples were matched through the five blood components. 24 fatty acids were analysed and their means are tabulated in the next table.

Table 4.1. Fatty acids of whole blood, red blood cells, plasma, plasma cholesterol ester, and plasma phospholipids (mean ±SD).

% of total fatty acids					
Fatty Acid	**WB**	**RBC**	**Plasma**	**CE**	**PL**
	Total n= 120	total n=131	total n= 131	Total n=128	total n=117
14:0	0.65 ± 0.28	0.21 ± 0.07	0.88 ± 0.36	0.83 ± 0.26	0.46 ± 0.15
15:0	0.42 ± 0.22	0.30 ± 0.14	0.38 ± 0.18	1.00 ± 0.53	0.78 ± 0.24
16:0	21.54 ± 1.83	19.35 ± 1.28	23.03 ± 2.42	12.51 ± 1.25	31.0 ± 3.13
17:0	0.30 ± 0.06	0.34 ± 0.13	0.30 ± 0.08	0.19 ± 0.12	0.65 ± 0.19
18:0	11.41 ± 1.08	19.82 ± 1.14	7.63 ± 0.95	1.69 ± 0.81	19.84 ± 3.08
20:0	0.17 ± 0.08	0.21 ± 0.06	0.11 ± 0.07	0.09 ± 0.10	0.23 ± 0.19
16:1n7	1.54 ± 0.54	0.40 ± 0.11	2.12 ± 0.70	2.63 ± 1.04	0.69 ± 0.20
18:1n9 trans	0.18 ± 0.07	0.22 ± 0.06	0.15 ± 0.05	0.25 ± 0.17	0.17 ± 0.05
18:1n7 trans	0.22 ± 0.08	0.24 ± 0.07	0.17 ± 0.07	traces	0.24 ± 0.08
18:1n9 Cis	19.21 ± 1.85	14.13 ± 0.91	21.04 ± 2.59	18.37 ± 2.20	8.72 ± 1.72
18:1n7 Cis	1.59 ± 0.21	1.32 ± 0.15	1.69 ± 0.25	1.21 ± 0.37	1.47 ± 0.29

Fatty Acid	WB	RBC	Plasma	CE	PL
20:1n9	0.36 ± 0.10	0.43 ± 0.10	0.33 ± 0.14	0.11 ± 0.08	0.21 ± 0.19
18:2n6 trans	0.12 ± 0.06	0.12 ± 0.09	0.13 ± 0.11	0.23 ± 0.18	0.21 ± 0.19
18:2n6 Cis	22.57 ± 2.37	10.34 ± 1.11	28.11 ± 3.61	49.47 ± 3.39	18.41 ± 2.44
18:3n6	0.31 ± 0.10	0.08 ± 0.08	0.43 ± 0.14	0.88 ± 0.32	0.15 ± 0.09
20:2n6	0.31 ± 0.07	0.34 ± 0.07	0.29 ± 0.13	0.34 ± 0.27	0.68 ± 0.20
20:3n6	1.86 ± 0.37	2.08 ± 0.42	1.72 ± 0.39	0.82 ± 0.22	2.61 ± 0.85
20:4n6	10.60 ± 1.49	17.23 ± 1.28	7.19 ± 1.46	6.39 ± 1.60	9.64 ± 1.57
22:4n6	1.29 ± 0.40	3.94 ± 0.66	0.36 ± 0.29	0.75 ± 0.51	0.29 ± 0.24
18:3n3	0.47 ± 0.18	0.18 ± 0.06	0.66 ± 0.17	0.62 ± 0.18	0.21 ± 0.07
20:5n3	0.71 ± 0.27	0.72 ± 0.25	0.73 ± 0.35	0.64 ± 0.33	0.58 ± 0.30
22:5n3	1.26 ± 0.24	2.77 ± 0.45	0.56 ± 0.17	0.17 ± 0.13	0.82 ± 0.29
22:6n3	2.77 ± 0.64	5.10 ± 1.00	1.82 ± 0.52	0.74 ± 0.37	1.80 ± 0.58
20:3n9	0.14 ± 0.05	0.13 ± 0.05	0.16 ± 0.07	0.08 ± 0.04	0.14 ± 0.05
SFA	34.48 ± 1.70	40.23 ± 1.51	32.33 ± 2.61	16.31 ± 1.81	52.95 ± 3.37
TFA	0.52 ± 0.16	0.59 ± 0.16	0.46 ± 0.17	0.47 ± 0.28	0.62 ± 0.23
Atherogenic FA	22.71 ± 2.08	20.14 ± 1.35	24.37 ± 2.71	13.82 ± 1.32	32.08 ± 3.04
∑ MUFA	23.10 ± 2.15	16.75 ± 0.94	25.51 ± 2.91	22.57 ± 2.92	11.51 ± 1.94
∑ PUFA	42.42 ± 3.05	43.02 ± 1.65	42.17 ± 4.17	61.11 ± 3.02	35.54 ± 2.56
USFA	64.99 ± 1.77	59.18 ± 1.56	67.21 ± 2.66	83.21 ± 1.90	46.43 ± 3.43
PUFA/SFA	1.23 ± 0.14	1.07 ± 0.07	1.32 ± 0.21	3.78 ± 0.50	0.67 ± 0.09
USFA/SFA	1.89 ± 0.14	1.47 ± 0.09	2.10 ± 0.24	5.17 ± 0.64	0.88 ± 0.12
LCP n6	14.06 ± 1.77	23.59 ± 1.70	9.56 ± 1.55	8.29 ± 1.66	13.22 ± 1.77
LCP n3	4.74 ± 0.91	8.59 ± 1.33	3.12 ± 0.82	1.55 ± 0.58	3.20 ± 0.76
∑ LCPUFA	18.80 ± 2.14	32.18 ± 1.84	12.68 ± 2.00	9.85 ± 1.92	16.42 ± 1.95
∑ n3	5.21 ± 0.91	8.76 ± 1.34	3.31 ± 0.82	2.17 ± 0.62	3.41 ± 0.77
∑ n6	36.94 ± 2.83	34.00 ± 1.72	38.23 ± 3.98	58.64 ± 3.09	31.78 ± 2.58
∑ n7	3.35 ± 0.63	1.96 ± 0.20	3.99 ± 0.79	3.84 ± 1.10	2.41 ± 0.38
∑ n9	19.89 ± 1.87	14.91 ± 0.93	21.68 ± 2.60	18.81 ± 2.22	9.24 ± 1.74
n6/n3	7.29 ± 1.34	3.99 ± 0.72	3.22 ± 0.77	29.31 ± 8.56	9.81 ± 2.41
n3/n6	0.14 ± 0.03	0.26 ± 0.05	0.33 ± 0.08	0.04 ± 0.01	0.11 ± 0.03
LA/ALA	52.51 ± 15.84	64.33 ± 21.74	45.64 ± 13.21	85.99 ± 22.13	97.01 ± 32.11
AA/ EPA	16.94 ± 6.32	26.79 ± 9.00	11.59 ± 4.72	1.83 ± 2.43	22.69 ± 16.17
DPA/ DHA	0.48 ± 0.13	0.56 ± 0.14	0.33 ± 0.12	0.28 ± 0.26	0.50 ± 0.24
Omega 3 index	3.48 ± 0.80	5.82 ± 1.13	2.55 ± 0.74	1.38 ± 0.56	2.38 ± 0.71
Holman Index	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.21 ± 0.25	0.02 ± 0.01
n3 HUFA score	0.25 ± 0.04	0.27 ± 0.04	0.25 ± 0.05	0.16 ± 0.05	0.20 ± 0.04

Fatty acid values of different blood components have been found different due to variation in the concentration of the FFA, PLs and steryl esters (Bailey-Hall, 2008).

The most abundant family of fatty acids in all fractions was PUFAs except for PL where more than half of the fatty acids were saturated (52.95 %). In WB, RBC and plasma the second abundant FAs family was SFA followed by MUFA, while CE fraction was the poorest in SFAs.

4.1.1. Saturated Fatty Acids SFA

PL fraction was the richest in SFAs as palmitic acid alone was approximately one third (31.0%) of the total fatty acids and Stearic acid was around 20% (19.84%). Among all FAs in Plasma PL, palmitic acid (16:0) was the highest proportion (Ogura et al., 2010). However, RBC was the poorest in palmitic acid. It was noticed that plasma CE had the lowest of the Stearic acid (1.69%) which was extremely different from the highest for the same FA in RBC and PL (19.82% and 19.84%, respectively). However; the presence of C 18:0 was higher in WB than in plasma (11.41% vs. 7.63%). CE was the poorest fraction in SFA (16 %).

4.1.2. Cis Monounsaturated Fatty Acids

PL fraction was the poorest in MUFA's family, because oleic acid was very low (8.72%) while in other fractions its level was in close range. Oleic acid (18:1n9) predominant in CE in comparison to PL and RBC (Riséa et al., 2007). Palmitoleic acid (16:1n7) in RBC was the lowest in comparison to other fractions followed by PL (0.4%, 0.69% respectively) while in CE was the highest followed by plasma then WB (2.63%, 2.12%, 1.54% respectively).

4.1.3. n-6 Polyunsaturated Fatty Acids

Amongst the tested systems, CE fraction was the richest in n-6 fatty acids as more than 58% of total fatty acids in CE were n-6 PUFA. LA concentrations are the highest in CE

(Arab, 2003) which was confirmed by our results; it was close to become half of all the fatty acids presence (49.47%). However, the lowest LA content was found in RBC (10.34%). A study done by Riséa in 10 healthy subjects showed that plasma is known to have a higher level of LA than in RBC while AA, DTAn-6 and DPAn-6 is higher in RBC than in plasma (Riséa et al., 2007) which is shown in this study's obtained results.

Although the level of 18:3n6 is low in all analysed fractions (<1.0%), the lowest was found in RBC (0.08%) while the highest found in CE (0.88%). AA was the highest in RBC (17.23%) while the lowest in CE (6.39%), plasma's level was close to CE's (7.19%), WB (10.6%) and PL (9.64%). The presence of n-6 was highest in CE (58.64%), lowest in PL (31.78%).

4.1.4. n-3 Polyunsaturated Fatty Acids

ALA was higher in plasma and CE as compared to other analysed fractions (0.66% and 0.62%, respectively) such as RBC (0.18%) and PL (0.21%). Usually ALA concentration in PL is not more than 0.5% (Holman, 1998). No trace for ALA in RBC (Riséa et al., 2007), in our results RBC had the lowest percentage of ALA.

RBC were the richest while CE had the lowest level of DHA level (5.1% vs. 0.74%). However; DHA was higher than EPA in all analysed fractions. Long term intake of vegetable oils that contain a high level of ALA and a low level of LA would increase the DHA levels in blood (Ratnayake and Galli, 2009). Plasma and RBC concentration of DHA is a better indicator of fish and n-3 intake than is EPA because the latter is affected by some factors. EPA is mobilized faster form the adipose tissue than DHA (Sun et al., 2008).

EPA presence in WB, RBC, and plasma were similar (0.71%, 0.72%, and 0.73%, respectively). For DPA, the richest fraction was RBC (2.77%) followed by WB (1.26%), while the lowest was CE fraction (0.17%). DPA (22-5n3) level in the tissue reflect the overall metabolic pathway because it mediates the conversion of EPA to DHA (Ratnayake and Galli, 2009).

In regards to omega 3, the highest was found in RBC (8.76%), followed by WB (5.21%) and the lowest in plasma CE (2.17%) which confirms the idea of that WB might substitute the RBC as test system for an omega-3 indicator. n-3 HUFA score were very close and in similar range in WB, RBC, Plasma, PL (0.25, 0.27, 0.25, 0.20 respectively) while in CE the ratio was lower (0.16%). n-3 HUFA (in the total HUFA pool) was considered to be the tissue biomarker of blood omega-3 fatty acids. It showed constantly (low variable) and strong correlation with animal tissues (Stark 2008). From our data, n-3 HUFA were similar in WB, RBC, plasma followed by PL while it was lower in CE.

4.1.5. Omega-3 Index

Omega-3 index was the highest in RBC (5.82%) followed by WB (3.48%), plasma (2.55%) and PL (2.38%) while the lowest was found in CE (1.38%). This supports that RBC would be the best compartment to establish omega-3 index followed by WB.

4.1.6. n6/n3 ratio

According to the high concentration of n-6 FAs in plasma CE thus the highest ratio of n6/n3 was in CE while the lowest was found in plasma and RBC (3.22 and 3.99). Vice versa holds for the lowest value of the ratio of n3/n6 was in CE while the highest found in plasma followed by RBC (0.33 and 0.29). 4:1 was the suggested ratio of n6:n3 for optimal functional by Yehuda and Carasso (Holman, 1998; Yehuda and Carasso, 1993).

AA/EPA+DHA might be used as an indicator of n-6/n-3 status which showed a high correlation between capillary WB, RBC and PL (Bailey-Hall et al., 2009). Differences in n6/n3 ratio would highly increase cell membrane composition of AA concentration and slightly EPA, consequently increase the production of eicosanoids and inflammatory mediators (Adams et al., 1996). A high n6/n3 ratio in plasma inversely correlates with health consequences which can be corrected by either increase the dietary n-3 intake and/or decrease n-6 intake (Harris et al., 2006). High n3/n6 ratio was correlated with low incidence of the cancer mortality (Adams et al., 1996). Low n3/n6 ratio might be an index for the risk of cognitive impairment and dementia (Heude et al., 2002).

4.1.7. n-7, n-9 Polyunsaturated Fatty Acids and Holman Index

The lowest n-7 was found in RBC, while the lowest for n-9 was found in PL. For the mead acid (an index of EFAD concentrations), levels were similar in WB, RBC, plasma, PL while in CE the concentration was lower (0.08%). Usually 20:3n9 (Mead Acid) has a very low concentration makes it difficult to be detectable (Strijbosch et al., 2008) and it's an indication of the EFAD if elevated (Holman, 1970). Holman index in RBC, WB, and plasma were equal while the highest found in CE (0.21). None of the five fractions showed any EFAD laboratory signs as Holman index was ≤ 0.2.

Holman has published that triene/tetraene ratio of > 0.2 to be abnormal and EFAD ratio is greater than 0.4 in PL fraction (Holman, 1960; Holman et al., 1979). Holman index is being used as a predictor of EFAD especially with the EFAD clinical signs such as growth retardation (Strijbosch et al., 2008) and it was suggested to consider 20:3n9/20:4n6 > 0.2 as cutoff point for the EFAD diagnosis (Jeppesen et al., 1998). The Holman index which used to be called (triene-to-tetraene) in previous works, is considered to determine the fat deficiency status in rats, swine and other species (Mohrhauer and Holman, 1963).

4.1.8. Total Polyunsaturated Fatty Acids

CE fraction was the richest in PUFA family. LA and ALA have the strongest correlation with dietary intake because those PUFAs are derived only from diet (Popitt et al., 2005). LA/ALA ratio was massively highest in PL (97.01) followed by CE (85.99) which might be explained to the high of LA in PL and CE. LA/ ALA ratio intake should be 14:1 for equal metabolized competitive (Holman, 1998). However; LA/ALA and ALA levels affect the conversion of ALA to its derivatives (Ratnayake and Galli, 2009).

