INTRODUCTION

Long-chain polyunsaturated fatty acids (PUFA) (arachidonic acid AA and docosahexaenoic acids DHA) are essential for optimum fetal growth and development.They represent major components of the developing central nervous system, and are essential for cognitive and visual functions ***(Boelsma et al., 2001).***

Their consumption may reduce the risk for a variety of diseases, including cardiovascular, neurological and immunological disorders, diabetes and cancer ***(Assisi et al., 2006)***.

There is increased demand for these fatty acids during pregnancy, and maternal supplies of AA and DHA are likely the major source of long-chain PUFA, determining fetal essential fatty acid and long-chain PUFA accretion ***(Wijendran et al., 1999)***.

Diabetes impairs the synthesis of both AA and DHA ***(Ghebremeskel et al., 2004 and Thomas et al.,2004)***

In poorly controlled maternal diabetes, it is conceivable that the relative insufficiency of AA and DHA may exacerbate

speech and reading impairments, behavioral disorders, suboptimal performance on developmental tests, and lower IQ, which have been reported in some children born to mothers with type 1 diabetes mellitus ***(Rizzo et al.,1991; Rizzo et al.,1997 and Yamashita et al.,1996)***.

Moreover, compromised levels of PUFA in infants of diabetic mothers may be severe enough to be a risk for congenital malformations ***(Reece et al.,1996)***.

This is to be added to the known fact that In infants of diabetic mothers, congenital anomalies occur about two-three times as often as in normal population ***(Wiznitzer et al., 1999)***.

AIM OF THE WORK

To assess whether infants of diabetic mothers (prepregnancy and gestational diabetics) have compromised arachidonic and docosahexaenoic acids in their plasma and whether this could be related (if present) to deficiency of the same compounds in their mothers.

Chapter [I]

LIPID METABOLISM

Basic Biochemistry:

Lipid is a general term that describes substances that are relatively water insoluble and extractable by nonpolar solvents. Complex lipids of humans fall into one of two broad categories; nonpolar lipids, such as triacylglycerols and cholesterol esters, and polar lipids, which are amphipathic i.e.they contain both a hydrophobic domain and a hydrophilic region in the same molecule ***(Glew, 2002)***.

Classification of Lipids:

(1) ***Simple lipids:*** Esters of fatty acids with various alcohols.

i. ***Fats:*** Esters of fatty acids with glycerol. A fat in the liquid state is known as oil.

ii. ***Waxes:*** Esters of fatty acids with higher molecular weight monohydric alcohols.

(2) ***Complex Lipids:*** Esters of fatty acids containing groups in addition to an alcohol and a fatty acid.

i. ***Phospholipids:*** Lipids containing, in addition to fatty acids and an alcohol, a phosphoric acid residue. They frequently have nitrogen-containing bases and other constituents, e.g., in glycerophospholipids the alcohol is glycerol and in sphingophospholipids the alcohol is sphingosine.

ii. ***Glycolipids (Glycosphingolipids):*** Lipids containing a fatty acid, sphingosine and carbohydrate.

iii. ***Other Complex Lipids:*** Such as sulfolipids and aminolipids. Lipoproteins may also be placed in this category.

(3) Precursor and Derived Lipids: These include fatty acids, glycerol, steroids, alcohols in addition to glycerol and sterols, fatty aldehydes, and ketone bodies, hydrocarbons, lipid-soluble vitamins, and hormones.

Because they are uncharged, acylglycerols (glycerides), cholesterol, and cholesterol esters are termed neutral lipids ***(Mayes, 2000)***.

Cholesterol :

Although every living organism has been found to contain sterols, cholesterol is found almost exclusively in animals, in which it is also the main sterol. Virtually all cells and body fluids contain some cholesterol. Like other sterols, cholesterol is a solid alcohol of high molecular weight that possesses the tetracyclic perhydrocyclo-pentanophenathrene skeleton. The molecule contain 27 carbon atoms ***(Bachorik et al., 1996)***.

