Lipids and fatty acid composition of early and term healthy placenta with a specific focus on foetal growth and development

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A thesis submitted in partial fulfilment of the requirements of London Metropolitan University for the degree of Doctor of Philosophy

Institute of Brain Chemistry & Human Nutrition

This research project was carried out in collaboration with the Reproductive Medicine Unit, Newham University General Hospital, NHS Trust & with Maternal & Fetal Research Unit, Department of Women's Health, Guys, King's College & St. Thomas' School of Medicine, King's College London, St Thomas' Hospital, London

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ABSTRACT

Diabetes is associated with abnormal nutrient metabolism particularly carbohydrates and lipids. In pregnancy, this diabetes-associated metabolic perturbation has been shown to alter, qualitatively and quantitatively, the uterine lipid environment and consequently the fatty acids delivered to the developing foetus. Perhaps not surprisingly, maternal consumption of a high saturated fat diet during pregnancy compounds the problem. *The primary aims of the studies reported in this thesis were to investigate the effect of: (a) maternal diabetes on placental fatty acid composition in human subjects, and (b) maternal diabetes and a high saturated fat intake on tissue composition of the offspring in laboratory animal model.*

Therefore, the key objectives of this thesis were to: (1) analyse human placental fatty acids from healthy pregnancies at two times in gestation, early (8-14 wk) and term (38-41wk), (2) compare placental fatty acids from pregnant women with gestational diabetes mellitus (GDM) and women who delivered preterm with the healthy term placenta, and (3) investigate the effects of maternal diabetes and/or a high fat diet during pregnancy on the fatty acid composition of the vascular tissues, particularly arachidonic (AA) and docosahexaenoic (DHA) acids of the offspring.

The main outcomes of these investigations were that: (1) AA was incorporated in high proportions (17.2-35.9%) in most phospholipids. At term, AA was reduced in choline (CPG), ethanolamine (EPG) and inositol (IPG) phosphoglycerides while di-hommo-γ-linolenic acid (DGLA) was markedly increased in CPG, EPG, IPG and serine (SPG) phosphoglycerides compared with early in gestation (8-14wk) placental membranes, (2) the GDM placenta had elevated AA and DHA in CPG and EPG while the preterm placenta had reduced DHA in CPG and SPG and AA in SPG, and (3) maternal diabetes reduced AA and arachidonic-to linoleic acid ratio (AA/LA) in the offspring. Similarly, feeding dams to a 32.9%-fat or 20%-fat (mainly lard) diet reduced DHA in liver and heart CPG and EPG or AA and DHA in aorta CPG and EPG of the offspring.

The high dominance of AA, particularly in the membranes of the early placenta, may suggest that AA has an important role for placental development – organogenesis and vascularisation. Conversely, the enrichment of placental membranes with DGLA at term is more consistent with a profile favouring optimal blood flow to nourish the fetal growth spurt. On the other hand, maternal diabetes and preterm delivery led to a compromised AA and DHA placental content. The experimental data strongly support the hypothesis that disturbance *in utero* environment through inadequate maternal essential fatty acid lipid nutrition impaired tissue synthesis DHA and/or AA in the offspring and, hence, may set the offspring at high risk for the development of cardiovascular disorders later in life.

AUTHOR'S STATEMENT

I certify that the data in this thesis entitled "Lipids and fatty acid composition of early and term healthy placenta with a specific focus on foetal growth and development", submitted for the degree of Doctor of Philosophy, is entirely original and my own work.

All technical and analytical aspects, including sample storage and processing, tissues fatty acid extraction and analysis, statistical analysis and interpretation of the raw data were carried out exclusively by me.

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Signed:.....

