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Diet Related Diseases & Lipidomics– A Diagnostic Tool!

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Abstract—*Lipidomics is an emerging field in which lipid analysis is done to analyze various diseases related to nutrition habit of an individual. The abundance of individual lipid molecular species in plasma may be indicative of the variety of specific human diseases like cardiovascular disease, diabetes mellitus, hypertension, obesity etc. It is essential to determine which lipid diet source reflects the nutritional status of each individual most accurately so that disease can be monitored at the nutrition level. Analysis of a patient's plasma lipidome provides a valuable approach for detecting and monitoring human diseases and their treatment efficacy. Analysis also reveals information on the functions and effects of particular lipid deficiencies on the whole organism. The analysis based on mass spectrometry has enabled high throughput quantitative profiling of the lipidome from minute amounts of samples and the identification of lipids as individual molecular species. Evidence for the beneficial effects of a diet with fish and omega 3 fatty acid-rich products has proved to be beneficial on various diseases.*

Keywords: *Lipidomics, plasma, lipidome, mass spectrometry*

1. INTRODUCTION

Lipids are important nutrients for cell existence and proper functioning. Lipids provide polyunsaturated fatty acids (PUFA) that are important for several cellular functions in the body [1-3] including ligands for transcription factors, precursors of signal molecules and building blocks in all cells of the body. The intracellular disturbance in the lipid metabolism is responsible for the cause of numerous metabolic diseases that have genetic and/or dietary and nutritional or unhealthy life style.

Many major diseases involve lipids. Examples include atherosclerosis, alzheimer's disease, diabetes, lipid storage disease and cancer [4, 5, 6]. Several acquired or secondary factors including genetic determinants, diabetes mellitus, obesity, and sedentary lifestyle can cause hypertriglyceridemia, a prevalent form of dyslipidemia that is frequently associated with premature coronary artery disease [7].

Lipidomics may have implications for the treatment of metabolic diseases including Type 2 diabetes and cardiovascular disease as well as autoimmune disorders including rheumatoid arthritis, neurodegenerative diseases, kidney and liver disorders, and numerous other lipodystrophys [8]. In patients with sitosterolemia, disorder, plasma cholesterol levels are also elevated, and xanthomatosis and premature atherosclerosis develop [9,10].

The increase in the number of patients with metabolic diseases including type 2 diabetes and obesity, which are associated with an elevated risk of cardiovascular disease demands more detailed lipid analyses both for diagnostic purposes and for monitoring the efficacy of prescribed therapy.

It will also be important to understand how to sample lipidomes. One has to determine which lipid diet source reflects the nutritional status of each individual most accurately.

Lipid analysis can be used as a diagnostic tool in molecular medicine. It can be used in nutritional research to increase the life expectancy as our health is clearly dependent on our diet. Research shows that by changing the eating habits in males in the eastern Finnish population, the life expectancy of males is becoming normalized to reach the European average. Because of intake of high saturated lipids diet, the mortality used to be unusually high because of cardiovascular disease in males in the eastern Finnish population [11]. Evidence for the beneficial effects of a diet with fish and omega 3 fatty acid-rich products has proved to be beneficial on coronary disease [12]. Physicians regularly prescribe lipid-lowering drugs to patients found to have dyslipidemia [8].

2. PLASMA LIPIDS IN THE METABOLIC SYNDROME

Abnormal levels of plasma lipids and lipoproteins are important risk factors for metabolic and cardiovascular diseases and are targets for therapeutic intervention. In circulating blood, chylomicrons, very low density lipoprotein (VLDL), triacyl glycerol (TAG) undergoes hydrolysis, catalyzed by lipoprotein lipase (LPL), to generate a pool of free fatty acids (FFAs) that is used as an energy source in tissues, including muscle. Excess FFAs are stored in adipocytes in the form of TAGs. Such caloric abundance leads to an unopposed expansion of adipose tissue and, ultimately, to obesity and associated metabolic complications

characterized by insulin resistance and diabetes. Stored TAG in adipocytes undergoes lipolysis on demand as a result of hormone-sensitive lipase (HSL), leading to an energy-balanced level of FFAs in plasma. In insulin resistance, adipocytes exhibit a high rate of lipolysis and are highly responsive to fat-mobilizing enzymes but respond poorly to lipolysis restraining insulin. Furthermore, insulin resistance depresses adipocyte LPL activity. In combination with increased lipolysis, this process generates abnormally high plasma levels of FFAs, allowing their increased uptake into hepatocytes in excess of metabolic requirements, which leads to storage as TAG and results in hepatic steatosis and inflammation. Some TAGs are exported as VLDL, contributing to hypertriglyceridemia. In general, saturated FFAs promote cardiac disorders and systemic inflammation, whereas n−3 FFAs prevent these effects. HDL helps remove excess LDL-derived cholesterol, in its free form (FC) by reverse cholesterol transport (RCT), with the formation of esterified form (CE) by lecithin cholesterol acyltransferase (LCAT), and subsequent uptake of the CE by the liver. High levels of HDL are correlated with low cardiovascular risk [8].