Fatty Acids:

Fatty acids can be classified according to their degree of saturation. Saturated fatty acids have no double bonds between carbon atoms, monounsaturated fatty acids contain one double bond, and polyunsaturated fatty acids contain more than one double bond. The double bonds in polyunsaturated fatty acids of both animal and plant origin are usually three carbon atoms apart ***(Carl et al., 1999)***.

In saturated fatty acids, the chain is extended and flexible (i.e., the carbon atoms can rotate freely around the longitudinal axis). Unsaturated fatty acids, however, have fixed 30-degree bends in their chains at each double bond. Depending on the plane in which this bend occurs, either the cis or trans isomer is

produced. In mammals, all naturally occurring unsaturated fatty acids are of cis variety. Trans fatty acids result from catalytic hydrogenation ***(Carl et al., 1999)***.

Long-chain fatty acids are oxidized in the mitrochondria and produce energy by a series of reactions that operate in a repetitive manner to shorten the fatty acid chain by two carbon atoms at a time from the –COOH terminal of the molecule, a process known as a β-oxidation. A fatty acid that occurs in a living system normally contains an even number of carbon atoms, and the hydrocarbon chain is usually unbranched. If there are carbon-carbon double bonds in the chain, the fatty acid is unsaturated; if there are only single bonds, the fatty acid is saturated ***(Campbell, 1999)***.

Table 1: Typical naturally occurring saturated fatty acids.

Acid	No. of Carbon Atoms	Formula	Melting Point ºC
Lauric	12	$CH_3(CH_2)_{10}CO_2H$	44
Myristic	14	$CH_3(CH_2)_{12}CO_2H$	58
Plamitic	16	$CH_3(CH_2)_{14}CO_2H$	63
Stearic	18	$CH_3(CH_2)_{16}CO_2H$	71
Arachidic	20	$CH_3(CH_2)_{18}CO_2H$	77