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AA; Arachidonic acid (20:4n-6)
AA/DHA; Arachidonic-to-docosahexaenoic Acid ratio
AA/LA; Arachidonic-to-linoleic acid ratio
AC; Abdominal circumference
Acsls; Long chain acyl-CoA synthetase (formerly known as ACS)
ADA; American Diabetes Association
AFP; alpha-fetoprotein
AGA; Appropriate for gestational age
ALA; α-Linolenic acid (18:3n-3)
ARVM; Adult rat isolated ventricular myocytes
BBM; Brush border membrane or MVM of syncytiotrophoblasts
BeWO; Human placental choriocarcinoma cell line
BHT; 2,6 - di - tert - butyl - p – cresol (Butylated hydroxy toluene)
BM; Basal membrane of syncytiotrophoblasts
BMI; Body Mass Index (Kg/m^2)
BPD; Biparietal diameter
BWT; Birthweight
CAD; Coronary artery diseased
CBMC; Cord blood mononuclear cell
CE, Cholesterol esters
CHD; Coronary heart disease
CRH; cornicotropin-releasing hormones
CNS; Central nervous system
COX; Cyclooxygenase
CPG, Choline phosphoglycerides
CVD; Cardiovascular diseases
CYP; Cytochrome P-450
D; Day
DG; Diacylglycerols
DGLA; di-Hommo-γ-linolenic acid (20:3n-6)
DHA; Docosahexaenoic acid (22:6n-3)
DM; Diabetes Mellitus
DPA n-3; Docosapentaenoic acid (22:5n-3)
DPA n-6; Docosapentaenoic acid (22:5n-6)
DPA n-6/DHA; Docosapentaenoic (n-6)-to-docosahexaenoic acid ratio
DPA/DTA n-6; Docosapentaenoic-to-docosatetraenoic acid n-6 ratio
DTA; Docosatetraenoic acid (22:4n-6)
ECL; Equivalent chain lengths
EDHF; Endothelial – derived hyperpolarization factors
EFA; Essential fatty acids
EL; Endothelial lipase
EPA; Eicosapentaenoic acid (20:5n-3)
EPG; Ethanolamine phosphoglycerides
ER; Endoplasmatic reticulum
FA; Fatty acid/s
FABP; Cytosolic fatty acid-binding protein or
FABPpm; Plasma membrane FA-binding protein
FAME; Fatty acid methyl esters
FAT/CD36; Fatty acid translocase protein
FAS; Fatty acid synthase
FATP; Fatty acid transport protein
FID; Flame ionisation detector
FFA; Free fatty acids
FL; Femur length
11,12-EET; 1,12-epoxyeicosatrienoic acid
GA; Gestational age
GC; Gas Chromatography
GCT; Glucose challenge test
GDM; Gestational Diabetes Mellitus
GLC; Gas - Liquid chromatography
GLUT4; glucose transporter type 4
H; Hour
HC; Head circumference
HCA; Human coronary arteries
hCG; Human chorionic gonadotropin
HDL; high density lipoprotein
12-HETE; 12-hydroxyeicosatetraenoic acid
HFC; High-fat controls
HFD; High-fat diabetics
HPA; Hypothalamic-pituitary-adrenal-axis
hPL; Human placental lactogen
HPLC; High performance liquid chromatography
HPV; Hypoxic pulmonary vasoconstriction
IDDM; Insulin Dependent Diabetes Mellitus
IGGT; Impaired gestational glucose tolerance
IIV; Immature intermediate villi
IPG; Inositol phosphoglycerides
IR; Insulin resistance
IUGR; Intrauterine growth retardation
LA; Linoleic acid (18:2n-6)
LBW; Low birth weight
LCAT; lecithin:cholesterol acyltransferase
LCPUFA; Long chain polyunsaturated fatty acids
LDL; low density lipoprotein
LFC; Low-fat controls
LFD; Low-fat diabetics
LGA; Large for gestational age
LMP; Last menstrual period
LO; Lipoxygenase
logRRT; Logarithmic value of relative retention time
LPL; Lipoprotein lipase
MONO; Monounsaturated fatty acids
MR; Methylating reagent
MVM; microvillous membrane or brush border membrane (BBM) of syncytiotrophoblasts; maternal side of the placenta
NDDG; National Diabetes Data Group
NIDDM; Non-Insulin Dependent Diabetes Melitus (Type 2 diabetes)
NL; Neutral lipids
OFN; Oxygen free nitrogen
OGTT; Oral glucose tolerance test
PCOS; Polycystic ovary syndrome
Petrol; Petroleum spirit
PG; Prostaglandins
PGE_1; Prostaglandin E1