The highest content of PUFAs (cis and total) was found in CE while the lowest was in PL. However, WB, RBC and Plasma were very similar, but the SD in RBC is the lowest which might be explained by the long term dietary intake reflection and low biological variability. WB and Plasma SD were higher than the RBC SD (plasma SD is also higher

than WB SD) which also be explained by the short term dietary intake variation (and individual variations).

The highest level of the USFA were found in CE (83.21%), WB and plasma were in close range (64.99%, 67.99%), not far RBC (59.18%) while PL had the lowest (46.43%). For LCPUFA, RBC had the highest concentration (32.18%) while the lowest was found in plasma CE (9.85%). However, sum of LCPUFA in WB, PL and Plasma somehow in close average (18.8%, 16.42%, and 12.68%). LCPUFA 'correlations are the main consideration in most studies since they indicate the dietary intake of them (as they are not biosynthesized in vivo) (Bailey-Hall et al., 2008).

DPA/ DHA ratio in RBC and PL were similar (0.56, 0.5) and it was low in CE (0.28). AA/EPA ratio was higher in RBC followed by PL (26.79, 22.69) while it was extremely low in CE (1.83). Plasma PL AA/EPA showed to have correlations with some chronic disorders and positively with clinical symptoms of depression while the AA/DHA ratio showed correlation with neuroticism (Holub 2009). AA/EPA level would reflect the depression grade. In plasma PL; AA/EPA ratio was strongly correlated with the Hamilton depression rating scale (Adams et al., 1996).

HUFA (20- to 22- carbon fatty acids with 3 to 6 double bonds) are precursors for the eicosanoids. In fish eating, eicosanoids derived from EPA while in non-fish eating they are derived from AA. Thus the AA/EPA ratio affects the biochemical and physiological responses to stress (Harris et al., 2006). In our results, RBC would be a good indicator as it shows the highest ratio followed by PL and not from plasma CE.

AA/homo-g-LA is considered as an index for the Δ5 desaturase enzyme which is responsible to insert the double bond to the ninth carbon starting from the COOH side. AA/LA (product/precursor) is an index for the AA metabolic pathway activity (Lewis-Barbed et al., 2000). The plasma P/S ratio indicates the risk of death from coronary heart disease (Anderson et al., 2009) since it reflects the double bond index (Lepage et al., 1989). Since the PUFA was the highest among CE thus the P/S ratio was also the highest in CE fraction, while the lowest was in PL since the SFA was predominant.

4.1.9. *trans* Fatty Acids TFA

Trans fatty acids were the highest in plasma PL (0.62%). Atherogenic SFA was found to be the highest in PL followed by plasma and WB while the lowest concentration was found in CE.

4.2. Comparison between fatty acids composition from our data and other studies

Our results of the five different blood components were compared with other studies which analysed same blood components and using the same method. Our results appear in the range of the other studies outcomes.

4.2.1. Comparison between fatty acids composition in whole blood

The percentage of total analysed fatty acids in WB from our results are mentioned in table (4.2.1.) in order to compare our results with other studies that used WB and same analysing method as well. Number of subjects from our data was 120 mixed genders (male and female) and the number of analysed fatty acids was 24 FA.

In Italy; Marangoni analysed FAs from fasting WB in 6 healthy subjects from both genders where their age ranged between 22 to 72 years who worked in the Department of Pharmacological Science in Milan. No differences were found between WB FAs from arm venous artery and fingertip blood drop (Marangoni et al., 2004). On the other hand, Marangoni in 2007 analysed 19 FAs from fasting fingertip WB in healthy 47 males and 53 females separately aged from 22 to 70 years who were attending to the Department of Pharmacological Science in Milan (Marangoni et al., 2007).

In 2008, Armestrong analysed 24 FAs from WB in 4 healthy males in Waterloo Ontario Canada (Armestrong et al., 2008). However; his colleagues Metherel in 2009 analysed 21 FAs from fasting venous artery WB in healthy 9 males and 7 females separately (Metherel et al., 2009).

In Costa Rica 2005, a study was done to analyse 31 FAs from fasting WB in healthy 99 males and 101 females aged between 56 and 62 years (Baylin et al., 2005). Chavarro analysed 8 FAs from fasting WB in 476 healthy males (aged between 53 to 63 years old) in a USA study that compared the FAs status differences between prostate cancer cases and control cases. In the following table control cases were stated (Chavarro et al., 2007).

Table 4.2.1. Comparison between **whole blood fatty acids composition** from our data with other References

Country	n (subjects)	gender	n of FA	14:0	15:0	16:0	17:0	18:0	20:0	[illegible]	18:1n9t	18:1n7tr	18:1n9c	18:1n7c	20:1n9	18:2n6tr	18:2n6c	18:3n6	20:2n6	20:3n6	20:4n6	22:4n6	18:3n3	20:5n3	22:5n3	22:6n3	SFA	MUFA	PUFA	n-6	n-3
Austria [o]	120	3	24	0.65	0.52	21.54	0.30	11.41	0.14	1.54	0.18	0.22	19.21	1.59	0.36	0.12	22.57	0.31	0.31	1.86	10.60	1.29	0.47	0.71	1.26	2.77	34.48	23.10	42.42	36.94	5.21
Italy[1]	47	1	19	-	-	25.17	-	13.42	0.55	3.06	-	-	21.34	2.09	0.32	-	16.40	-	-	1.10	6.68	1.19	0.70	0.61	1.08	1.75	41.86	28.33	29.81	24.89	4.11
Italy[2]	34	2	19	-		25.17	-	13.64	0.59	2.75	-	-	19.63	2.10	0.23	-	17.99	-	-	1.21	7.05	0.94	0.62	0.59	0.71	1.97	42.11	26.42	31.46	27.57	3.89
Italy[3]	6	3	-	-	-	-	-	11.46	-	-	-	-	21.93	-	-	-	18.38	-	-	-	10.46	-	0.28	1.25	1.36	4.59	32.33	27.84	39.83	-	-
Canada[4]	4	1	24	1.01	-	23.07	-	8.87	0.19	0.89	-	-	17.02	1.53	0.29	-	23.14	0.29	0.10	0.73	8.75	1.22	0.65	0.53	1.11	1.17	35.09	22.95	37.82	34.37	3.45
Canada[5]	9	1	21	0.76	-	21.33	-	10.17	0.18	1.60	-	-	17.53	1.83	0.33	-	21.72	0.20	-	1.62	9.22	1.42	0.39	0.39	1.15	1.68	35.25	22.53	38.33	34.73	3.60
	7	2	21	0.94	-	23.57	-	9.23	0.16	1.82	-	-	18.72	1.90	0.23	-	22.09	0.19	-	1.38	8.36	1.16	0.41	0.30	0.72	2.06	35.92	23.82	37.21	33.72	2.06
Costa Rica[6]	200	3	31	0.52	0.14	21.92	0.28	9.42	0.26	1.86	0.24	0.25	18.23	1.52	0.17	0.12	22.38	0.28	-	1.75	9.08	1.28	0.37	0.81	0.90	2.39	34.07	23.32	39.87	35.10	4.54
USA[7]	476	1	8	-	-	-	-	-	-	-	-	-	-	-	-	-	25.30	0.26	-	1.36	10.10	-	0.35	1.79	0.97	2.19	-	-	-	-	4.96

Values = % of total fatty acids.

Gender: 1= Male, 2= Female, 3= Mix

n of FA= number of total fatty acids analyzed.

[1 and 2] Marangoni et al., 2007, [3] Marangoni et al., 2004, [4] Armestrong et al., 2008, [5] Metherel et al., 2009, [6] Baylin et al., 2005, [7] Chavarro et al., 2007, [o] our data 2010

4.2.2. Comparison between fatty acids composition in Red Blood Cells

The percentage of total analysed fatty acids in RBC from our results is mentioned in table (4.2.2.) in order to compare our results with other studies that analysed RBC using the same analysing method. Number of subjects from our data was 131 mixed genders (male and female) and the number of analysed fatty acids was 24 FA.

In South Africa, Koorts analysed 22 FAs from RBC in 10 healthy subjects from both genders (6 males and 4 females), the mean age was 38 years (Koorts et al., 2002). King analysed 21 FAs from RBC in 30 healthy postmenopausal females aged 50-79 years (King et al., 2006). Armestrong analysed 24 FAs from RBC in 4 healthy males in Waterloo Ontario Canada (Armestrong et al., 2008) while his colleagues Metherel analysed 21 FAs from RBC in healthy 9 males and 7 females separately (Metherel et al., 2009).

Table 4.2.2. Comparison between **red blood cells fatty acids composition** from our data with other References

Country	n	gender	n of FA	14:0	16:0	18:0	20:0	16:1n7	18:1n7c	20:1n9	18:2n6c	18:3n6	20:2n6	20:3n6	20:4n6	22:4n6	18:3n3	20:5n3	22:5n3	22:6n3	SFA	MUFA	PUFA	n-6	n-3
Austria[0]	131	3	24	0.21	19.35	19.82	0.21	0.40	1.32	0.43	10.34	0.08	0.34	2.08	17.23	3.94	0.18	0.72	2.77	5.10	40.23	16.75	43.02	34.00	8.76
South Africa[1]	10	3	22	0.39	21.21	16.64	0.38	0.17		0.22	12.11	0.03	0.42	1.62	15.92	0.39	0.13	0.28	1.56	4.26					
USA, Washington Seattle[2]	30	2	21	0.26	18.96	13.97	0.39	0.37	0.99	0.24	8.56		0.25	1.57	14.17	2.93	0.14	0.68	2.29	5.05	40.09	16.81	35.68	27.51	8.17
Canada Waterloo[3]	4	1	24	0.45	24.59	13.06	0.33	0.89	1.53	0.36	11.35	0.08	0.30	0.75	13.23	3.06	0.18	0.53	2.22	1.99	41.92	18.54	34.14	29.22	4.92
Canada Ontario Waterloo[4]	9	1	21	0.63	22.27	13.62	0.25	0.32	1.45	0.26	9.82	0.01		1.60	12.30	3.12	0.12	0.38	2.05	2.51	42.90	17.65	32.65	27.60	5.07
	7	2	21	0.74	23.50	12.79	0.26	0.39	1.49	0.28	9.83	0.01		1.36	12.01	2.99	0.12	0.31	1.48	3.44	42.74	17.85	32.35	27.00	5.35

Values = % of total fatty acids.

Gender: 1= Male, 2= Female, 3= Mix

n of FA= number of total fatty acids analyzed.

[1]Koorts et al., 2002, [2] King et al., 2006, [3] Armestrong et al., 2008, [4] Metherel et al., 2009, [o] our data 2010

4.2.3.Comparison between fatty acids composition in plasma

The percentage of total analysed fatty acids in plasma from our results are mentioned in table (4.2.3.) in order to compare our results with other studies that used plasma and the same analysis method as well. Number of subjects from in this experiment was 131 mixed genders (male and female) and the number of analysed fatty acids was 24 FA.

However, Akiko and his colleagues analysed 9 FAs from plasma in healthy subjects from different Asian ethnic groups (Japan, Korea, and Mongolians); 411 healthy Japanese (194 males and 217 females) aged 30-60 years, 418 healthy Koreans (240 males and 178 females) aged 30-60 years, and 215 Mongolians (100 males and 152 females) (Akiko et al., 2007).

In Costa Rica 2005, a study was done to analyse 31 FAs from plasma in healthy 99 males and 101 females aged between 56 and 62 years old (Baylin et al., 2005). In Canada, Metherel analysed 21 FAs from plasma in healthy 9 males and 7 females separately (Metherel et al., 2009).

Table 4.2.3. Comparison between **Plasma fatty acids composition** from our data with other References

Country	n	gender	n of FA	14:0	16:0	18:0	20:0	16:1n7	18:1n9c	18:1n7c	20:1n9	18:2n6c	18:3n6	20:3n6	20:4n6	22:4n6	18:3n3	20:5n3	22:5n3	22:6n3	SFA	MUFA	PUFA	n-6	n-3
Austria[0]	131	3	24	0.88	23.03	7.63	0.11	2.12	21.04	1.69	0.33	28.11	0.43	1.70	7.19	0.36	0.66	0.73	0.56	1.82	32.33	25.51	42.17	38.23	3.31
Japanese[1]	411	3	9		24.40	7.80			18.50			33.70			6.30		0.50	2.50	0.90	5.50	32.10	18.50	48.50	39.90	8.50
Koreans[1]	418	3	9		23.60	6.70			19.60			37.20			6.40		0.60	1.40	0.50	4.00	30.20	19.60	50.10	43.60	6.50
Mongolians[1]	215	3	9		23.90	8.30			20.70			34.60			6.30		0.70	1.00	1.10	3.50	32.20	20.70	46.00	40.80	5.10
Canada Ontario Waterloo[2]	9	1	21	0.97	21.66	6.53	0.13	2.14	20.66	1.95	0.15	29.39	0.32	1.43	6.17	0.19	0.57	0.39	0.42	1.07	30.51	25.43	40.29	37.85	2.45
	7	2	21	1.10	23.72	6.12	0.12	3.03	20.95	2.09	0.21	26.96	0.31	1.51	5.50	0.21	0.56	0.30	0.30	1.34	32.11	26.81	37.44	34.94	2.51
Costa Rica[3]	200	3	31	0.74	22.78	6.76	0.19	2.64	20.74	1.71	0.14	28.09	0.30	1.68	6.02	0.26	0.49	0.34	0.41	1.49	31.85	25.91	38.39	36.74	2.62

Values = % of total fatty acids.

Gender: 1= Male, 2= Female, 3= Mix

n of FA= number of total fatty acids analyzed.

[1]Akiko et al., 2007, [2] Metherel et al., 2009, [3] Baylin et al., 2005, [o] our data 2010

4.2.4. Comparison between fatty acids composition in plasma phospholipid

The percentage of total analysed fatty acids in PLs from our results are mentioned in table (4.2.4.) in order to compare our results with other studies that used plasma phospholipids (PL) and used the same analysing method. Number of subjects from our data was 117 mixed genders (male and female) and the number of analysed fatty acids was 24 FA.

Wang analysed 11 FAs from PL in 3309 subjects in middle age adults (45- to 64- years) in a study was held to investigate the relation of PL composition with incidence of CHD. They compared subjects with CHD incident vs. no CHD. In the following table, no CHD subjects PL's FAs profile was stated (Wang et al., 2003).

King analysed 21 FAs from PL in 30 healthy postmenopausal females aged 50-79 years (King et al., 2006). Armestrong analysed 24 FAs from PL in 4 healthy males in Waterloo Ontario Canada (Armestrong et al., 2008).

However, a recent study was conducted to examine the association between breast cancer risk and the FA composition of PLs. PLs profile was analysed from 257 postmenopausal women aged 50-69 years (Takata et al., 2009).