The plasma pool of free fatty acids is also an important source of lipid for hepatocytes, and any excess of free fatty acids is likely to be converted to TAGs and stored in the liver or incorporated into lipoproteins and secreted into the circulatory system [13]. Predictably, raising plasma free fatty acid levels will lead to an accumulation of TAGs in the liver, which not only triggers hepatic insulin resistance but may also cause hepatic steatosis [14, 15]. Thus, specific free fatty acids are responsible for several serious health problems associated with obesity [16].

Chronic inflammation in liver and adipose tissue is common in obesity. Whereas saturated fatty acids amplify the proinflammatory action of lipopolysaccharides, many polyunsaturated fatty acids have anti inflammatory effects.

Cancer cells have aberrant glycerophosphocholine metabolism, leading to an elevation of phosphocholine, an intermediate in glycerophosphocholine biosynthesis and other choline containing phospholipids [31, 32]. Elevated phosphocholine levels have been reported in several types of cancers and have been evaluated as a target for anticancer therapy [34-36].

Enhanced autotaxin expression and overproduction of lysophosphatidic acid from lysoglycerophosphocholine by autotaxin, have been noted in numerous types of cancer, including ovarian cancer [37].

Many of the chronic conditions—cardiovascular disease, diabetes, cancer, obesity, autoimmune diseases, rheumatoid arthritis, asthma, and depression— are associated with increased production of thromboxane A2, leukotriene B4, IL-1b, IL-6, TNF (tumor necrosis factor), and C-reactive protein [38]. These factors increase by increases in omega-6 fatty acid intake and decrease by increases in omega-3 fatty acid intake, either ALA (Alpha-linolenic acid) or EPA (eicosapentaenoic acid) and DHA (decosahexaenoic acid). Omega-6 and omega-3 both are polyunsaturated fatty acids (PUFA).

Table I: Plasma Lipids in the metabolic syndrome

Glycerolipids	Syndrome	Regulation
Triacylglycerols (TAGs),	Obesity	Up
Diacylglycerols (DAGs),		Up
Ether-linked glycerolipids		Up
Phospholipids		
Lysoglycerophosphocholine		Up
Ether glycerophospholipids[65]		Down
Docosahexaenoic acid (DHA 22:6 n−3)–containing glycerophosphocholine		Down
Ether glycerophospholipids	Hypertension	
Docosahexaenoic acid (DHA 22:6 n−3) containing ether glycerophospholipids		Down
Arachidonic acid (20:4 n−6) containing ether glycerophospholipids		Down
TAG molecular species containing odd-chain-length fatty acids [17]	Dilated cardiomyopathy	Down
Saturated TAG	Schizophrenia[86]	Up
Plasma free fatty acids (saturated and monounsaturated fatty acids)	Sudden cardiac death [18]	Up
	Cancer-induced cachexia	Up
	may impair lymphocyte function [19, 20]	Up
	Ventricular arrhythmia	Up
n−6 to n−3 polyunsaturated fatty acids in the serum	Neurologic disorders	
	Alzheimer's Disease and other dementia disorders, [21, 22]	Up
	Major depression, [23]	Up
	Bipolar affective disorder [24].	Up
LDL cholesterol in blood	Coronary artery disease and stroke	Up
Plant sterols in plasma & cholesterol level	Sitosterolemia, xanthomatosis, premature atherosclerosis	UP
Glucosylceramide and Ceramide in blood plasma	Gaucher's disease	Up
Sphingomyelins in blood plasma	coronary heart disease [25]	Up

Ceramides in blood plasma	Type 2 diabetes[1] [26] and Alzheimer's disease [27]	Up
Increased maternal serum ratios of sphinganine to sphingosine	neural-tube defects in offspring [28]	Up

Long-term elevations of plasma free fatty acid levels lead to insulin resistance in muscle, desensitization of adipocytes to the lipogenic effects of insulin, and steatosis in the liver [14, 29]. Epidemiologic studies indicate that fat rich in saturated fatty acids promotes insulin resistance, whereas monounsaturated and polyunsaturated fatty acids reduce it [30].