PGE_2; Prostaglandin E2
PGF_{2a}; Prostaglandin F2-alpha
PGI_2; Prostacyclin
PKC; Protein kinase C
PL; Phospholipids
PLA_2; Phospholipase A_2
PLC; Phospholipase C
PPARs; Perixosome proliferators-activated receptors
Premix; Chloroform / Methanol. CH_3Cl:MeOH (2 : 1 v/v)
PUFA; Polyunsaturated fatty acids
LCPUFA; Long chain PUFA
RBC; Red blood cells
RDS; Respiratory distress syndrome
ROP; Retinopathy of prematurity
ROS; Reactive oxygen species
RRT; Relative retention time
RT; Retention time
SGA; Small for gestational age
SFA; Saturated fatty acids
SPG; Serine phosphoglycerides
SPM; Sphingomyelin
SR; Sarcoplasmic reticulum
STZ; streptozotocin
T; Temperature
TG; Triacylglycerols
TLC; Thin Layer chromatography
TXA_2; Thromboxane A_2
UV; Ultraviolet radiation
SMC; Smooth muscle cells
VLBW; Very low birth weight
VLDL; Very low density lipoprotein
VLPUFA; Very long chain PUFA
WHO; World Health Organisation
Wk; Weak
Wt; Weight
Y; Years
Σ; Total
∑(AA precursors)/AA; Total arachidonic precursors-to-arachidonic acid ratio
Σmet n-3; Total metabolites n-3
Σmet n-6; Total metabolites n-6
∑met n-6/∑met n-3; Total metabolites n-6 PUFA-to-total metabolites n-3 PUFA
∑mono; Total monounsaturated FA
Σn-3; Total n-3
Σn-6; Total n-6
∑n-6/ ∑n-3; Total n-6 PUFA-to-total n-3 PUFA ratio
∑SFA; Total saturated FA

PUBLICATIONS ARISING FROM THIS THESIS

Abstracts

E. Koukkou, **D. Bitsanis**, K. Ghebremeskel, C. Lowy and MA Crawford (1997) Both diabetes and maternal diet rich in saturated fatty acids alters fetal liver lipid composition. Proceedings of the 4th International Congress on Essential Fatty acids and their Eicosanoids. J. Prostaglandins, Leucotrienes EFAs 57: 268

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K. Ghebremeskel, **D. Bitsanis**, E. Koukkou, C. Lowy, L. Poston and M.A. Crawford (1998) Abnormal Partitioning of Liver fatty Acid in Diabetic Rats and their Pups. Proceedings of 3rd ISSFAL Congress, International Society for the Study of Fatty Acids and Lipids, Lyon-France.

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Other Presentations

M.A. Crawford, Y. Min, I. Golfetto, **D. Bitsanis,** T. Moodley, K. Ghebremeskel and L. Poston. Arachidonate and Docosahexaenoate in Perinatal Neurovascular Development. Plenary Lecture. International Society of the Study of fatty Acids and Lipids (ISSFAL) in Montreal in Canada, May 2002.

D.Bitsanis, T. Moodley, Ghebremeskel, O.B. Djahanbakhch and M.A. Crawford. Arachidonate and Docosahexaenoate Composition of First Trimester and Term Placenta. International Society of the Study of fatty Acids and Lipids (ISSFAL) in Montreal in Canada, May 2002.

D. Bitsanis, K. Ghebremeskel, I.Y. Khan, P.D. Taylor, L. Poston and M.A. Crawford. Arachidonate and Docosahexaenoate Levels in Heart of Adult Rats are Age and Gender Dependent. 6th Congress of the International Society for the study of Fatty Acids and Lipids (ISSFAL) in Brighton, UK, June 2004.

D.Bitsanis, T. Moodley, Ghebremeskel, O.B. Djahanbakhch and M.A. Crawford. Placental Arachidonate and Docosahexaenoate Levels are Enhanced by Gestational Diabetes. 6th Congress of the International Society for the study of Fatty Acids and Lipids (ISSFAL) in Brighton, UK, June 2004.

D.Bitsanis Gestational Diabetes increases Placental and Reduces Maternal Red Cell Arachidonic and Docosahexaenoic Acids. 6th Congress of the International Society for the study of Fatty Acids and Lipids (ISSFAL) in Brighton, UK, June 2004.

D. Bitsanis, K. Ghebremeskel, M.A. Crawford, I.Y. Khan, P.D. Taylor & L. Poston. Arachidonic and Docosahexaenoic Acid Composition of Thoracic Aorta of Adult Rats. 6th Congress of the International Society for the study of Fatty Acids and Lipids (ISSFAL) in Sidney, Australia, July 2006.