Table 4.2.4. Comparison between **Plasma phospholipids fatty acids composition** from our data with other References

Country	n	gender	n of FA	14:0	16:0	18:0	20:0	16:1n7	18:1n9c	18:1n7c	18:2n6c	18:3n6	20:3n6	20:4n6	22:4n6	18:3n3	20:5n3	22:5n3	22:6n3	SFA	MUFA	PUFA	n-6	n-3
Austria[0]	117	3	24	0.46	31.00	19.84	0.23	0.69	8.72	1.47	18.41	0.15	2.61	9.64	0.29	0.21	0.58	0.82	1.80	52.95	11.51	35.54	31.78	3.41
Minneapolis USA[2]	3309		11		25.40	13.30		0.64	8.60		22.00	0.11	3.32	11.50		0.15	0.56		2.80	40.60	9.97	42.70	38.20	4.42
Seattle USA [3]	30	2	21	0.25	25.56	12.92	0.56	0.65	8.37	1.41	18.62		3.45	11.19	0.45	0.21	0.99	0.98	4.14	42.48	12.96	40.42	34.10	6.11
Canada Waterloo[4]	4	1	24	0.77	30.44	12.09	0.33	0.84	8.26	1.51	22.17	0.07	1.57	9.40	0.41	0.28	0.81	0.94	1.52	46.30	12.37	37.87	34.28	3.59
USA[5]	257	2	39	0.29	26.20	13.30	0.41	0.57	8.06	1.27	19.60	0.09	3.21	11.0	0.42	0.10	0.65	0.82	2.77	43.50	13.00		34.00	4.33

Values = % of total fatty acids.

Gender: 1= Male, 2= Female, 3= Mix

n of FA= number of total fatty acids analyzed.

[1]Wang et al., 2003, [2] King et al., 2006, [3]Armestrong 2008, [4]Takata et al., 2009, [o] our data 2010

4.2.5. Comparison between fatty acids composition in plasma cholesterol ester

The percentage of total analysed fatty acids in CE from our results are mentioned in table (4.2.5.) in order to compare our results with previous studies that determined the fatty acid pattern in CE using the same analysing method. Number of subjects from our data was 128 mixed genders (male and female) and the number of analysed fatty acids was 24 FA.

Wang analysed 11 FAs from CE in 3309 subjects in middle age adults (45- to 64- years) in a study was held to investigate the relation of CE composition with incidence of CHD. They compared subjects with CHD incident vs. no CHD. In the following table, no CHD subjects CE's FAs profile was stated (Wang et al., 2003).

King analysed 21 FAs from CE in 30 healthy postmenopausal females aged 50-79 years (King et al., 2006).

Table 4.2.5. Comparison between **Plasma cholesterol ester fatty acids composition** from our data with other References

CE (plasma)	n	gender	n of FA	14:0	16:0	18:0	20:0	16:1n7	18:1n9c	18:1n7c	20:1n9	18:2n6c	18:3n6	20:2n6	20:3n6	20:4n6	22:4n6	18:3n3	20:5n3	22:5n3	22:6n3	SFA	MUFA	PUFA	n-6	n-3
Austria[0]	128	3	24	0.83	12.51	1.69	0.09	2.63	18.37	1.21	0.11	49.47	0.88	0.34	0.82	6.39	0.75	0.62	0.64	0.17	0.74	16.31	22.57	61.11	58.64	2.17
Minneapolis USA[1]	3309		11		10.00	0.89		2.58	16.00			54.10	1.02		0.76	8.25		0.41	0.54		0.44	11.60	18.60	65.70	64.20	1.41
Seattle USA[2]	30	2	21	0.63	11.14	0.96	0.62	3.57	17.81	1.29	N/A	49.53		N/A	0.88	7.99	N/A	0.61	0.91	0.06	0.72	13.76	23.21	60.86	58.56	2.30

Values = % of total fatty acids.

Gender: 1= Male, 2= Female, 3= Mix

n of FA= number of total fatty acids analyzed.

[1]Wang et al., 2003, [2] King et al., 2005, [o] our data 2010

Tabels 4.2.1. to 4.2.5. show evaluation of our results with other published studies that used folch method in analysing FAs.

In table 4.2.1. FAs% in WB were compared from our data (Austria) with WB FAs% from Italy, Canada, USA and Costa Rica. SFA and MUFA from Austira were in the range of Canada, USA, and Costa Rica while PUFA and n-3 from our data were higher. However, Italy showed higher SFA, MUFA lower PUFA and n-3 as the case for Austria.

In table 4.2.2. FAs% in RBC from our data (Austria) were compared with RBC FA% from South Africa, USA, and Canada. While USA Washington Seattle showed very similar n-3, SFA, and MUFA with Austria, Canada appears to have lower n-3.

In table 4.2.3. FAs % in plasma from Austria were compared with FA% in plasma from Japan, Korea, Mongolia, Canada, and Costa Rica. Japan had the highest n-3 followed by Korea while Canada and Costa Rica had the lowest. SFA and PUFA were in similar range between Austria and other countries.

In table 4.2.4. FAs % in PLs from Austria were compared with FA% in PLs from USA and Canada. Austria appeared to be similar to Canada Waterloo in n-3 and PUFA however, SFA looked higher in Austria than other compared countries.

In table 4.2.5. FAs % in CE from Austria were compared with FA% in CE from USA (Minneapolis and Washington Seattle). Austria's n-3 was similar to Seattle but higher than Minneapolis. However, SFA in Austria was higher.

4.3. Correlation coefficient between the five blood components

Bivariate correlations were measured between each FA and Index among WB, RBC, plasma, CE, and PL. Table 4.3 shows summary of the correlation coefficients for each fatty acid between the tested systems.

Table 4.3: Summary of the correlation coefficients between each fatty acid between the tested system

Fatty Acid	WB, RBC	WB, Plasma	WB ,CE	WB, PL	RBC, plasma	RBC, CE	RBC, PL	Plasma, PL	Plasma, CE	PL, CE
14:0	0.374**	0.686**	0.306**	0.406**	0.345**	0.424**	0.245**	.348**	.243**	.455**
15:0	0.342**	0.197**	0.258**	-0.004	-0.038	0.012	-0.026	0.040	0.066	.430**
16:0	0.460**	0.720**	-0.014	0.207*	0.348**	-0.120	0.277**	.265**	0.111	0.041
17:0	0.692**	0.496**	0.435**	0.485**	0.515**	0.318**	0.406**	.404**	.409**	.372**
18:0	0.270**	0.644**	0.271**	0.358**	0.188*	0.175	0.401**	.389**	.390**	.451**
20:0	0.217*	0.544**	0.331**	0.303**	0.238**	0.108	0.113	.332**	.204*	.234*
16:1n7	0.523**	0.821**	0.752**	0.691**	0.428**	0.521**	0.543**	.731**	.831**	.769**
18:1n9trans	0.217*	0.216*	0.208*	0.318**	0.083	-0.016	0.073	0.127	0.075	.344**
18:1n7trans	0.441**	0.593**	N/A	0.522**	0.189*	N/A	0.253**	.511**	N/A	N/A
18:1n9Cis	0.365**	0.768**	0.084	0.200*	0.392**	0.138	0.098	.332**	-0.002	.506**
18:1n7Cis	0.551**	0.667**	0.000	0.484**	0.388**	0.092	0.238*	.524**	.418**	.359**
20:1n9	0.247**	0.615**	0.134	0.388**	0.209*	0.049	0.087	.301**	-0.02	.194*
18:2n6 trans	0.296**	0.345**	0.225*	0.256*	0.199*	0.079	0.167	0.107	.252**	.433**
18:2n6 Cis	0.511**	0.732**	0.552**	0.371**	0.583**	0.435**	0.455**	.397**	.576**	.357**
18:3n6	0.408**	0.820**	0.719**	0.300**	0.504**	0.407**	0.207*	.391**	.712**	.334**
20:2n6	0.518**	0.537**	0.125	0.374**	0.396**	0.166	0.385**	.241*	.186*	.447**
20:3n6	0.752**	0.829**	-0.033	0.434**	0.578**	-0.124	0.234*	.489**	0.032	0.097
20:4n6	0.419**	0.732**	0.121	0.046	0.307**	-0.057	-0.022	0.028	.206*	0.123
22:4n6	0.389**	0.16	-0.03	0.223*	0.184*	0.041	0.020	-0.050	0.091	-0.083
18:3n3	0.269**	0.727**	0.618**	0.581**	0.163	0.468**	0.270**	.558**	.656**	.715**
20:5n3	0.616**	0.796**	0.606**	0.449**	0.597**	0.413**	0.247**	.412**	.471**	.475**
22:5n3	0.572**	0.515**	0.154	-0.017	0.231*	0.137	0.071	0.116	0.059	-0.014
22:6n3	0.746**	0.841**	0.496**	0.490**	0.687**	0.468**	0.454**	.567**	.535**	.456**
20:3n9	0.234*	0.566**	0.352**	0.398**	0.102	0.178	0.241*	.338**	.179*	0.172

Continue table 4.3: Summary of the correlation coefficients between each fatty acid between the tested system

Fatty Acid	WB, RBC	WB, plasma	WB ,CE	WB, PL	RBC, plasma	RBC, CE	RBC, PL	Plasma, PL	Plasma, CE	PL, CE
SFA	0.152	0.597**	0.025	-0.059	0.211*	-0.081	0.091	-0.071	-0.038	0.128
Atherogenic FA	0.415**	0.698**	0.021	0.010	0.305**	-0.102	0.246**	0.237*	0.099	-0.003
TFA	0.304**	0.508**	0.240*	0.198	0.246**	0.131	0.270**	0.245*	0.156	.447**
cis-MUFA	0.291**	0.762**	0.399**	0.292**	0.335**	0.082	0.101	0.411**	.464**	.588**
∑ MUFA	0.264**	0.763**	0.401**	0.289**	0.295**	0.060	0.071	0.402**	.464**	.576**
cis-PUFA	0.253**	0.768**	0.317**	0.178	0.220*	-0.070	0.170	0.252**	.418**	0.131
∑ PUFA	0.251**	0.770**	0.323**	0.197	0.139	-0.061	0.171	0.265**	.428**	0.132
USFA	0.14	0.577**	0.04	-0.068	0.195*	-0.078	0.104	-0.079	-0.038	0.143
LCP n6	0.457**	0.694**	0.403**	0.015	0.290**	-0.169	0.027	0.026	.553**	-0.096
LCP n3	0.702**	0.790**	0.518**	0.386**	0.542**	0.480**	0.378**	0.568**	.639**	.614**
Omega 3 index	0.770**	0.856**	0.522**	0.526**	0.640**	0.494**	0.468**	0.593**	.679**	.656**
∑ LCPUFA	0.291**	0.689**	0.366**	0.141	0.247**	0.174	0.230*	0.174	.568**	0.116
∑ n6	0.385**	0.730**	0.387**	0.231*	0.212*	0.083	0.131	0.280**	.546**	0.146
∑ n3	0.730**	0.799*	0.539**	0.411**	0.550**	0.500**	0.340**	0.572**	.592**	.587**
∑ n7	0.315**	0.845**	0.723**	0.515**	0.207*	0.226*	0.206*	0.474**	.787**	.470**
∑ n9	0.362**	0.778**	0.357**	0.213*	0.394**	0.144	0.074	0.351**	.389**	.522**
n6/n3	0.792**	0.720**	0.594**	0.400**	0.641**	0.523**	0.306**	0.284**	.495**	.483**
n3/n6	0.831**	0.704**	0.641**	0.459**	0.662**	0.544**	0.369**	0.284**	.515**	.574**
PUFA/SFA	0.186*	0.826**	0.092	0.077	0.189*	-0.101	0.132	0.085	0.091	0.058
USFA/SFA	0.141	0.725**	0.049	-0.059	0.203*	-0.070	0.086	-0.090	-0.019	0.103
LA/ALA	0.394**	0.614**	0.546**	0.519**	0.112	0.333**	0.302**	0.564**	.612**	.643**
AA/ EPA	0.610**	0.730**	0.176	0.290**	0.553**	0.099	0.192*	0.395**	.208*	0.099
DPA/ DHA	0.824**	0.809**	0.308**	0.396**	0.726**	0.275**	0.262**	.204*	.213*	.198*
Holman Index	0.263**	0.263**	0.113	0.342**	1.000**	0.017	0.130	0.130	0.017	.193*
n3 HUFA score	0.883**	0.834**	0.578**	0.245*	0.678**	0.510**	0.277*	0.376**	.579**	.341**

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

From FAs means value in table 4.1. two types of correlation were calculated, parametric Pearson correlation and nonparametric Spearman's rho. Correlations via the 5 blood components would give a good estimate about the connection of one FA in a blood component with the same FA in other blood component. This would allow the worker to choose the blood component according to the FA in interest.

No negative correlations were found between WB and RBC, WB and plasma, WB and CE (except for 16:0, 20:3n6 and 22:4n6), RBC and plasma except for 15:0 (r= -0.038), RBC and PL except for (15:0 and AA), plasma and PL except for (22:4n6, SFA, and U/S) and between PL and CE except for (DPA, LCPn-3 and atherogenic FA). The most fractions were noticed to have negative correlation were between RBC and CE.

The correlations between WB and CE were noticed to have negative correlations for the fatty acids of 16:0 (r= -0.014), 20:3n6 (r= -0.033), and 22:4n6 (r= -0.03). The correlations between WB and PL were negative among the fatty acids of 15:0 (r= -0.004), DPA (r= -0.017), SFA (r= -0.059), USFA (r= -0.068) and for the ratio of U/S (r= -0.059). However, the correlations between RBC and plasma were all positive except for the fatty acid 15:0 (r= -0.038) being negative.

With regards to the CE fraction; the correlation with RBC method was noticed to have the most negative linear relationship which would be explained in that CE reflects fatty acids of the very short term intake while RBC reflect longer intake term; (SFA index, the Atherogenic indexes, PUFA, LCPn-6, P/S, U/S, fatty acid of 16:0, 18:1n9t, 20:3n9 and AA). Correlations with plasma were also found to have many negative correlations among (SFA, USFA, U/S, oleic acid, and 20:1n9). In addition, the correlations with PL were negative among TFA, LCPn-6, and DPA.

Correlation coefficients were weak, absent or negative between RBC and PL, CE especially among (15:0, 16:0, 20:0, 18:1n9t, 18:1n9c, 20:3n9, 18:2n6t, DPA, AA, SFA, MUFA, PUFA, USFA, P/S, U/S, and Holman index).

The strongest significant positive correlations (at p. value < 0.01 level (2-tailed) and r < 0.05 strong degree of linear relationship) were found mostly between WB and plasma except for 22:4n6, while a medium significant positive correlation was found for (15:0, 17:0, 18:1n9t, 18:2n6t and holman index) since the correlation were positive but middling than the other FAs (r < 0.05).

Although the entire correlation coefficients were positive for the LA in all the 5 fractions, the strength degree between WB and plasma was the highest in comparison to others (r= 0.732 at p. value < 0.01 level).

According to ALA, the strongest correlation was found between WB and plasma followed by CE and PL, the weakest correlation was between RBC and plasma. While for EPA the strongest correlation was between WB and plasma; the weakest was between RBC and PL. DHA strongest correlation were noticed between WB and Plasma followed by WB and RBC. Two negative correlations were noticed for the DPA between WB and PL, and PL with CE.

For SFA, many negative correlations were noticed. In general the only positive correlations were between WB and plasma followed by RBC and plasma.

WB and plasma showed the strongest correlations for MUFA, PUFA, USFA, LCPUFA, n-6, n-3, n-7, n-9, Omega-3 index and P/S ratio. However, the next highly significant correlations were found between WB and RBC after WB and plasma.

For the omega-3 index, the correlations were positive and close to ($r \geq 0.5$ at p. value < 0.01 level) but the strongest as mentioned before was between WB and plasma (r= 0.856, p-value 0.01 level followed by WB and RBC (r=0.770 at p. value < 0.01 level).