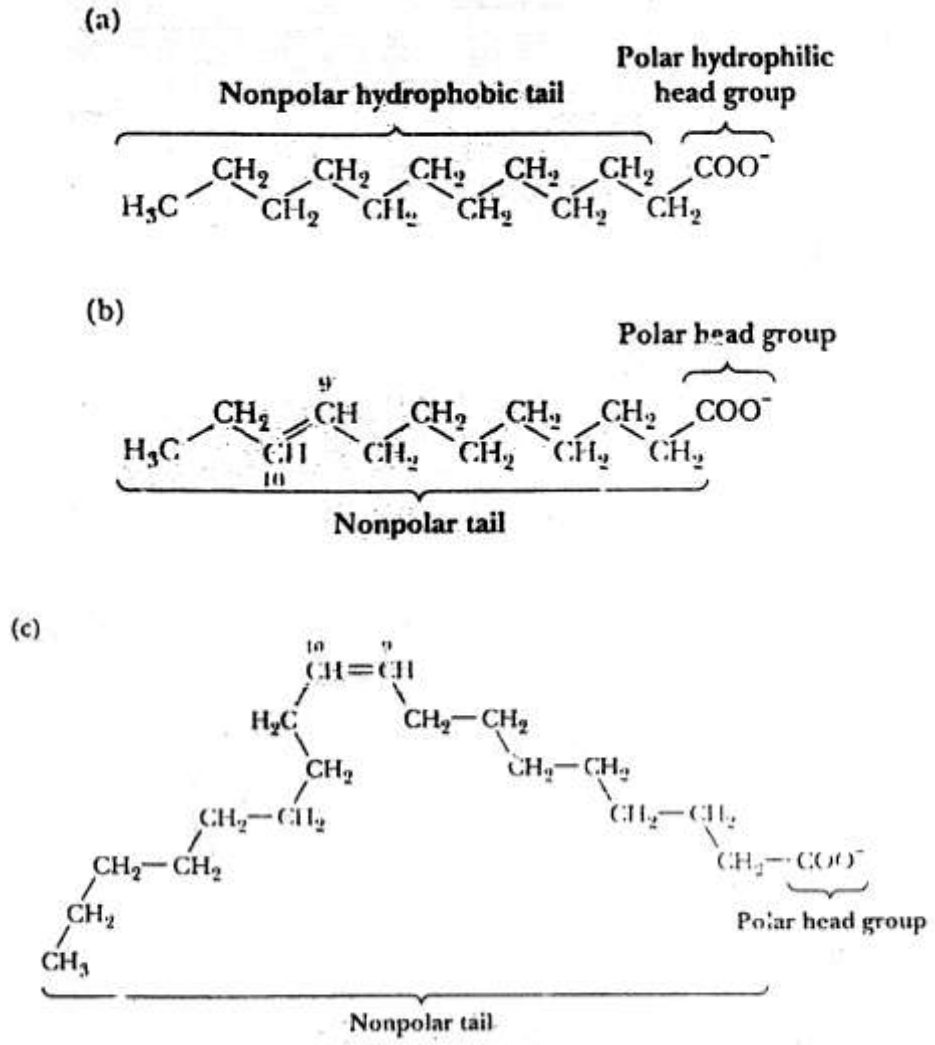

Figure (1): Structures of representative fatty acids. a) Dodecanoate, the ionized form of the saturated fatty acid dodecanoic acid (lauric acid); b) Dodecenoate, the ionized form of the unsaturated fatty acid **dodecenoic** acid, showing the effect of a trans double bond; c) Oleate, the ionized form of oleic acid, another unsaturated fatty acid. Note that the double bound introduces a kink in the hydrocarbon chain. The double bonds in both unsaturated fatty acids are at the ninth carbon atom from the carboxyl end ***(Campbell, 1999)***.

Table 2: Typical naturally occurring unsaturated fatty acids.

Acid	No. of Carbon Atoms	Degree of unsaturation*	Formula	Melting Point ºC
Palmitoleic	16	16:1-Δ^9	$CH_3(CH_2)_5$ $CH=CH(CH_2)_7CO_2H$	- 0.5
Oleic	18	18:1-Δ^9	$CH_3(CH_2)_7$ $CH=CH(CH_2)_7CO_2H$	16
Linoleic	18	18:2-$\Delta^{9,12}$	$CH_3(CH_2)_4$ $CH=CH(CH_2CH=CH$ $(CH_2)_7CO_2H$	-5
Linolenic	18	18:3-$\Delta^{9,12,15}$	$CH_3(CH_2CH=CH)_3=$ $(CH_2)_7CO_2H$	-11
Arachidonic	20	20:4-$\Delta^{5,8,11,14}$	$CH_3(CH_2)_4$ $CH_2CH=CH)4(CH2)2$ CO2H	-50

*: Degree of unsaturation refers to the number of double bonds. The superscript indicates the position of duble bonds, for example, Δ^9 refers to a double bond at the ninth carbon atom from the carboxyl end of the molecule.

(Campbell, 1999).

Tables (1, 2) list a few examples of the two classes. Note that the double bonds are isolated from one another by several singly bonded carbons; fatty acids do not normally have conjugated double bond systems. The notation used for fatty acids indicated the number of carbon atoms and the number of double bonds. In this system 18:0 denotes an 18-carbon saturated fatty acid (no double bonds), and 18:1 denotes an 18-carbon fatty acid with one double bond. Note that in the unsaturated fatty acids in table (2) (except arachidonic acid) there is a double bond at the ninth carbon atom from the carboxyl end. The position of the double bond results from the way unsaturated fatty acids are synthesized in organisms. Unsaturated fatty acids have lower melting points than saturated ones. Plant oils are liquid at room temperature because they have higher proportions of unsaturated fatty acids than do animal fats, which tend to be solids. Conversion of oils to fats is a commercially important process. It involves hydrogenation, the process of adding hydrogen across the double bond of unsaturated fatty acids to produce the saturated counterpart ***(Campbell, 1999)***.

Fatty Acid Oxidation:

The oxidation of fatty acids is the chief source of energy in the catabolism of lipids. In fact, lipids that are sterols are not catabolized as a source of energy. Both triacylglycerols and phosphoacyl glycerols have fatty acids as part of their covalently bonded structures. In both types of compounds, the bond between the fatty acid and the rest of the molecule can be hydrolyzed (Fig.2), with the reaction catalyzed by suitable groups of enzymes (Lipases and phospholipases respectively) ***(Wakil and Barnes, 1971)***.