CHAPTER 1. INTRODUCTION

1.1. Lipid Biochemistry

Lipids are a diverse group of biomolecules that differ in structure and function (Bausch, 1993; Merrill & Schroeder, 1993; McKee & Mc Kee, 1996; Dow et al, 1997; Thabrew & Ayling, 2001). They can be categorised into structural, storage or metabolic; but some lipids can perform more than one of these functions (Garrow & James, 1993). The major lipid components in the body are triacylglycerols (TG), fatty acids (FA), cholesterol and cholesterol esters (CE) and phospholipids (PL) (Dow et al, 1997). These can be provided in the diet, but can also be synthesised in the cell to be used for energy production or to be converted into other biological important compounds (Thabrew & Ayling, 2001).

The FA of a lipid vary in length (short-, medium- or long acyl chains) and classified according to the presence of double bonds in their molecule as saturated (SFA; lacking double bonds), monounsaturated (mono; one double bond) and polyunsaturated fatty acids (PUFA; two or more double bonds). The latter are found in appreciable amounts in the lipids of higher organisms with double bonds of the *cis*-configuration separated by a single methylene group ($-CH=CH-CH_2-CH=CH-$).

1.2. Essential fatty acids

Essential fatty acids (EFA) are polyunsaturated fatty acids (PUFA) containing a double bond at positions beyond C9 that can be synthesised from plant sources but not by the mammalian body (Brenner, 1984; Garrow & James, 1993; Dow et al, 1997; Lauritzen et al, 2001). EFA are the two parent compounds of the n-6 and the n-3 PUFA, linoleic acid (LA; 18:2n-6 or 9-,12-octadecadienoic acid) and α-linolenic acid (ALA; 18:3n-3 or 9,12,15-octadecatrienoic acid), respectively. These PUFA can only be provided in the diet and the biochemical and biophysical mechanisms leading to their essentiality are related to their structure and the double bonds in their molecules (Brenner, 1984; Minich et al, 1997). LA and ALA are important precursors for the synthesis of eicosanoids affecting a wide range of diverse body functions (i.e. immune system or blood pressure).

EFA were first discovered in 1929 in a study by Burr & Burr (Brenner, 1984; Uauy et al, 1989; Ruyle et al, 1990; Garrow & James, 1993; Uauy-Dagach & Mena, 1995; Lauritzen et al, 2001)

that described how acute deficiency states (dermatitis, retardation, infertility) produced in rats fed fat-free diets, could be eliminated or even prevented by adding specific FA. The FA related to LA were the most effective and by that time were termed 'vitamin F', and now are known as n-6 PUFA. It was not till early 1970s, that the primary role of the n-3 PUFA for the retina and the brain was determined (Anderson & Maude, 1972; Crawford & Sinclair, 1972). In early 80s, the first case of ALA deficiency was reported (Holman et al, 1982). A six years old girl who lost 300cm of her intestines was maintained for 5 months on total parenteral nutrition on an emulsion containing LA, but poor on ALA (Holman et al, 1982; Minich et al, 1997). She experienced weakness, blurred vision, paresthesia, inability to walk and pain in the legs. Serum FA analysis revealed significant ALA deficiency and when the emulsion changed to include soyabean oil, the neurological symptoms were disappeared (Holman et al, 1982; Minich et al, 1997).

On the other hand, arachidonic acid (AA; 20:4n-6 or 5,8,11,14-eicosatetraenoic acid) it becomes essential if its precursor, LA is missing from the diet (Nelson et al, 1997a; Thabrew & Ayling, 2001). Additionally, it is known to be an important n-6 PUFA for the fetus, since the sheep model possesses a weak Δ-6 desaturase activity (Lafond et al, 2000). Dietary AA has a growth promoting effect, which could be related to its eicosanoic precursor or to its structural function in membrane lipids (Koletzko & Braun, 1991). AA is involved in cell membrane biogenesis, particularly in brain, kidney and spinal cord and represents a large proportion of the syncytiotrophoblast lipids (Lafond et al, 2000).