The highest correlation among all the 5 methods were found for the 16:1n7, LA, EPA, DHA, LCPn-3, omega 3 index, n-3, and n-3 HUFA score. However the perfect linear relationship r=1.00 was found for holman index between RBC and plasma.

Bailey-Hall concluded from their study done to validate capillary WB with RBC, PLs LCPUFA status, that DHA level in RBC is higher than in WB since RBC is rich in PL. They also reported the weak correlation between WB AA and AA from RBC and PLs (Bailey-Hall et al., 2008).

4.4. Linear plot relationship

To illustrate the correlations of the FAs between blood components, scatter plot Bivar linear relationship were computed. In the following sections; omega-3 index, LA/ALA and EFA (LA with ALA) correlations are demonstrated.

4.4.1. Linear plot of omega-3 index between fractions

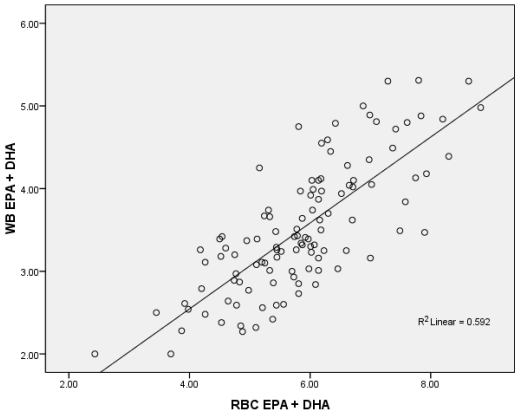

Fig 4.4.1.1 Correlation between WB Omega-3 index and RBC n-3 index r^2= 0.592, p. value < 0.01

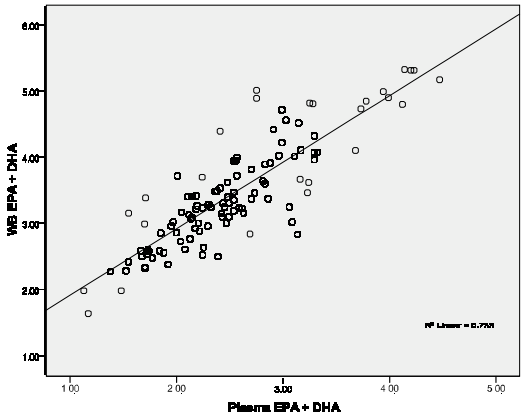

Fig 4.4.1.2 Correlation between WB Omega-3 index and plasma n-3 index r^2= 0.733, p. value < 0.01

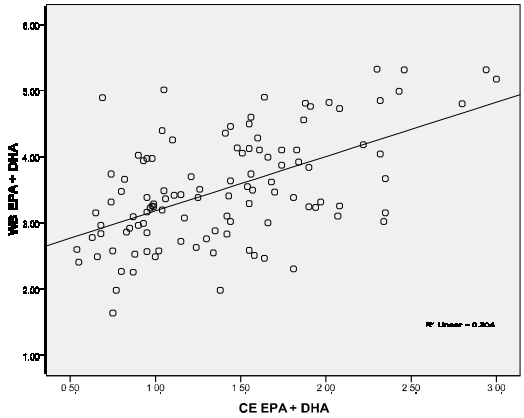

Fig 4.4.1.3 Correlation between WB Omega-3 index and CE n-3 index r^2= 0.304, p. value < 0.01

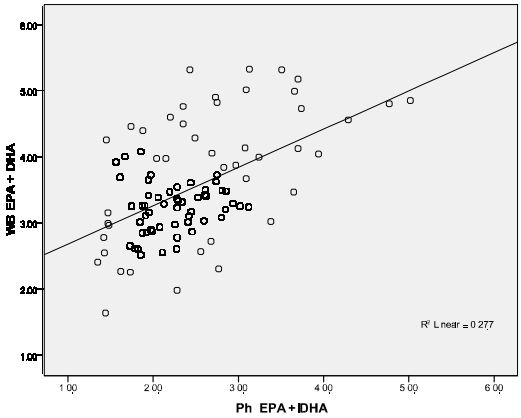

Fig 4.4.1.4. Correlation between WB Omega-3 index and PL n-3 index r^2= 0.277, p. value < 0.01

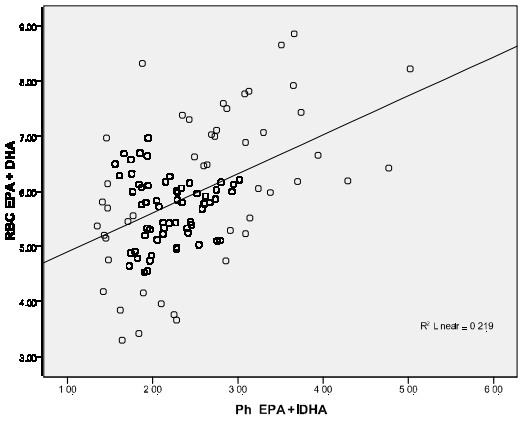

Fig 4.4.1.5 Correlation between RBC Omega-3 index and PL n-3 index

r^2= 0.219, p. value < 0.01

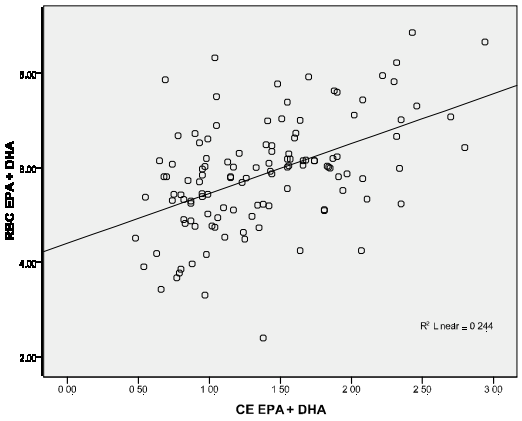

Fig 4.4.1.6 Correlation between RBC Omega-3 index and CE n-3 index

r^2= 0.244, p. value < 0.01

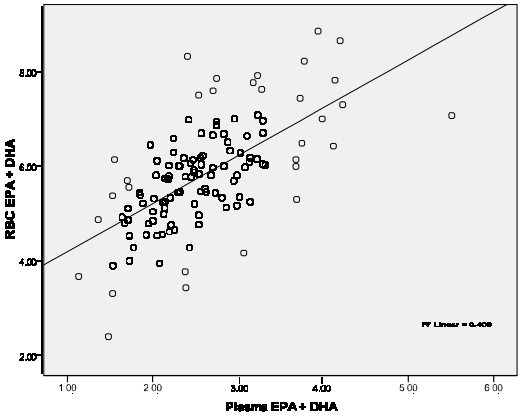

Fig 4.4.1.7 Correlation between RBC Omega-3 index and Plasma n-3 index

r^2= 0.409, p. value < 0.01

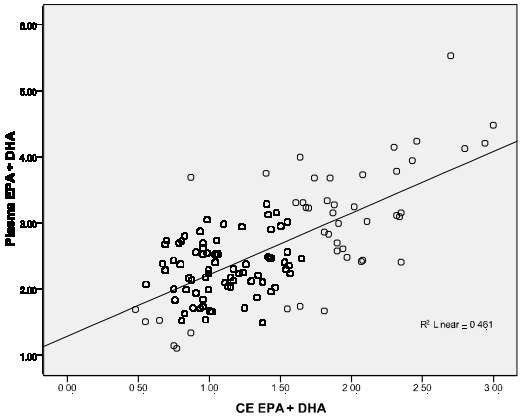

Fig 4.4.1.8 Correlation between Plasma Omega-3 index and CE n-3 index

r^2= 0.461, p. value < 0.01

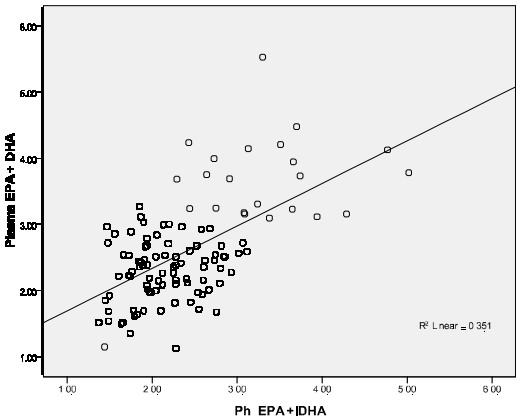

Fig 4.4.1.9 Correlation between Plasma Omega-3 index and PL n-3 index

r^2= 0.351, p. value < 0.01

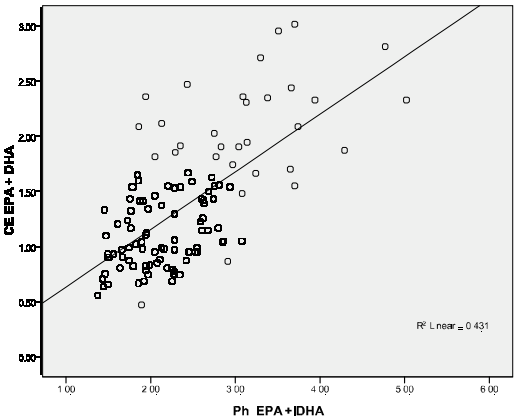

Fig 4.4.1.10 Correlation between PL Omega-3 index and CE n-3 index

r^2= 0.431, p. value < 0.01

From the previous figures, the scatter plot Bivar linear relationship between omega-3 index (EPA+ DHA) concentrations through the 5 fractions show positive correlations. However, linear plot relationships matched that correlation coefficient.

Omega-3 index of WB and plasma showed the highest correlation r^2= 0.733 at p. value 0.01 (fig 4.4.1.1) followed by WB and RBC r^2= 0.592 at p. value 0.01 (fig 4.4.1.2), while the lowest were between omega-3 index RBC and PL r^2= 0.219 at p. value 0.01 (fig 4.4.1.5), RBC and CE r^2= 0.244 at p. value 0.01 (fig 4.4.1.6).

These results strengthen selecting WB blood components as an omega-3 index biomarker along with RBC and plasma according to the high correlations between those compartments. In analogous, choosing PL and CE is not favourable to validate the status.

4.4.2. Linear plot relationship of ratio (LA/ALA) between fractions

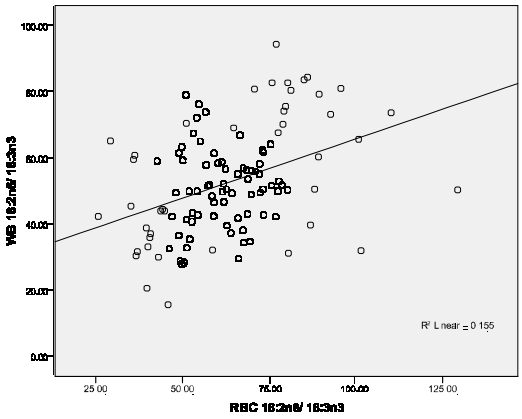

Fig 4.4.2.1 Correlation between WB LA/ALA and RBC LA/ALA

r^2= 0.155, p. value < 0.01

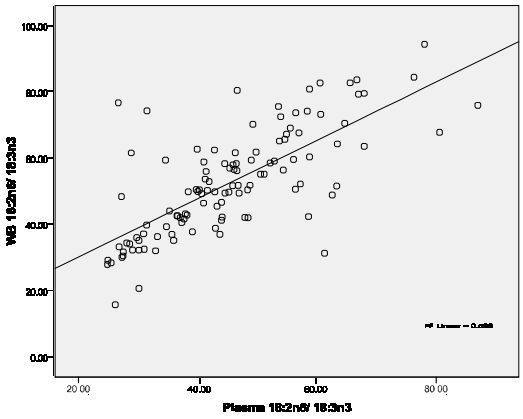

Fig 4.4.2.2 Correlation between WB LA/ALA and Plasma LA/ALA

r^2= 0.533, p. value < 0.01

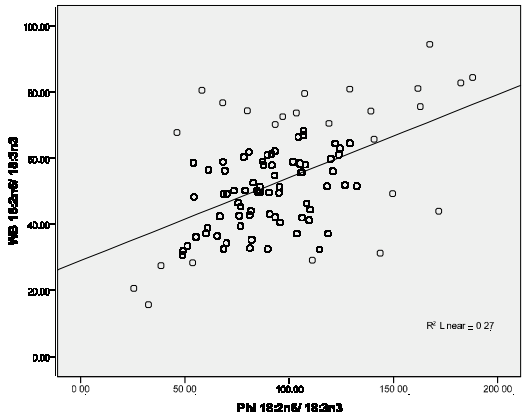

Fig 4.4.2.3 Correlation between WB LA/ALA and PL LA/ALA

r^2= 0.27, p. value < 0.01

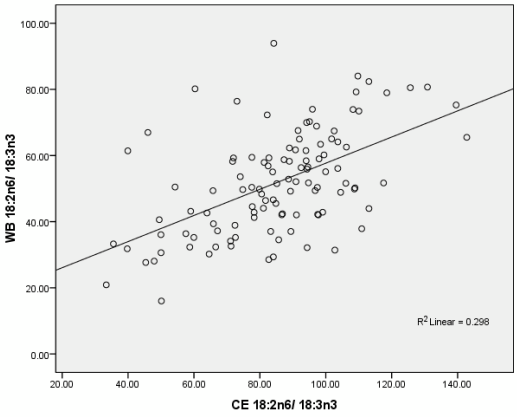

Fig 4.4.2.4 Correlation between WB LA/ALA and CE LA/ALA

r^2= 0.298, p. value < 0.01

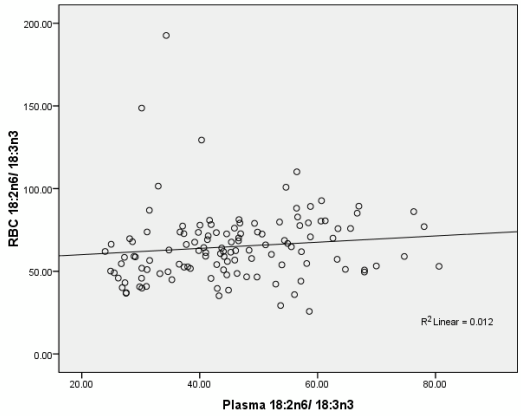

Fig 4.4.2.5 Correlation between RBC LA/ALA and plasma LA/ALA r^2= 0.012,

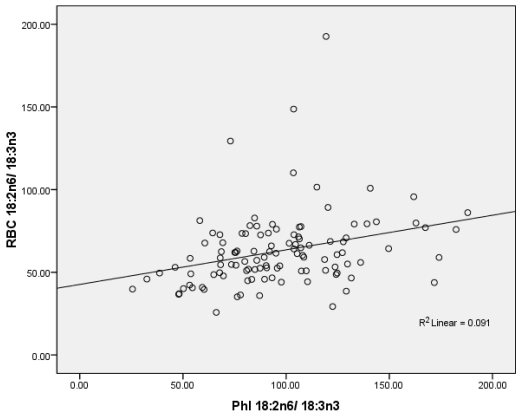

Fig 4.4.2.6 Correlation between RBC LA/ALA and PL LA/ALA r^2= 0.091, p. value < 0.01