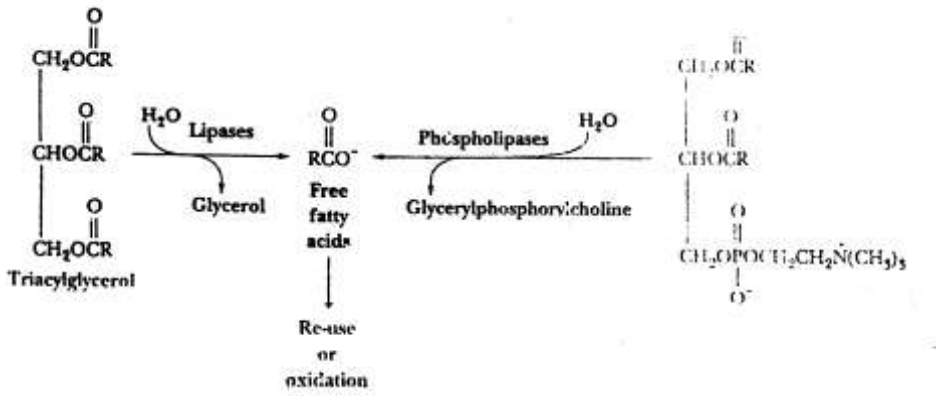

Figure (2): The release of fatty acids for future use. The sources of fatty acids can be triacylglycerol (left) or a phospholipids such as phosphatidylcholine (right) ***(Campbell, 1999)***

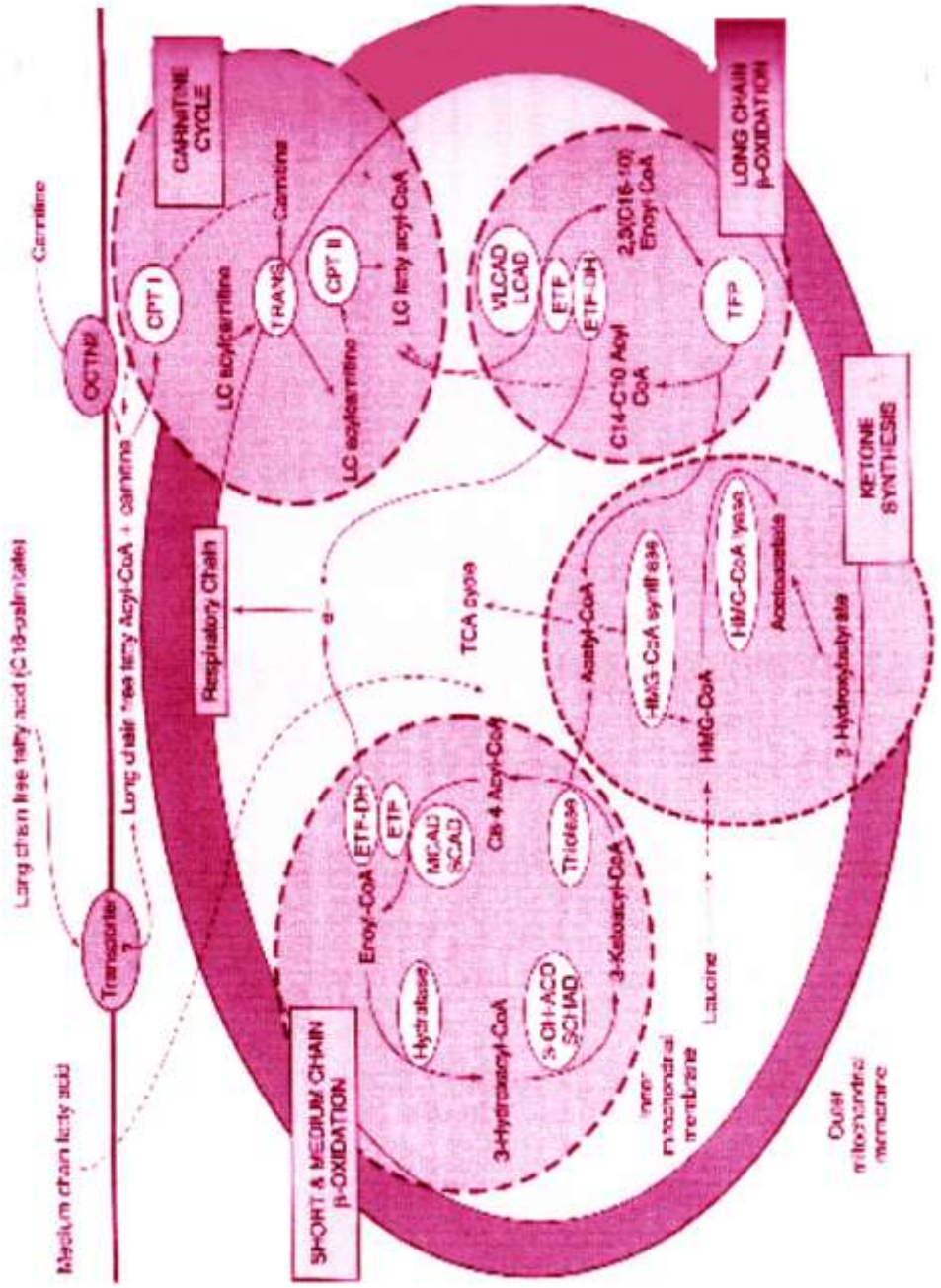
CARNITINE CYCLE
LONG CHAIN β-OXIDATION
KETONE SYNTHESIS
SHORT & MEDIUM CHAIN β-OXIDATION
Carnitine
OCTN2
CPT I
CPT II
TRANS
LC acylcarnitine
LC fatty acyl-CoA
VLCAD LCAD
ETF
ETF-DH
2,3(D16-10) Enoyl CoA
C14-C10 Acyl CoA
TFP
Respiratory Chain
TCA cycle
Acetyl-CoA
HMG-CoA synthase
HMG-CoA lyase
Acetoacetate
3-Hydroxybutyrate
HMG-CoA
Enoyl-CoA
ETF-DH
ETF
MCAD SCAD
C6-4 Acyl-CoA
Thiolase
3-Ketoacyl-CoA
Hydratase
3-OH-ACD SCHAD
3-Hydroxyacyl-CoA
Leucine
Inner mitochondrial membrane
Outer mitochondrial membrane
Long chain free fatty acid (C16-palmitate)
Long chain fatty Acyl-CoA + carnitine
Transporter ?
Medium chain fatty acid