3. DIETARY SOURCES OF LIPIDS

Lipids are obtained in diet in raw form from both plants and animals. Lipids of clinical importance are omega-3 and omega-6, the balanced ratio of which is important for various metabolic functions and cell integrity.

Table II: Food sources of omega-3 and omega-6 fatty acids [39]

Fatty acids	C-atoms	Double bonds	Sources
Linoleic acid	18:2	n-6	Vegetable oils, margarines, grain
α-linolenic acid (ALA)	18:3	n-3	Green leaves, linseed, soybean and canola oil
Eicosapentaenoic acid (EPA)	20:5	n-3	Marine animals, cod liver oil and fish oil
Docosapentanoic acid (DPA)	22:5	n-3	Marine animals, cod liver oil and fish oil
Docosahexaenoic acid (DHA)	22:6	n-3	Marine animals, cod liver oil and fish oil

Omega-3 fatty acids are derived from α-linolenic acid (ALA, C18:3 omega-3, also designated 18:3n-3) found mostly in vegetable oils. Linseed oil, canola oil and soybean oil contain approximately 57%, 8% and 7% α-linolenic acid, respectively, but these oils are without any eicosapentaenoic acid (EPA, C20:5n-3) or docosahexaenoic acid (DHA, 22:6n-3) (Table II). Humans, like all mammals, cannot make omega -6 and omega-3 and must obtain them in their diet.

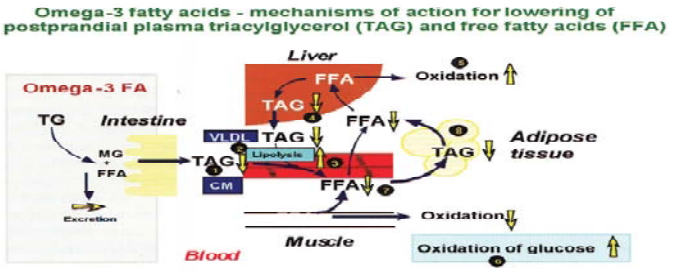
Table III: Fatty acid composition of vegetable oils, marine oils (% of fatty acids) and grain (g/100 g) [40, 4]

Sources of Oil Saturates	Saturates	Monoene	Polyene	N-3	N-6
Soy	16	23	61	8	53
Corn	13	27	60	1.3	58
Sunflower	12	24	64	1	63
Rape seed	6	64	29	9	20
Cod liver	16	51	29	27	2
Krill	26	24	49	31	4
Tuna fish	31	23	45	38	7
Palm oil	51	39	10	0.5	10
Coconut oil	91	7	3	0	3
Oatmeal	1	2	2	2	0.1
Wheat flour	0.2	0.3	1	1	0.1
Rye flour	0.3	0.2	1	1	0.1
Rice, natural	0.6	0.6	1	1	0

We see that very long-chain omega-3 fatty acids are obtained more from fatty fish (herring, mackerel, salmon, trout, eel, anchovies, sardines, etc), in addition to fish oil, cod liver oil, tuna fish oil and krill oil than from plant species (Table III). The omega-3 fatty acids in fatty fish or cod liver are not synthesized in the fish itself but in very small organisms called phytoplankton before the marine fatty acids are transferred through the food chain to the respective fishes, seals and whales. In most other marine oils the majority of omega-3 fatty acids are found in triglycerides [39].

4. OMEGA-3 FATTY ACIDS AND LIPOPROTEINS AND MECHANISM OF ACTION

Omega-3 fatty acids promote a striking reduction of TAGs in VLDL and chylomicron particles [42,43,44]. With daily intake of 2-4 g/day of very long-chain omega-3 fatty acids, the plasma concentration of TAGs is reduced by 20-30% [45], and the hepatic synthesis of triacylglycerols is decreased, probably because omega-3 fatty acids inhibit esterification of other fatty acids in addition to being poor substrates for TAG-synthesising enzymes [46]. Very long-chain omega-3 fatty acids may also reduce TAG production by increasing fatty acid oxidation via peroxisomal β-oxidation [47]. EPA as well as DHA cause enhanced clearance of postprandial plasma TAG via lipoprotein lipase [48,49].