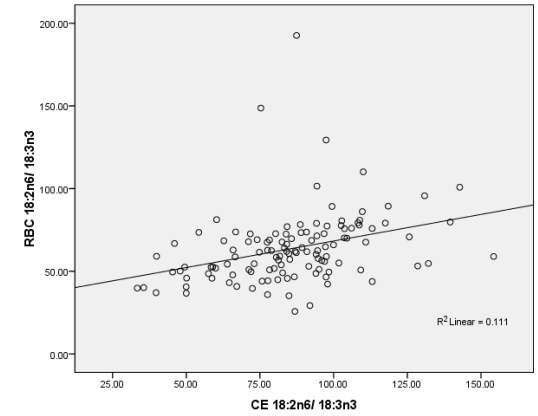

Fig 4.4.2.7 Correlation between RBC LA/ALA and CE LA/ALA r^2= 0.111, p. value < 0.01

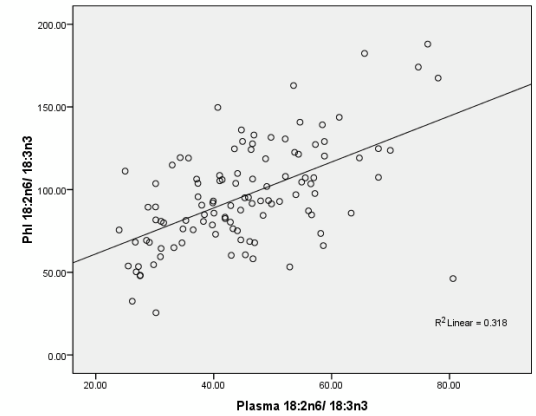

Fig 4.4.2.8 Correlation between Plasma LA/ALA and PL LA/ALA r^2= 0.318, p. value < 0.01

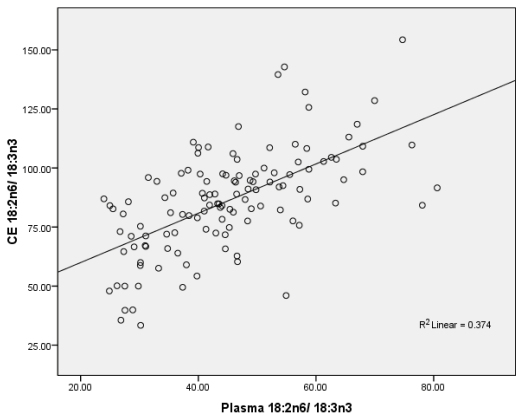

Fig 4.4.2.9 Correlation between Plasma LA/ALA and CE LA/ALA r^2= 0.374, p. value < 0.01

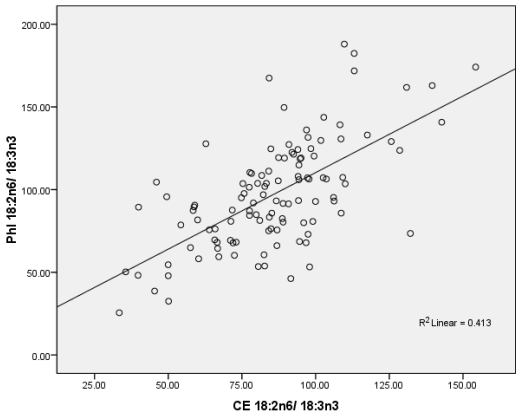

Fig 4.4.2.10 Correlation between CE LA/ALA and PL LA/ALA r^2= 0.413, p. value < 0.01

From the previous figures, the scatter plot Bivar linear relationship between the EFA LA/ALA ratios through the 5 fractions shows positive correlations. However, the highest correlations were between WB and plasma followed by PL and CE, while the lowest were between RBC and Plasma, RBC and PL.

LA/ALA of WB and plasma showed the highest correlation r^2= 0.533 at p. value 0.01 (fig 4.4.2.2) followed by PL and CE r^2= 0.413 at p. value 0.01 (fig 4.4.2.10), while the lowest were between RBC and PL r^2= 0.019 at p. value 0.01 (fig 4.4.2.6), RBC and plasma r^2= 0.012 (fig 4.4.2.5).

These results strengthen selecting WB blood components as an LA/ALA biomarker along with plasma according to the high correlations between those compartments. In analogous, choosing RBC is not favourable.

4.4.3. Linear plot relationship of concentrations of LA and ALA within tested systems

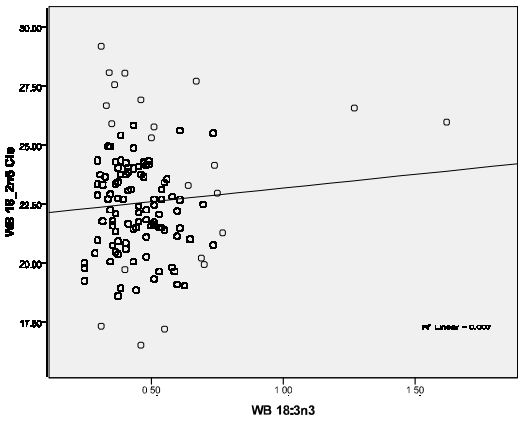

Fig 4.4.3.1 Correlation between WB LA and ALA

R^2= 0.007

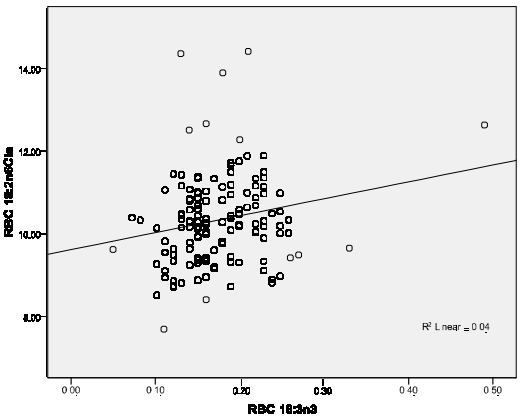

Fig 4.4.3.2 Correlation between RBC LA and ALA

R^2= 0.04, at p.value < 0.05

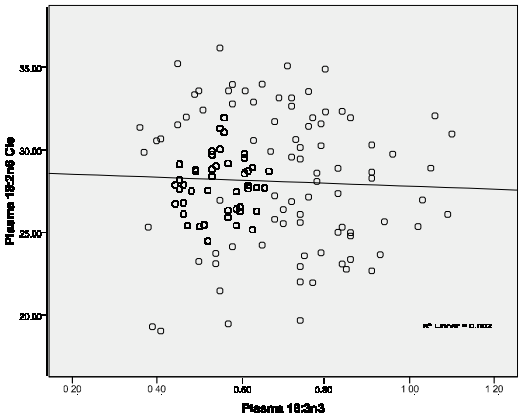

Fig 4.4.3.3 Correlation between plasma LA and ALA

R^2= 0.002

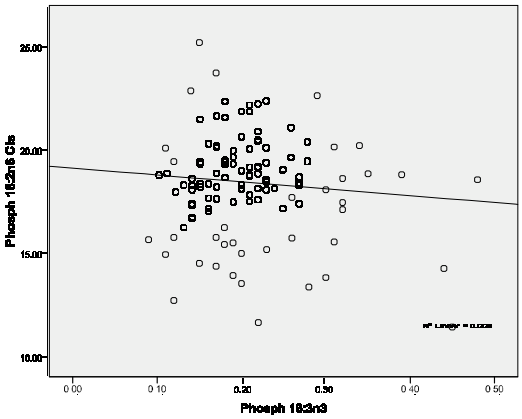

Fig 4.4.3.4 Correlation between PL LA and ALA

R^2= 0.009

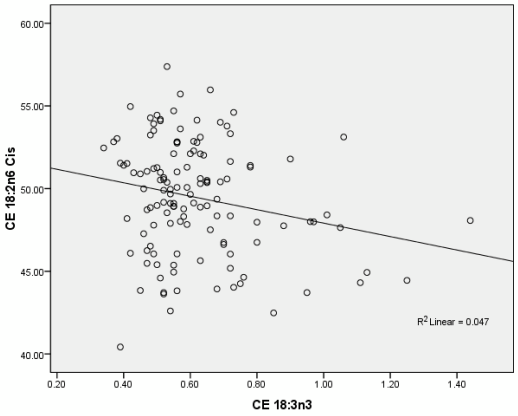

Fig 4.4.3.5 Correlation between CE LA and ALA

R^2= 0.047, at p. value < 0.05

From the above figures, the scatter plot Bivar linear relationship between the EFA presence among the 5 blood components LA and ALA within the 5 fractions shows weak positive correlations. However, the strongest significant correlations were for RBC r^2= 0.04, at p.value < 0.05 (fig 4.4.3.2) followed by CE r^2= 0.047, at p. value < 0.05 (fig 4.4.3.5), while the weakest was for plasma r^2= 0.002.

4.5. Fatty acid analysis in correlation to individuals characters

The plasma FA composition of a person was found to be influenced by age, body weight, sex and health status as well as habitual life smoking status, alcohol drinking (MA et al., 1995).

Tables 4.5 1.to 4.5.5 show the analyzed fatty acids in relation to the following: sex, age, smoking status, blood glucose and blood lipids.

4.5.1. **Gender differences**

Distribution amongst the five parameters:

Table 4.5.1.1. Subjects characters according to gender.

	Male	Female
WB	59	61
RBC	64	67
PLAMSA	65	66
CE	62	66
PL	51	66

The following table summarize the significant and none significant values amongst the five fractions. For example, within the WB fraction, the comparison was between the total fatty acids mean of 59 males and 61 females.

Table 4.5.1.2. Comparison of the fatty acids status according to the gender.

fatty acids	WB	RBC	Plasma	CE	PL
14:0	n.s.	0.004	n.s.	n.s.	n.s.
15:0	n.s.	n.s.	0.000	n.s.	n.s.
16:0	n.s.	n.s.	n.s.	n.s.	n.s.
17:0	0.008	n.s.	n.s.	n.s.	0.004
18:0	0.006	0.001	0.010	0.000	0.000
20:0	n.s.	0.001	n.s.	n.s.	n.s.
16:1n7	0.000	n.s.	0.012	0.001	0.001
18:1n9trans	n.s.	0.040	n.s.	n.s.	n.s.
18:1n7trans	n.s.	n.s.	n.s.		n.s.
18:1n9Cis	n.s.	0.000	0.000	n.s.	n.s.
18:1n7Cis	n.s.	n.s.	n.s.	n.s.	n.s.
20:1n9	n.s.	n.s.	n.s.	n.s.	n.s.
18:2n6 trans	0.040	n.s.	0.009	n.s.	n.s.
18:2n6 Cis	0.050	n.s.	n.s.	n.s.	n.s.
18:3n6	n.s.	n.s.	n.s.	n.s.	n.s.
20:2n6	n.s.	n.s.	n.s.	n.s.	0.005
20:3n6	0.000	n.s.	0.007	n.s.	0.031
20:4n6	n.s.	n.s.	n.s.	n.s.	n.s.
22:4n6	0.000	n.s.	n.s.	n.s.	0.000
18:3n3	n.s.	0.034	n.s.	n.s.	n.s.
20:5n3	n.s.	n.s.	n.s.	n.s.	n.s.
22:5n3	0.001	0.001	n.s.	n.s.	n.s.
22:6n3	0.019	0.001	0.000	n.s.	0.021

Continue table 4.5.1.2.

fatty acids	WB	RBC	Plasma	CE	PL
20:3n9	0.036	n.s.	0.017	n.s.	n.s.
SFA	n.s.	n.s.	n.s.	0.045	0.011
Atherogenic FA	n.s.	n.s.	n.s.	n.s.	n.s.
TFA	n.s.	n.s.	0.028	n.s.	n.s.
∑ MUFA	n.s.	0.000	0.006	n.s.	0.054
∑ PUFA	n.s.	0.000	n.s.	n.s.	n.s.
USFA	n.s.	n.s.	n.s.	n.s.	0.010
LCP n6	n.s.	n.s.	n.s.	n.s.	n.s.
LCP n3	n.s.	n.s.	0.013	n.s.	0.051
Omega 3 index	0.037	0.003	0.003	n.s.	0.013
∑ LCPUFA	n.s.	0.024	n.s.	n.s.	n.s.
∑ n6	n.s.	0.020	n.s.	n.s.	n.s.
∑ n3	n.s.	n.s.	0.048	0.034	0.037
∑ n7	0.000	n.s.	0.055	0.003	0.006
∑ n9	n.s.	0.000	0.000	n.s.	n.s.
n6/n3	n.s.	n.s.	0.013	n.s.	n.s.
n3/n6	n.s.	n.s.	0.010	0.047	n.s.
P/S	n.s.	0.004	n.s.	n.s.	0.022
U/S	n.s.	n.s.	n.s.	n.s.	0.013
LA/ALA	n.s.	0.025	n.s.	n.s.	n.s.
AA/ EPA	n.s.	n.s.	n.s.	n.s.	n.s.
DPA/ DHA	0.000	0.000	0.000	n.s.	0.051
Holman Index	n.s.	n.s.	n.s.	n.s.	n.s.
n3 HUFA score	n.s.	n.s.	0.008	n.s.	0.034

n.s. not significant at p >0.05

Due to the differences in hormonal balance, age, dietary intake or disease status (cholesterol gallstones which showed significant lower EPA levels) gendre differences occurred (Ogura et al., 2010). In healthy women, an inverse correlation was found between the LA intake and serum homo-g-LA (Lweis-Barned et al., 2000).

There were no significant differences between men and women among all fractions of the fatty acids of 16:0, 18:1n7t,c , 20:1n9, GLA, AA, EPA, Atherogenic FA, LCPn-6, AA/EPA, and holman index.

However, in FA 18:0 and omega-3 index the differences between men and women were highly significant among all the fractions. For DHA, and DPA/DHA the differences

were significant among all the fractions except in CE. For n-7, and their ratio (16:1n7/ 18:1n7) the differences were significant among all the fractions except in RBC. Oleic acid was highly significantly differing in RBC and plasma.

In adipose tissue, LA and DHA were shown to be higher in Scottish women than in Scottish men while in Japan a study by Ogura showed no significant differences between genders in PUFAs of the plasma PL (Ogura et al., 2010). In our study LA found to be different among WB, only in the other fractions no significant differences were noticed.

Estrogen effects might lower the conversion of the ALA to EPA and DHA in women than in men in the same age (Ratnayake and Galli, 2009). Although no significant differences in their concentrations were found in our PL fractions.

Welch noticed that DPA concentration was higher in men than in women which agreed with our results; while plasma fatty acids concentrations were higher in women than in men. In addition, fatty acids of plasma phospholipids concentrations are higher in women than in men even with same amount of fish intake. That might be explained according to the body size and plasma volume differences. In addition, among non dietary n-3 PUFA consumers, women still have higher n-3 PUFAs than men which could be returned to the oestrogen concentration (Welch et al., 2006).

In the study of MA et al.,1995 study, plasma PL and CE SFA and MUFA were higher in men than in women while PUFA were lower (MA et al., 1995). In this study it was noticed that men had higher SFA and lower MUFA and PUFA than women. Men had higher % of 16:0 in RBC PL than in women (Heude et al., 2002). However; in our data it was found that both RBC and plasma PL were higher in females than males although no significant differences were noticed.