Figure (3): Mitochondrial fatty acid oxidation. Carnitine enters the cell through the action of the organic cation/carnitine transporter (OCTN2). Palmitate, a typical 16-carbon long-chain fatty acid, is transported across the plasma membrane and can be activated to form a long chain (LC) fatty acyl-CoA. It then enters into the carnitine cycle, where it is transesterified by carnitine palmitoyltransferase-I (CPT-I), translocated across the inner mitochondrial membrane by carnitine/acylcarnitine translocase (TRANS), and then reconverted into a long-chain fatty acyl CoA by carnitine palmitoyltransferase-II (CPT-II) to undergo β-oxidation. Very long chain acyl-CoA dehydrogenase (VLCAD/LCAD) leads to the production of (C16-10) 2,3 enoyl-CoA. Trifunctional protein (TFP) contains the activities of enoyl-CoA hydratase (hydratase), 3-OH-hydroxyacyl CoA dehydrogenase (3-OH-ACD), and β-ketothiolase (thiolase). Acetyl CoA, FADH, and NADH are produced. Medium-chain acyl-CoA dehydrogenase (MCAD), short-chain acyl-CoA dehydrogenase (SCAD), and short-chain hydroxyl acyl-CoA dehydrogenase (SCHAD) are required. Acetyl-CoA can then enter the Krebs (TCA) cycle. Electrons are transported from FADH to the respiratory chain via the electron-transfer flavoprotein (ETF) and the electron-transfer flavoprotein dehydrogenase (ETF-DH). NADH enters the electron transport chain through complex I. Acetyl-CoA can be converted into hydroxymethylglutaryl-CoA (HMG-CoA) by β-hydroxy-β-methylglutaryl-CoA synthase (HMG-CoA synthase) and then the ketone body acetoacetate by the action of β-methylglutaryl-CoA lyase (HMG-CoA lyase).***(Venditti and Stanley, 2004).***