Model showing how omega-3 fatty acids interact with lipid metabolism as indicated with yellow arrows [39]. The plasma level of triacylglycerol in chylomicrons (CM) (1) and very low density lipoproteins (VLDL) (2) is decreased in non-fasting and fasting conditions, respectively. CM triacylglycerol are probably reduced because of higher activity of lipoprotein lipase (3), whereas VLDL triacylglycerol is reduced because of reduced synthesis and secretion from hepatocytes (4), and possibly because hepatic peroxisomal fatty acid oxidation is increased (5), in addition to whole body glucose oxidation being increased (6). Plasma concentration of free fatty acids and glycerol is reduced for unknown reasons (7), whereas some adipose tissues decrease in size in rats during feeding with omega-3 fatty acids (8), (ref 165).

5. RECOMMENDED INTAKE OF ESSENTIAL FATTY ACIDS

The clinical studies in patients with cardiovascular disease, arthritis, asthma, cancer, and mental illness clearly indicate the need to balance the omega-6/omega-3 fatty acid intake for prevention, and during treatment, of disease. The optimal dose or ratio of omega-6/omega-3 varies from 1/1 to 4/1, depending on the disease under consideration [38]. Because many chronic diseases prevalent are multigenic and multifactorial, it is not surprising that the dose or the ratio differs. Studies show that the background diet, when balanced in omega-6/omega-3, decreases the drug dose. It is therefore essential to decrease the omega-6 intake while increasing the omega-3 in the prevention and management of chronic disease.

If 1-2 g/day of EPA and DHA is eaten in combination with proper amounts of fruits and vegetables and limited amounts of saturated and trans fatty acids, most people will probably benefit with better health [39]. For pregnant women there are some data demonstrating beneficial effects of 2.5 grams marine omega-3 fatty acids daily for the mother as well as for the child [50, 51]. For patients with hypertension or rheumatoid arthritis the dosage of omega-3 fatty acids needed for clinically meaningful effects is from 2 to 4 grams daily.

6. LIPIDS AS BIOMARKERS OF DISEASE

Detailed lipidomic analyses may serve as biomarkers for the early detection of acquired obesity hypertension, cardiac disease, cancer and neurological disorders (table1). A detailed lipidomic analyses performed independently in 14 pairs of young-adult monozygotic twins showed TAG 50:2, 52:2, 52:3, and 52:4 the most abundant glycerolipid species. When the twin study was controlled for patient age and genetic background, it showed that several (but not all) TAG molecular species, including 56:4, correlated significantly with body mass index and measurements of subcutaneous fat and may serve as biomarkers for the early detection of acquired obesity, especially when applied to children [52]. A separate lipidomic analysis of 19 hypertensive persons and 59 normotensive control subjects showed that hypertension was also associated with a decrease in ether glycerophospholipids — specifically, the ones containing arachidonic acid (20:4 n−6) and docosapentaenoic acid (DPA 22:5 n−3) [53].

A very minor species in plasma such as sphinganine has been useful as a biomarker since increased maternal serum ratios of sphinganine to sphingosine correlate with the occurance of neural-tube defects in offsprings. Lathosterol is also present in normal plasma and can be of diagnostic value as an indicator of whole-body cholesterol synthesis [54].

Obesity is a global and rapidly increasing disease [55] that is closely associated with development of inflammatory markers like enhanced plasma concentration of TNFα, IL-6, CRP, sialic acid, orosomucoid and alpha1-antichymotrypsin [56]. These markers are adipokines (protein hormones secreted from the adipose tissue like TNFα, IL-6 and CRP) or acute phase proteins (like CRP, sialic acid, orosomucoid and a1-antichymotrypsin). Although there is data suggesting a relation between inflammation and obesity [57], the inflammatory markers are often hard to detect.

7. MASS SPECTROMETRY IN LIPIDOMICS

Lipids can be identified by mass spectrometry in many ways [58, 59]. Species of some lipid classes have unique elemental compositions and could therefore be directly identified, even in crude total extracts, solely by their masses determined and with higher than parts per million accuracy [60]. Such 'top-down' lipidomics approaches require high-resolution mass spectrometers, such as Orbitraps [61]. However, it is more common for lipid molecular ions to be subjected to tandem mass spectrometry analysis (MS/MS) to produce structure-specific fragment ions that help to distinguish the species — an approach termed 'bottom-up' lipidomics [62]. The most common types of tandem mass spectrometers are triple quadrupole, ion trap and quadrupole time-of-flight, among others [63]. They take advantage of different physical principles of selecting, fragmenting and detecting ions, and the machines differ by features such as sensitivity, speed of spectra acquisition, mass resolution, precursor isolation window and dynamic range. Importantly, even lipids of the same class might produce different fragment ions, depending on the type of tandem mass spectrometer.

By providing absolute quantities of the lipid molecular species, lipidomics is bound to become an integral part of systems analysis for sustainable health development.

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