As for the serum CE fraction, females' concentration of LA decreases with age faster than males while AA increases in males faster than females. However, it was found that PUFA and n-6 fatty acids have different falling curve, they decrease faster in

females than in males with getting old which reflect the decreasing in EFA status. Males had higher USFA indexes than females (Holman et al., 1979) while in this study, PL shows significant high USFA level in women.

4.5.2. **Age differences**

The ranges of age used in this work were 19-30 for youth subjects, and 40-62 for adult subjects.

Table 4.5.2.1. Shows the age groups characters categorized according to gender.

	Female (n)		**Male (n)**	
	19- 39	**40- 62**	**19- 39**	**40- 62**
WB	32	29	31	28
RBC	34	33	36	28
plasma	33	33	36	29
CE	32	34	37	25
PL	33	33	30	21

The following table summarizes the differences between age groups within each fraction. For example in WB, fatty acids levels were compared between females aged from 19 to 39 yrs old and females aged from 40 to 62 yrs old, similar within male.

Table 4.5.2.2: Comparison of the fatty acids in regards to age and differentiated according to Gender.

	Female					Male				
Fatty Acids	**WB**	**RBC**	**plasma**	**CE**	**PL**	**WB**	**RBC**	**plasma**	**CE**	**PL**
14:0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.017	n.s.	n.s.	n.s.
15:0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.020	n.s.
16:0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.024	n.s.	n.s.	n.s.
17:0	n.s.	n.s.	n.s.	n.s.	0.046	n.s.	n.s.	n.s.	n.s.	n.s.
18:0	n.s.	n.s.	n.s.	n.s.	n.s.	0.053	n.s.	n.s.	n.s.	0.017
20:0	n.s.	n.s.	n.s.	n.s.	n.s.	0.007	n.s.	0.014	n.s.	0.023
16:1n7	n.s.	n.s.	n.s.	n.s.	n.s.	0.005	0.053	0.018	0.016	0.012
18:1n9trans	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.003	n.s.
18:1n7trans	n.s.	0.016	n.s.	N/A	n.s.	n.s.	n.s.	n.s.	N/A	n.s.
18:1n9Cis	n.s.	0.008	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.015
18:1n7Cis	n.s.	n.s.	n.s.	0.016	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
20:1n9	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
18:2n6 trans	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.013	n.s.
18:2n6 Cis	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
18:3n6	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
20:2n6	0.009	0.056	0.049	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
20:3n6	0.001	0.030	0.002	0.002	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
20:4n6	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
22:4n6	n.s.	0.014	n.s.	n.s.	n.s.	0.009	0.025	n.s.	n.s.	n.s.
18:3n3	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
20:5n3	0.018	n.s.	0.021	0.054	n.s.	0.010	0.004	n.s.	0.011	n.s.
22:5n3	0.029	0.004	0.000	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
22:6n3	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.042
20:3n9	n.s.	n.s.	0.009	n.s.	n.s.	n.s.	0.005	0.044	n.s.	n.s.
SFA	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Atherogenic FA	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.023	n.s.	n.s.	n.s.
TFA	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.003	n.s.
∑ MUFA	n.s.	0.012	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.013
∑ PUFA	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
USFA	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.050	n.s.
LCP n6	n.s.	0.012	n.s.	n.s.	n.s.	0.028	0.007	n.s.	n.s.	n.s.
LCP n3	n.s.	n.s.	0.021	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Omega 3 index	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.012	n.s.
∑ LCPUFA	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
∑ n6	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.000	n.s.	n.s.	n.s.

Continue table 4.5.2.2.

	Female					Male				
Fatty Acids	**WB**	**RBC**	**plasma**	**CE**	**PL**	**WB**	**RBC**	**plasma**	**CE**	**PL**
∑ n3	n.s.	n.s.	0.042	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
∑ n7	n.s.	n.s.	n.s.	n.s.	n.s.	0.051	n.s.	0.054	0.031	0.030
∑ n9	n.s.	0.010	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.016
n6/n3	n.s.	n.s.	0.004	0.055	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
n3/n6	n.s.	n.s.	0.006	n.s.	n.s.	n.s.	0.021	n.s.	0.041	n.s.
P/S	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
U/S	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
LA/ALA	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
AA/ EPA	0.002	n.s.	0.001	n.s.	n.s.	0.006	0.001	n.s.	n.s.	n.s.
DPA/ DHA	n.s.	0.018	0.038	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Holman Index	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.003	0.002	n.s.	0.064
n3 HUFA score	0.014	0.063	0.005	n.s.	n.s.	0.034	0.023	n.s.	0.055	n.s.

n.s. not significant. N/A not available

Within serum CE fraction, females' concentration of LA decreased with age faster than males while AA increased in males faster than females. However, it was found that PUFA and n-6 fatty acids have different falling curve, they decreased faster in females than in males with getting old which reflect the decreasing in EFA status (Holman et al., 1979). This shows that age and sex have an evident effect on fatty acid composition. Aging reduces the activity of Δ6 desaturase which is important for the formation of GLA, DGLA, AA, EPA, and DHA (Undurti 2008).

Omega-3 index is affected by age (+0.50% per decade) (von Schacky, 2010) which was noticed to be significant in the CE fractions among males. Although no significant differences were noticed within the other fractions, the values were higher in older groups than younger groups in both genders amongst the 5 fractions. Rabini noticed a significant increase ($p < 0.05$) in RBC membrane U/S ratio amongst younger group (aged 21-40 yrs old) than in older group (aged > 41 yrs old) (Rabini et al., 2002).

Among Females there were no significant differences in regard to age categorizes among all fractions for 14:0, 15:0, 16:0, 20:0, 16:1n7, 18:1n9t, 18:2n6t, 18:2n6, 18:3n6, 20:4n6, ALA, DHA, SFA, TFA, PUFA,EFA, omega-3 index, LCPUFA, n-6, n-7, P/S, U/S, LA/ALA, and the Holman index. In Males there were no significant differences in regard to age categorizes in 17:0, 18:1n7t, 18:1n7c, 20:1n9, 18:2n6, 18:3n6, 20:2n6, 20:3n6, 20:4n6, ALA, DPA, LCPUFA, n-3, U/S, LA/ALA, and DPA/DHA.

Amongst fractions:

WB: it was found that DGLA, EPA, and DPA increased significantly with age in females while AA/EPA decreased. In males 16:1n7 and atherogenic acids significantly increased with age while 18:0, 20:0, AA, USFA, n-7, LCPn-6, and AA/EPA decreased.

RBC: oleic acid, DPA, MUFA, n-9, DPA/DHA and n-3 HUFA score significantly increased in females with age while DGLA, 22:4n6, and LCPn-6 decreased. However, in males, 14:0, 16:0, 16:1n7, 22:4n6, EPA, and atherogenic acids significantly increased with age while PUFA, n-6, LCPn-6 and AA/EPA decreased.

Plasma: in females, EPA, DPA, DHA, mead acid, n-3 and LCPn-3 significantly increased with age while AA/EPA decreased. However, in males, 16:1n7, mead acid and n-7 significantly increased with age while 20:0 and LA decreased.

CE: in females, adult women showed higher levels of 18:1n7, EPA, DHA and lower level of 22:4n6 than younger women. In males, adult males showed lower level of 15:0, 18:1n9trans, 18:2n6trans, atherogenic acid, TFA and higher level of 16:1n7, EPA, USFA, LCPn-3 and omega-3 index than younger males.

PLs: significant increasing with age was noticed in females for 17:0 and 18:0 fatty acids. In males, significant increasing with age had been noticed for 16:1n7, OA, DHA, MUFA, USFA, n-7 and n-9 and significant decreasing for 18:0, 20:0, and SFA.

Although age (19 - 64 years) has an influence on the serum fatty acids' absorption, synthesis, and metabolism; it is not associated with the marine origin n-3 PUFA in serum PL (Kobayashi et al., 2001).

In a study done in Japan among 75 adults (≤ 49 till 70≥ years old), correlations were noticed between age and PUFAs as a result of the dietary factors. While this correlation was positive with n-3 PUFA, it was negative with n-6 PUFAs (Ogura et al., 2010). AA in PLs was higher in young than in elderly even with identical AA intake. However, fish consumption increased significantly with age but no difference was notice with AA intake with age (Kawabata et al., 2011).

4.5.3. **BMI differentiation:**

Normal BMI stands for subjects with body mass index lay between 19- 24.99 kg/m^2 while overweight BMI stands for over 25 kg/m^2.

Table 4.5.3.1. Shows the BMI groups characters categorized according to gender.

	Female		**Male**	
	Normal BMI	**Overweight**	**Normal BMI**	**Overweight**
WB	47	14	34	25
RBC	53	14	40	24
plasma	51	15	39	26
CE	52	14	40	22
PL	52	14	35	16

The following table summarizes the differences between BMI groups within each fraction. For example in WB, fatty acids levels were compared between normal weight females with overweight females, similar within male.

Table 4.5.3.2. Comparison of the fatty acids in regards to BMI and categorized according to genders.

	BMI Differences in Female					BMI Differences in Male				
Fatty acid	**WB**	**RBC**	**plasma**	**CE**	**PL**	**WB**	**RBC**	**plasma**	**CE**	**PL**
14:0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
15:0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.007	n.s.
16:0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
17:0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.037	n.s.
18:0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
20:0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.017	n.s.
16:1n7	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
18:1n9 trans	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
18:1n7 trans	n.s.	n.s.	n.s.	N/A	n.s.	n.s.	n.s.	n.s.	N/A	n.s.
18:1n9 Cis	n.s.	n.s.	0.040	n.s.	n.s.	n.s.	n.s.	n.s.	0.056	n.s.
18:1n7 Cis	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
20:1n9	n.s.	n.s.	n.s.	n.s.	n.s.	0.042	n.s.	n.s.	n.s.	n.s.
18:2n6 trans	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
18:2n6 Cis	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
18:3n6	0.034	n.s.	0.039	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
20:2n6	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
20:3n6	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
20:4n6	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
22:4n6	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
18:3n3	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
20:5n3	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
22:5n3	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
22:6n3	0.059	n.s.	0.047	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
20:3n9	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
SFA	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Atherogenic FA	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
TFA	n.s.	n.s.	n.s.		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
∑ MUFA	n.s.	n.s.	0.017	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
∑ PUFA	0.040	n.s.	0.018	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Continue table 4.5.3.2.

	BMI Differences in Female					BMI Differences in Male				
Fatty acid	**WB**	**RBC**	**plasma**	**CE**	**PL**	**WB**	**RBC**	**plasma**	**CE**	**PL**
USFA	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
LCP n6	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
LCP n3	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Omega 3 index	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
∑ LCPUFA	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
∑ n6	n.s.	n.s.	0.037	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
∑ n3	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
∑ n7	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
∑ n9	n.s.	n.s.	0.031	n.s.	n.s.	n.s.	n.s.	n.s.	0.056	n.s.
n6/n3	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
n3/n6	n.s.	n.s.	n.s.	0.027	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
P/S	n.s.	n.s.	0.056	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
U/S	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
LA/ALA	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
AA/ EPA	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.032	n.s.
DPA/ DHA	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Holman Index	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
n3 HUFA score	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s. not significant , N/A not available.

Amongst females, males and both BMI categories; there were no significant differences in LA, ALA, AA, EPA, DPA, mead acid, SFA, TFA, USFA, LCPn-6, LCPn-3, EFA, omega 3 index, n-7, and holman index.

Although BMI has an influence on the serum fatty acids' absorption, synthesis, and metabolism; it has no association with the marine origin n-3 PUFA in serum PL (Kobayashi et al., 2001). Omega-3 index is affected by BMI (-0.30 % per three units) (von Schacky 2010) although that wasn't noticed in this study since no obese participant was included.

The relationship between quality of fat intake and obesity is still controversial. A study done in Spain reported no role of the fat intake and obesity, another study found a positive association between fat intake (rather than vegetable fats) and BMI. However, it has been reported that BMI was not correlated with any FAs. Amongst Australians, a negative association was found between n-3 PUFA and obesity. Plasma cholesterol ester fatty acids composition showed significant positive association between BMI and EPA and AA, and negative association with LA (Ogura et al., 2010). However, this study might suggest that females' fatty acids composition would be affected by BMI more than males since fatty acids amongst males WB, RBC, plasma, PL and most CE didn't differ significantly between both BMI groups.

Since no obese (BMI > 30 kg/m^2) subjects were enrolled in this study, little significant differences were found between categories. Plasma MUFA and OA significantly increased in female overweight than normal weight while plasma n-6 and PUFA in plasma and WB decreased.

4.5.4. **Smoking differentiation**

Table 4.5.4.1. Shows the smoking groups characters.

	Smoker	None Smoker
WB	67	42
RBC	72	48
Plasma	72	48
CE	71	45
PL	66	40

Smoker indicates current smoker subjects, while none smoker can be either former smoker subjects or subjects who never smoked.

The following table summarizes the differences between smoking groups within each fraction. For example in WB, fatty acids levels were compared between smoker and none smoker subjects.

Table 4.5.4.2. Comparison of the fatty acids levels according to the smoking status.

Fatty acids	WB	RBC	Plasma	CE	PL
14:0	n.s.	n.s.	n.s.	n.s.	n.s.
15:0	n.s.	n.s.	n.s.	n.s.	n.s.
16:0	n.s.	n.s.	n.s.	n.s.	n.s.
17:0	n.s.	n.s.	n.s.	0.034	n.s.
18:0	n.s.	n.s.	n.s.	n.s.	n.s.
20:0	n.s.	n.s.	n.s.	0.048	n.s.
16:1n7	n.s.	n.s.	n.s.	n.s.	n.s.
18:1n9trans	n.s.	n.s.	n.s.	n.s.	n.s.
18:1n7trans	0.006	n.s.	0.010	N/A	n.s.
18:1n9Cis	n.s.	n.s.	n.s.	n.s.	n.s.
18:1n7Cis	n.s.	n.s.	n.s.	n.s.	n.s.
20:1n9	0.005	n.s.	n.s.	n.s.	0.057
18:2n6 trans	n.s.	n.s.	n.s.	n.s.	n.s.
18:2n6 Cis	n.s.	n.s.	n.s.	n.s.	n.s.
18:3n6	n.s.	0.024	n.s.	n.s.	n.s.
20:2n6	n.s.	0.026	n.s.	n.s.	n.s.
20:3n6	n.s.	n.s.	n.s.	n.s.	n.s.
20:4n6	n.s.	n.s.	n.s.	n.s.	0.044
22:4n6	n.s.	n.s.	n.s.	n.s.	n.s.
18:3n3	n.s.	n.s.	n.s.	n.s.	n.s.
20:5n3	n.s.	n.s.	n.s.	0.028	0.028
22:5n3	n.s.	0.004	n.s.	n.s.	n.s.
22:6n3	n.s.	0.033	n.s.	n.s.	n.s.
20:3n9	n.s.	n.s.	n.s.	n.s.	n.s.
SFA	n.s.	n.s.	n.s.	n.s.	n.s.
Atherogenic FA	n.s.	n.s.	n.s.	n.s.	n.s.

Continue table 4.5.4.2.