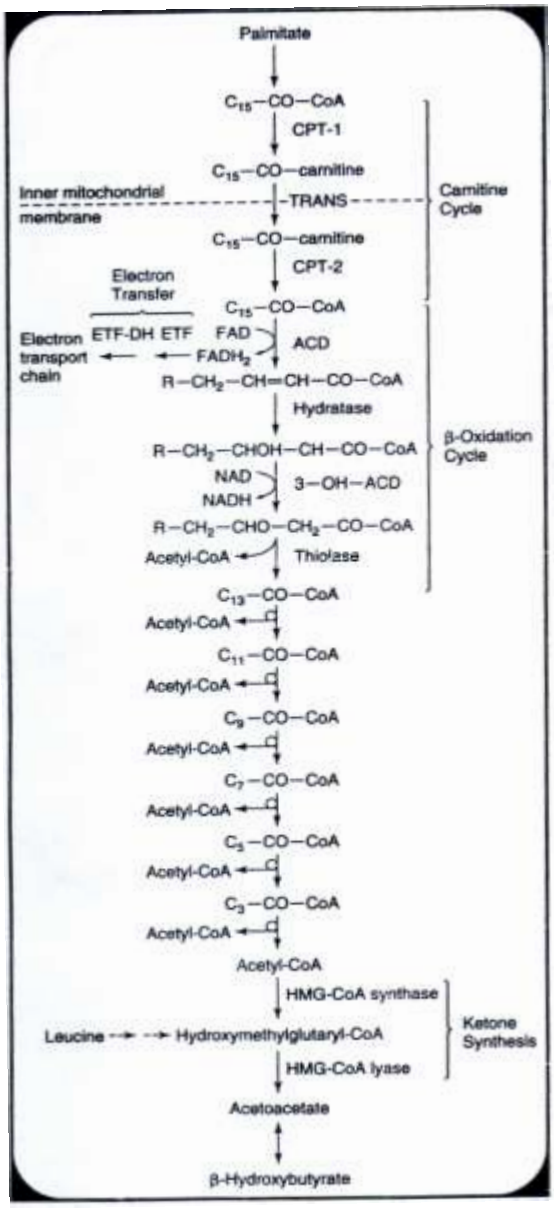

Figure (4): Pathway of mitochondrial oxidation of palmitate, a typical 16-carbon long-chain fatty acid. Enzyme steps include carnitine palmitoyltransferase (CPT) 1 and 2, carnitine/acylcarnitine translocase (TRANS), electron transfer flavoprotein (ETF), ETF-dehydrogenase (ETF-DH), acyl-CoA dehydrogenase (ACD), enoyl-CoA hydratase (hydratase), 3-hydroxy-acyl-CoA dehydrogenase (3-OH-ACAD), β-hydroxy-B-methylglutaryl-CoA (HMG-CoA) synthase, and lyase. ***(Venditti and Stanley, 2004).***

During periods of fasting, fatty acids become the predominant substrate for energy production via oxidation in the liver, cardiac and skeletal muscles. The brain does not directly utilize fatty acids for oxidative metabolism but readily oxidizes ketone bodies (derived from the partial oxidation of fatty acids by the liver). During prolonged aerobic exercise, fatty acid oxidation accounts for 60% of muscle energy consumption ***(Ahlborg et al., 1974)***.

Glucose differs from fatty acids as a fuel because it can provide high energy phosphate molecules under anaerobic conditions. Muscle oxygen delivery often becomes rate-limiting during heavy exercise so that glycolysis becomes the main or only ATP source ***(Snodgrass, 1992)***.

To be used, fatty acids are mobilized from adipose tissue stores and transported in the circulation mainly bound to albumin. Fatty acids are taken up by the liver and by other tissues by concentration-dependent mechanisms which may include both saturable carrier-mediated uptake and non-saturable diffusion ***(Schulz, 1985)***.

Once inside the cell (Fig. 5), fatty acids are activated to form coenzyme A (CoA) esters through the action of the cytoplasmic enzymes acyl-CoA synthetases. The acyl CoA