Fatty acids	WB	RBC	Plasma	CE	PL
TFA	n.s.	n.s.	n.s.	n.s.	n.s.
∑ MUFA	n.s.	n.s.	n.s.	n.s.	n.s.
∑ PUFA	n.s.	n.s.	n.s.	n.s.	n.s.
USFA	n.s.	n.s.	n.s.	n.s.	n.s.
LCP n6	n.s.	n.s.	n.s.	n.s.	0.043
LCP n3	n.s.	0.008	n.s.	n.s.	n.s.
Omega 3 index	n.s.	0.043	n.s.	n.s.	0.047
∑ LCPUFA	n.s.	0.009	n.s.	n.s.	0.012
∑ n6	n.s.	n.s.	n.s.	n.s.	n.s.
∑ n3	0.057	0.008	n.s.	0.048	n.s.
∑ n7	n.s.	n.s.	n.s.	n.s.	0.047
∑ n9	n.s.	n.s.	n.s.	n.s.	n.s.
n6/n3	0.027	0.004	n.s.	0.023	n.s.
n3/n6	0.032	0.019	n.s.	n.s.	n.s.
P/S	n.s.	n.s.	n.s.	n.s.	n.s.
U/S	n.s.	n.s.	n.s.	n.s.	n.s.
LA/ALA	n.s.	n.s.	n.s.	n.s.	n.s.
AA/ EPA	n.s.	n.s.	n.s.	n.s.	n.s.
DPA/ DHA	n.s.	n.s.	n.s.	n.s.	n.s.
Holman Index	n.s.	n.s.	n.s.	n.s.	n.s.
n3 HUFA score	n.s.	0.047	n.s.	n.s.	n.s.

n.s. not significant , N/A not available

Although smoking status might have an influence on the serum fatty acids' absorption, synthesis, and metabolism; it is not associated with the marine origin n-3 PUFA in serum PL (Kobayashi et al., 2001). Fatty acids composition in both cigarette smoker and non-smoker has been described. Tobacco is thought to have an inhibitory effect on the essential fatty acids metabolism (Simon et al., 1996).

WB, plasma and CE n-3 fatty acids concentration decreased significantly in smoker subject. PUFAs concentrations (both n-3 and n-6) in the membrane were found to be depleted as a result of smoking (Chiu et al., 2003).

In a study done in 2002 on male physicians in Boston, it was noticed that current smokers have significantly lower level or LCPUFAn-3 (Albert CM, 2002) which conformed to our RBC LCPn-3 levels. However, RBC fraction showed the more significant differences than other fraction which may raise a hypothesis that smoking would affect the FA long-term composition. 18:3n6, 20:2n6, and n-3:n6 ratios

increased significantly in smoker than non smoker, while DPA, DHA, n-3, LCPn-3, omega-3 index, sum of LCPUFA decreased. EPA in both CE and PL showed significant reduction in their concentrations in smoker subjects than non-smokers. Similar results were shown in another study; where smokers had lower percentage of EPA in RBC PL than none smoker (Heude et al., 2002).

Male smokers have high BMI (overweight or obesity) and alcohol consumption leads to high SFA in plasma PL and CE with low LA. That would be explained by the hypothesis of conversion alcohol to SFA endogenously or that those conditions would affect FA metabolism. SFA in plasma PL and CE were higher and 18:2n6 was lower in overweight, male smokers than non-smoking with normal BMI males (MA et al., 1995).

4.5.5. Glycaemic status and fatty acids composition

Table 4.5.5.1.Subjects characteristics according to glycaemic status.

	Hypoglycemia	Normal blood glucose
WB	16	93
RBC	18	100
plasma	18	100
CE	16	100
PL	15	90

When fasting, blood glucose (no caloric intake within last 8 hours) ranges between 4-6 mmol/L (60 -110 mg/dl), it reflects normal blood glucose. If the range exceeds 110 mg/dl, it reflects hyperglycemia (Earl et al., 2002) ranged from 6 to 11 mmol/l (Preissig and Rigby, 2010), while beneath 60 mg/dl it reflects hypoglycemia (Miller et al., 2001) alike to blood glucose ≤2·2 mmol/l (Vlasselaers et al., 2009).

Table (4.9.2.) summarizes the differences between normal glycaemic and hypoglycemic groups within each fraction. For example in WB, fatty acids levels were compared between normal- with hypo-glycaemic.

Table 4.5.5.2. Comparison of fatty acids composition between hypo- and normal-glycaemic subjects.

Fatty acids	**WB**	**RBC**	**Plasma**	**CE**	**PL**
14:0	n.s.	n.s.	n.s.	n.s.	n.s.
15:0	n.s.	n.s.	n.s.	n.s.	n.s.
16:0	n.s.	n.s.	n.s.	0.049	n.s.
17:0	n.s.	n.s.	n.s.	n.s.	n.s.
18:0	n.s.	n.s.	n.s.	n.s.	n.s.
20:0	n.s.	n.s.	n.s.	n.s.	n.s.
16:1n7	n.s.	n.s.	n.s.	n.s.	n.s.
18:1n9trans	n.s.	n.s.	n.s.	n.s.	0.052
18:1n7trans	n.s.	n.s.	n.s.	N/A	n.s.
18:1n9Cis	n.s.	n.s.	n.s.	n.s.	n.s.
18:1n7Cis	0.034	n.s.	n.s.	n.s.	n.s.
20:1n9	0.023	n.s.	n.s.	0.043	0.015
18:2n6 trans	n.s.	n.s.	n.s.	n.s.	n.s.

Continue table 4.5.5.2.

Fatty acids	WB	RBC	Plasma	CE	PL
18:2n6 Cis	n.s.	n.s.	n.s.	n.s.	n.s.
18:3n6	n.s.	0.035	n.s.	n.s.	0.000
20:2n6	n.s.	n.s.	n.s.	n.s.	0.057
20:3n6	n.s.	n.s.	n.s.	n.s.	n.s.
20:4n6	n.s.	n.s.	n.s.	n.s.	n.s.
22:4n6	0.021	n.s.	n.s.	n.s.	0.000
18:3n3	n.s.	n.s.	n.s.	n.s.	n.s.
20:5n3	n.s.	0.000	n.s.	n.s.	n.s.
22:5n3	n.s.	0.042	n.s.	n.s.	n.s.
22:6n3	n.s.	n.s.	n.s.	n.s.	n.s.
20:3n9	n.s.	0.021	n.s.	n.s.	n.s.
SFA	n.s.	n.s.	n.s.	n.s.	n.s.
Atherogenic SFA	n.s.	n.s.	n.s.	n.s.	n.s.
Atherogenic FA	n.s.	n.s.	n.s.	n.s.	n.s.
TFA	n.s.	n.s.	n.s.	n.s.	0.051
∑ MUFA	n.s.	n.s.	n.s.	n.s.	n.s.
∑ PUFA	n.s.	n.s.	n.s.	n.s.	n.s.
USFA	n.s.	n.s.	n.s.	n.s.	n.s.
LCP n6	n.s.	n.s.	n.s.	n.s.	n.s.
LCP n3	n.s.	0.054	n.s.	n.s.	n.s.
Omega 3 index	n.s.	n.s.	n.s.	n.s.	n.s.
∑ LCPUFA	n.s.	n.s.	n.s.	n.s.	n.s.
∑ n6	n.s.	0.009	n.s.	n.s.	n.s.
∑ n3	n.s.	0.054	n.s.	n.s.	n.s.
∑ n7	n.s.	n.s.	n.s.	n.s.	n.s.
∑ n9	n.s.	n.s.	n.s.	n.s.	n.s.
n6/n3	n.s.	0.029	n.s.	n.s.	n.s.
n3/n6	0.051	0.021	n.s.	n.s.	n.s.
LCPn6/ LCPn3	0.015	0.043	n.s.	n.s.	n.s.
P/S	n.s.	n.s.	n.s.	n.s.	n.s.
USFA/SFA	n.s.	n.s.	n.s.	n.s.	n.s.
LA/ALA	n.s.	n.s.	n.s.	n.s.	n.s.
AA/ EPA	0.005	0.000	n.s.	n.s.	n.s.
DPA/ DHA	n.s.	n.s.	n.s.	n.s.	n.s.
Holman Index	n.s.	n.s.	0.058	0.037	n.s.
161n7/181n7	n.s.	n.s.	0.004	n.s.	n.s.
n3 HUFA score	0.013	0.029	n.s.	n.s.	n.s.

n.s. not significant, N/A not available

No significant differences were found between the groups amongst all the fractions for OA, LA, ALA, AA, SFA, MUFA, PUFA, USFA, omega-3 index, n-7 and n-9.

EPA, DPA, n-3 and n-3 HUFA in RBC fraction were found to be significantly lower in hypoglycemic subjects than normal blood glucose subjects, and the opposite with n-6 and n6/n3.

Holman index was found to be significantly higher in hypoglycemic group in plasma CE fraction. 20:1n9 were significantly lower in normal blood glucose subject than hypoglycemic in WB, CE, and PL.

The AA/EPA ratio in WB, RBC were significantly lower in normal blood glucose subject than the hypoglycemic group (p=0.005 and p= 0.000, respectively).

A threshold and modest association was observed between omega 3 index (EPA and DHA) intake and type 2 diabetes incidents. However, no association as observed with ALA intake (Djoussé et al., 2011).

4.5.6. Lipidemia status and fatty acids composition

Table 4.5.6.1. Subjects characteristics according to lipidemia status.

	Hyperlipidemia	Normal blood TG
WB	8	105
RBC	8	114
Plasma	9	113
CE	9	110
PL	8	100

The American Heart Association had established guidelines for triglyceride levels in which Normal range, low risk < 150 mg/dl (<1.7 mmol/l), borderline high 150- 199 mg/dl (1.7- 2.2 mmol/l), and high level of TG when higher than 200 mg/dl (>2.2 mmol/l) (American heart association, 2010; Genest et al., 2009).

Table 4.5.6.2. Comparison of fatty acids composition between normal- and hyper-lipidemia subjects.

Fatty acids	WB	RBC	Plasma	CE	PL
14:0	0.005	n.s.	0.051	n.s.	n.s.
15:0	n.s.	n.s.	n.s.	n.s.	n.s.
16:0	0.007	n.s.	0.008	n.s.	n.s.
17:0	n.s.	n.s.	n.s.	n.s.	n.s.
18:0	n.s.	n.s.	n.s.	n.s.	n.s.
20:0	n.s.	n.s.	n.s.	n.s.	0.016
16:1n7	n.s.	n.s.	n.s.	n.s.	n.s.
18:1n9trans	n.s.	n.s.	n.s.	n.s.	n.s.
18:1n7trans	n.s.	0.004	n.s.	N/A	n.s.
18:1n9Cis	0.000	0.002	0.000	n.s.	n.s.
18:1n7Cis	n.s.	n.s.	n.s.	0.009	0.002
20:1n9	n.s.	n.s.	n.s.	n.s.	n.s.
18:2n6 trans	n.s.	n.s.	n.s.	n.s.	n.s.
18:2n6 Cis	n.s.	n.s.	0.000	n.s.	n.s.
18:3n6	0.008	0.057	n.s.	0.035	n.s.
20:2n6	n.s.	n.s.	n.s.	n.s.	n.s.
20:3n6	0.019	n.s.	0.013	n.s.	n.s.
20:4n6	0.001	n.s.	0.000	n.s.	n.s.

Continue table 4.10.2.					
Fatty acids	**WB**	**RBC**	**Plasma**	**CE**	**PL**
22:4n6	n.s.	n.s.	0.001	n.s.	n.s.
18:3n3	0.029	n.s.	n.s.	n.s.	n.s.
20:5n3	n.s.	n.s.	n.s.	n.s.	n.s.
22:5n3	n.s.	n.s.	0.001	n.s.	n.s.
22:6n3	0.002	n.s.	0.001	n.s.	n.s.
20:3n9	n.s.	n.s.	n.s.	n.s.	n.s.
SFA	0.017	n.s.	0.007	n.s.	n.s.
Atherogenic FA	0.004	n.s.	0.006	n.s.	n.s.
TFA	n.s.	n.s.	n.s.	n.s.	n.s.
∑ MUFA	0.001	0.003	0.000	n.s.	n.s.
∑ PUFA	0.000	n.s.	0.000	n.s.	n.s.
USFA	0.021	n.s.	0.009	n.s.	n.s.
LCP n6	0.000	n.s.	0.000	n.s.	n.s.
LCP n3	0.007	n.s.	0.015	n.s.	n.s.
Omega 3 index	0.009	n.s.	0.018	n.s.	n.s.
∑ LCPUFA	0.000	n.s.	0.000	n.s.	n.s.
∑ n6	0.001	n.s.	0.000	n.s.	n.s.
∑ n3	0.016	n.s.	0.018	n.s.	n.s.
∑ n7	n.s.	n.s.	n.s.	n.s.	0.028
∑ n9	0.000	0.002	0.000	n.s.	n.s.
n6/n3	n.s.	n.s.	n.s.	n.s.	n.s.
n3/n6	n.s.	n.s.	n.s.	n.s.	n.s.
P/S	0.001	0.056	0.000	n.s.	n.s.
U/S	0.020	n.s.	0.011	n.s.	n.s.
LA/ALA	0.054	0.046	0.020	n.s.	n.s.
AA/ EPA	n.s.	0.015	n.s.	n.s.	n.s.
DPA/ DHA	0.033	n.s.	n.s.	n.s.	n.s.
Holman Index	n.s.	n.s.	n.s.	n.s.	0.044
n3 HUFA score	n.s.	n.s.	n.s.	n.s.	n.s.

n.s. not significant, N/A not available

Sum of SFA concentration and atherogenic FA increased significantly in hyperlipidemic subjects in both WB and plasma. Fatty acid 14:0 significantly increased in hyperlipidemic WB and plasma. While 16:0 significantly increased in hyperlipidemic plasma, 18:0 significantly decreased in WB.

OA increased significantly in WB, RBC, and plasma in hyperlipidemic subjects. 18:3n6 were significantly higher in hyperlipidemic subjects in WB, RBC, and CE. While ALA was significantly higher in WB hyperlipidemic subjects, LA was found to be significantly higher in plasma normal TG level subjects. Similarly the case was with AA as its concentration was significantly higher among normal TG subjects in WB and Plasma. DPA and DHA were significantly higher in plasma normal TG.

MUFA levels were significantly higher in hyperlipidemic of WB, RBC and plasma, while both PUFA and USFA levels were significantly lower in WB and plasma.

WB and plasma n-3, n-6, LCPn-3, LCPn-6, omega 3 index, P/S, U/S, LA/ALA were significantly higher in normal TG subject while n-9 was significantly lower.

However, it was noticed that WB and plasma were the most affected fractions by the TG levels variation, while CE and PL were the lowest.

5. Discussion

The objective of this study is to evaluate the best blood component for assessing essential fatty acids and omega-3 index status that can give accurate profiles, easy-to-do, consumes less time, less material, less agents and less sample size.

5.1. Essential fatty acid status

The fatty acid analysis in the venous blood fractions demonstrated that the five blood components have lead to different concentrations of SFAs, MUFAs, PUFAs although some fractions were close in some fatty acids but in general they were not alike.

The most abundant family of fatty acids in all fractions was PUFAs except for PL where more than half of the fatty acids were saturated. In WB, RBC and plasma the second abundant FAs family was SFA followed by MUFA, while CE had the lowest content of SFAs.

The concentrations of the SFAs in the fractions were: PL> RBC> WB> Plasma > CE. Of the MUFA, the distributions were, plasma> WB> CE> RBC> PL while for the PUFA, CE> RBC> WB> Plasma> PL. In regard to USFA, the proportions were, CE> Plasma> WB> RBC> PL.

The percentage of EFA (LA + ALA) was not equal in the different fractions which were noticed in the ratio of LA/ALA. The percentage of LA was much higher in all fractions than ALA. However, LA (18:2n6c) was abundant in CE followed by plasma, WB, PL and the lowest was in RBC. ALA (18:3n3) showed similar series although the highest proportion of ALA was found in plasma followed by CE, then WB, PL and the lowest was in RBC.

Usually ALA concentration in plasma phospholipids is not more the 0.5% (Holman 1998) while no trace for ALA in RBC (Riséa et al., 2007). RBC might contain the lowest levels of EFA since EFA aren't stored in their intake forms but they are stored in their derivations forms. CE would be the best to assess EFA intake as their concentrations (ALA + LA) were found to be the highest in CE while the lowest were in RBC ,as RBC does not

influenced by day to day dietary intake according to its longer half life shelf (4 to 6 more time longer than plasma) (Sun et al., 2007).

As DHA concentrations were found to be the highest in RBC followed by WB therefore, the omega-3 index was highest in RBC followed by WB. n-3 and LCPn-3 fatty acids were also highest in RBC followed by WB whereas the lowest was in CE. n-6 (since it includes LA) concentrations were highest in CE followed by WB and the lowest in PL, while LCPn-6 (since it doesn't include LA) concentrations were the highest in RBC (according to the high level of AA) followed by WB whereas the lowest was found in CE. However, sum of LCPUFA were highest in RBC followed by WB and the lowest was in CE.

Omega-3 index in plasma might be affected with the most recently consumed meal. A meal poor in n-3 FAs and contain other FAs will enrich the plasma FA pool with other FAs but not n-3 FAs, which will decrease n-3 FAs content consequently. This will not happen in RBC FAs pool as RBC is not affected by recently consumed meals whatever were the FAs content of those meals. According to the stability of RBC Omega-3 index (EPA+ DHA) and since it isn't affected by the last meal, RBC are considered to be better biomarker than plasma or plasma PL (Harris and Thomas, 2009). Omega-3 index is found higher in coastal population than in land communities (3.8% Vs 3.2%) (Dewaillya et al., 2003).

Although there were significant differences between the different groups that were analysed, it was recognized from this study that blood triglycerides are more affected by the fatty acids than glycaemic status. No significant differences were found between glycaemic groups while SFAs, MUFAs, PUFAs, USFAs, omega-3 index, LCPUFA significantly differ between lipidemia groups. BMI had little effect after the exclusion of obese subjects; however, females' fatty acids composition would be affected by BMI more than males.

The interesting results will be the fatty acids and the indexes that relate to the EFA as they have been correlated with the risk for CVD and CHD. The major fatty acids that contribute to the total concentration of n-3 FA in human blood components are

EPA+DHA+DPA while ALA being the minor, AA +LA were the major components to the total n-6 FA.

5.2. Importance of omega-3 index for human health

n-3 fatty acids were established to assess the fatty acid pattern and the risk of death from CHD. In western populations, high concentrations of n-3 fatty acids (especially omega-3 index EPA+ DHA) in dietary intake and blood levels decrease the risk of sudden cardiac death. However, EPA+ DHA lower triacylglycerols, they also work as anti-hypertensive, and have anti-inflammatory roles in lowering CHD risk. Therefore, omega-3 index is hypothesized to be predictor for CHD mortality (Stark 2008).

One of the first studies was DART (the Diet and Reinfarction Trial) which investigated the association between dietary intake of n-3 fatty acids and secondary prevention of myocardial infarction. GISSI-Prevemzione Trial is one of the largest recent studies that concluded toward omega-3 fatty acids to reduce 45% of sudden death and 20% reduction in all cause mortality (Covington 2004).

n-3 fatty acids were found to be low in patients with bipolar disorder, schizophrenia (Ranjekar et al., 2003), depression (Sontrop and Campell, 2006), child hyperactivity (Chen et al., 2004) and autism (Vancassel et al., 2001).

5.3. Correlations between fatty acids status among methods

Comparisons between fatty acids were limited. Several blood biomarkers of omega-3 fatty acids intake have been reported; including the percentage of EPA + DHA omega-3 index in erythrocytes (Harris and Von Schacky, 2004), in plasma phospholipids (Prisco et al., 1996), EPA/AA ratio (Rupp et al.,2004), n-6/n-3 ratios (Harris et a., 2006), and n-3 HUFA score (Stark et al., 2005).

5.3.1. Choosing the biomarker depends on many considerations

1. Fatty acids or indexes that are the focus of the study.
2. Sensitivity of the biomarker; so if the interest was the short-term changes in n-3 status then plasma, PLs, CE will be more favourable than RBC. However, in the steady state all the medians would be fine according to their highly correlated between their values (Harris and Thomas, 2009).
3. For large observational and clinical trials, blood based biomarkers are more favourable than adipose tissue (Stark 2008) especially when dealing with large number of participants (Ogura et al., 2010) as participants would be more compliant if the samples are taken as blood more than an adipose tissue biopsy (MA et al., 1995).

5.3.2. Parallelism between fractions

The intake correlation is better in n-3 RBC than in n-3 adipose tissue. However, RBC is better as a biomarker for the intake than in plasma which might make RBC a replacement of the adipose tissue especially for the long term evaluation (Sun et al., 2007).

Plasma PL and CE might not give an exact reflection of the intake of fat and fatty acids. RBC cannot be used as a biomarker for SFA in short-term studies (3 wks) (Popitt et al., 2005).

In a study done by Qi Sun, RBC DHA concentration was better as a biomarker of the DHA intake than plasma DHA, although the correlation between both biomarkers and DHA's intake were very strong. The same was noticed with total *trans* fatty acids. While the associations between dietary MUFA and SFA with plasma and RBC were weak according to their endogenously synthesized from carbohydrates. Thus this study concluded that for long-term intake, RBC n-3 FA and TFA are suitable biomarkers (Sun et al., 2007).

5.3.3. Whole blood: favourable method

Since total blood fatty acids are analyzed in venous whole blood, thus values obtained from WB give a general overview of all dietary intake terms (short, medium, and long term). Whole blood method saves many steps that must be done for the other methods:

1. No need for the separation of WB into RBC, plasma (usually done by centrifugation) which also might save sample amount.
2. RBC needs washing to remove the white cells and plasma contamination.
3. Plasma PL and CE need separation by TLC which need around 3-5 hours (Bailey-Hall et al., 2008).
4. Since WB necessitate one step only to be analyzed, therefore it requires less agents, less time, and save materials.

WB represents two different LCPUFAn-3 pools, short term (plasma) and stored term (RBC), which would reflect the complementary profiles (Albert et al., 2002). Through the linear omega-3 index graphs, it was clear that WB had a stronger positive correlation with RBC and with plasma than between other fractions, which strengthen considering WB also as a system for an omega-3 index biomarker. The highest concentration of omega-3 index was in RBC (5.82%) followed by WB (3.48%), then plasma (2.55%), and PL (2.38%) while the lowest was found in CE (1.38%). This shows that RBC is the best according to the highest concentration followed by WB. Thus WB would be also a good indicator as the omega-3 index is also high and the analyzing process is the easiest amongst other methods.

Skeaff suggested whole blood for fatty acids analysing as it gives adequate approach (Skeaff et al., 2006) in addition, Riséa reported that blood fatty acids profile are mainly determined from plasma and RBC phospholipids but not for EPA. Therefore he suggested plasma and whole blood to be the best two methods for assessing FA status (Riséa et al., 2007). However, the whole blood method is characterized as simple, rapid (Ratnayake and Galli 2009) and useful for fatty acids status from large populations (Agostoni et al., 2011).

6. Summary

The objective of the study was to set up the appropriate system for assessing fatty acids profiles especially essential fatty acids and omega-3 index status that can give accurate profiles, easy-to-do, consumes less time, less material, less agents and less sample size.

The present study included 140 Austrian adults of both genders of which 69 female, 71 male. Five blood components had been analysed to assess 24 fatty acids and to calculate omega 3 index. Whole blood, red blood cells, plasma total lipids, plasma phospholipids and plasma cholesterol ester were all assessed from fasting venous blood.

The interesting fatty acids those that are related to essential fatty acids, since they have been correlated to CVD and CHD risks. The major fatty acids that contribute to the total concentration of n-3 fatty acids in human blood components are EPA and DHA and DPA while ALA being the minor; AA and LA are the major components to the total n-6 fatty acids

Many papers suggested RBC to be the best indicator for omega-3 index as RBC has the highest concentration with low variability. However, WB is the second highest parameters with omega-3 index especially that analysing omega-3 index from WB is easier than RBC since it does not need any extractions. Through the five tested systems, the best correlations were between WB and plasma followed by WB and RBC especially for the omega-3 index.

WB parameter was found to be the most favourable blood component to assess the omega-3 index and essential fatty acids due to the following aspect:

1. Reflects general indications of the short, medium and long term dietary intake.
2. Saves the separation steps which save:
.2.1 Time.
.2.2 Sample size.
.2.3 Materials and agents.
3. Simple
4. Rapid.
5. Gives accurate values.
6. Easy to do and does not require professional worker such as PL and CE methods.

According to this study, assessing EFA and omega-3 index from venous whole blood parameter are recommended.

7. Fazit:

Das Ziel der Studie war die Etablierung eines geeigneten Systems zur Bewertung des Fettsäuremusters, insbesondere essentieller Fettsäuren und des Omega-3-Index, welches ein genaues Profil reflektiert, einfach durchzuführen ist, weniger Zeit, Material und finanzielle Mittel sowie ein geringes Proteinvolumen benötigt.

Die vorliegende Studie umfasste 140 österreichische Erwachsene beiderlei Geschlechts davon 69 weiblich, 71 männlich. Fünf Blutkomponenten wurden verwendet um 24 Fettsäuren zu bewerten und den Omega-3-Index zu berechnen. Vollblut (VB), rote Blutkörperchen (RBZ), Plasma, Plasma-Phospholipide und Plasma-Cholesterinester wurden im venösen Nüchternblut untersucht.

Die essentiellen Fettsäuren, die in Verbindung mit dem Risiko für CVD und KHK stehen, bildeten den Schwerpunkt der Arbeit. Hierzu zählen die n-3-Fettsäuren EPA und DHA, DPA und ALA; sowie AA und LA sind die wichtigsten n-6-Fettsäuren im menschlichen Körper.

RBZ werden aufgrund der Fettsäuren-Konzentration und der geringen Variabilität vielfach als bestes Kompartiment zur Errechnung des Omega-3-Index angesehen. VB ist jedoch ebenfalls zur Bestimmung des Omega-3-Index geeignet, wobei die Analytik einfacher ist als in den RBZ, da keine Extraktionen durchgeführt werden müssen. Bei den fünf getesteten Komponenten wurden die besten Korrelationen zwischen VB und Plasma, gefolgt von VB und RBZ speziell für den Omega-3-Index gefunden.

VB konnte basierend auf folgenden Aspekten als bevorzugte Blutkomponente zur Beurteilung des Omega-3-Index und essentieller Fettsäuren erfasst werden:

1. Reflektiert die kurz-, mittel- und langfristige Aufnahme an Fett über die Nahrung.
2. Speichert die Trennungsschritte und spart daher:
.2.1 Zeit
.2.2 Stichprobenumfang
.2.3 Materialien und Reagenzien
3. Einfach
4. Schnell
5. Ergibt genaue Werte

6. Einfach durchzuführen und erfordert keine speziellen Fachkräfte wie PL und CE-Methoden

Laut dieser Studie, kann die Beurteilung der EFA und des Omega-3-Index in venösem Vollblut empfohlen werden.

8. References

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CURRICULUM VITAE

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DOB	06/03/1979
Gender	Female
Martial Status	married
Nationality	Palestinian

Education & Qualification

Since Oct 2008	**Doctoral Student:** Nutritional Science. ***University of Vienna - Austria***
2004-2005	**M.Med.Sci**: Human Nutrition. ***Sheffield University, United Kingdom***
2002-2003	Jeddah Economic Room **Diploma** in Management and Administration.
1996-2001	**BSc:** Nutrition and Food Science Department – Agriculture Engineering College ***Damascus University- Syria***

Career Goals

To apply my knowledge of **Human Nutrition** in a context which will use my management skills, client communication, and academic background.

Human Nutrition Master

I read for my MSc in Human Nutrition Department, at Sheffield University UK.

Majoring credits:

1-Biochemistry.
2-Epidemiolog.
3-Physiolog.
4-Third World Nutrition.
5-Clinical Nutrition.

Essays:

- AIDS/HIV Physiology changes in European adult.
- Comparison between Fast and Starvation.
- Food policy for infant in Sub-Saharan African.
- African Nutrition deficiency.
- Food policy history.
- Lab report: Resting Energy Requirement, Maximum heart rate.
- Nigerian children nutrition.
- Hormonal effective on Baby Growth

MSc Research

Investigation the effects of frequent feeding on overall bone turnover in healthy adults. Dealing with volunteers by measuring human urine biomarkers following particular meals in particular times.

The BSc

I read for my BSc in Nutrition and food Science Department, at Agriculture Engineering College at Damascus University- Syria.

Majoring

Food manufacture and Analysis, Food preservation and Nutrition and Food security strategy.

Research:

Chocolate Manufacture and Chemistry, Microbiology and Physical Analysis.

Visited Syrian Chocolate Factories and analyse Micro flora and Bacteria's Environment.

Work Experience

Jan-July 01	Nutritional Lecturer And Educator, **ABBOTT** Company for Babies, Lactating and Pregnancy Women's Food in Jeddah's Hospitals and Health Clinics, ***Saudi Arabia.***
Sep-05 to Nov 06	Dietician and Nutritional Consultant in **Nutrition and Diet Centre**. ***Jeddah, Saudi Arabia.***
Jan- 2007 to Sep 2008	Member in Nutrition and food science Department / Health Science College at **Al-Kalamoon University**, Full Time Lecturer, Assistance in Management Matters, Teach Nutritional Courses (Introduction to Human Nutrition, Community Nutrition Courses, Protein Biochemistry Course, Therapeutic Nutrition (Theory and Lab Sessions), Nutritional Assessment, and Food Safety Lab Session), Supervise Graduated Thesis, Schedules Control, Supervise Students' Scientific Training in Hospitals, Students' Tutorial. ***Deratiah, Damascus- Syria.***
28th to 30th June 2007	***Participation in The Conference*** of "Chronic Constipation In Infant And Toddlers" With Lecture Titled "The Role Of Fiber Dietary Intake In Treating Constipation In Infant And Toddlers" ***Tartous City- Syria.***
21st Of Feb 2008	***Participation In The Conference*** Of "Promotion of Breast Feeding In Syria" With Lecture Titled "The Nutritional Needs Of Lactating Women"; Under UNICEF Sponsored. ***Tadmur City- Syria.***
24TH Of June 2008	***Participation In The Conference*** Of "Arab Doctors Pan" With Lecture Titled "MyPyramid for kids"; ***Damascus city- Syria***

Skills

Languages

Fluent in **Arabic, English**.

IELTS 6.5 from Canterbury, UK.