Conversely, the essentiality of n-3 PUFA is related to the high content of 4,7,10,13,16,19-docosahexaenoic acid (DHA; 22:6n-3), in the human brain and retina (Anderson & Maude, 1972; Crawford & Sinclair, 1972; Sanders, 1993; Uauy & Hoffman, 2000). DHA makes up about 30-50% of the lipid chains in the retinal outer segment disc membranes and also a similarly large proportion of the neuronal membrane lipids (Everts & Davis, 2000; Uauy & Hoffman, 2000). This high concentration of DHA can increase membrane fluidity and modify the mobility of proteins and the activities of enzymes critical to transduction of visual segments (Uauy & Hoffman, 2000). In addition, approximately 50% of the dry weight of the brain is composed of lipids, of which up to 50% are long chain PUFA (Garrow & James, 1993), chiefly AA, DHA and 7,10,13,16-docosatetraenoic acid (DTA; 22:4n-6).

Studies in strict vegetarians, whose diet devoid 5,8,11,14,17-eicosapentaenoic acid (EPA; 20:5n-3) and DHA, and infants fed on artificial formulas demonstrated that humans can convert ALA to EPA but the capacity to make DHA is limited (Sanders, 1993; Brenna, 2002). More currently, in vivo stable isotope studies in animals showed that labeled EPA was more

efficiently used for DHA synthesis compared to labeled ALA in the rat plasma (Lin & Salem, 2005). Thus, preformed DHA appears to be more important in determining the levels in tissues than DHA derived from ALA (Sanders, 1993), particularly during fetal and neonatal brain and retina development. All the above have led to the current hypothesis that maternal and fetal and neonatal lipid nutrition is a determinant of the health of the child and the development of diseases later in life (Dutta-Roy, 2000a&b).

1.2.1. Dietary sources of n-3 and n-6 polyunsaturated fatty acids

Of the n-6 family, the parent and EFA, LA is present in high quantities in plant oils, corn, sunflower and safflower oils (Beynen & Katan, 1989; Garrow & James, 1993; Uauy & Hoffman, 2000), in less amounts in avocado, peanut and rape oils and in small amounts in olive, palm and coconut oils (Beynen & Katan, 1989; Garrow & James, 1993). Of the n-6 long chain polyunsaturated fatty acids (LCPUFA), di-homo-γ-linolenic acid (DGLA; 20:3n-6 or 8,11,14-eicosatrienoic acid) is found in large concentrations in evening primrose oil and blackcurrant and AA in meat and eggs.

On the other hand, the parent ALA is of terrestrial origin (Brenna, 2002), first isolated in 1887 from hempseed oil (Tinoco, 1982) and is found in high concentrations in linseed (flaxseed), canola and soyabean oils at approximately 57%, 8 and 7% , respectively (Burdge & Calder, 2005). ALA can be found also in rapeseed oil and in green leaves (Tinoco, 1982) whereas its n-3 LCPUFA derivatives, EPA (20:5n-3), 7,10,13,16,19-docosapentaenoic acid (DPA n-3, 22:5n-3) and DHA are present in high quantities in fish oils and algae (Sanders, 1993). The latter are found in the TG of phytoplankton and are consumed and identified in highest concentrations in ocean fish and animals (i.e. whale, mackerel, seal and salmon) (Malasanos & Stacpoole, 1991; Bitsanis et al, 2001).

1.2.2. Fatty acid synthesis: Elongation and desaturation

The process of desaturation, by which a double bond is introduced into fatty acid acyl chain occurs in the endoplasmatic reticulum (ER) and the desaturase complex required, it involves cytochrome b5, NADH-cytochrome b5 reductase and the specific desaturase (i.e Δ-9 or -6 desaturases) (Thabrew & Ayling, 2001; Brownie & Kernohan, 2005).

In the case of EFA, mammals lack the ability to introduce double bonds in fatty acids beyond carbons 9 and10 and therefore the parent LA and ALA must be supplied by the diet (Garrow & James, 1993; Thabrew & Ayling, 2001; Lauritzen et al, 2001; Brownie & Kernohan, 2005). Within the human organism, ingested LA and ALA are metabolized (primarily in the liver) respectively to 4,7,10,13,16-docosapentaenoic acid (DPA n-6; 22:5n-6) and DHA (22:6n-3) via an alternative sequence of position specific desaturases and chain elongation steps (Figure 1.1) (Sprecher, 1992, 1993).

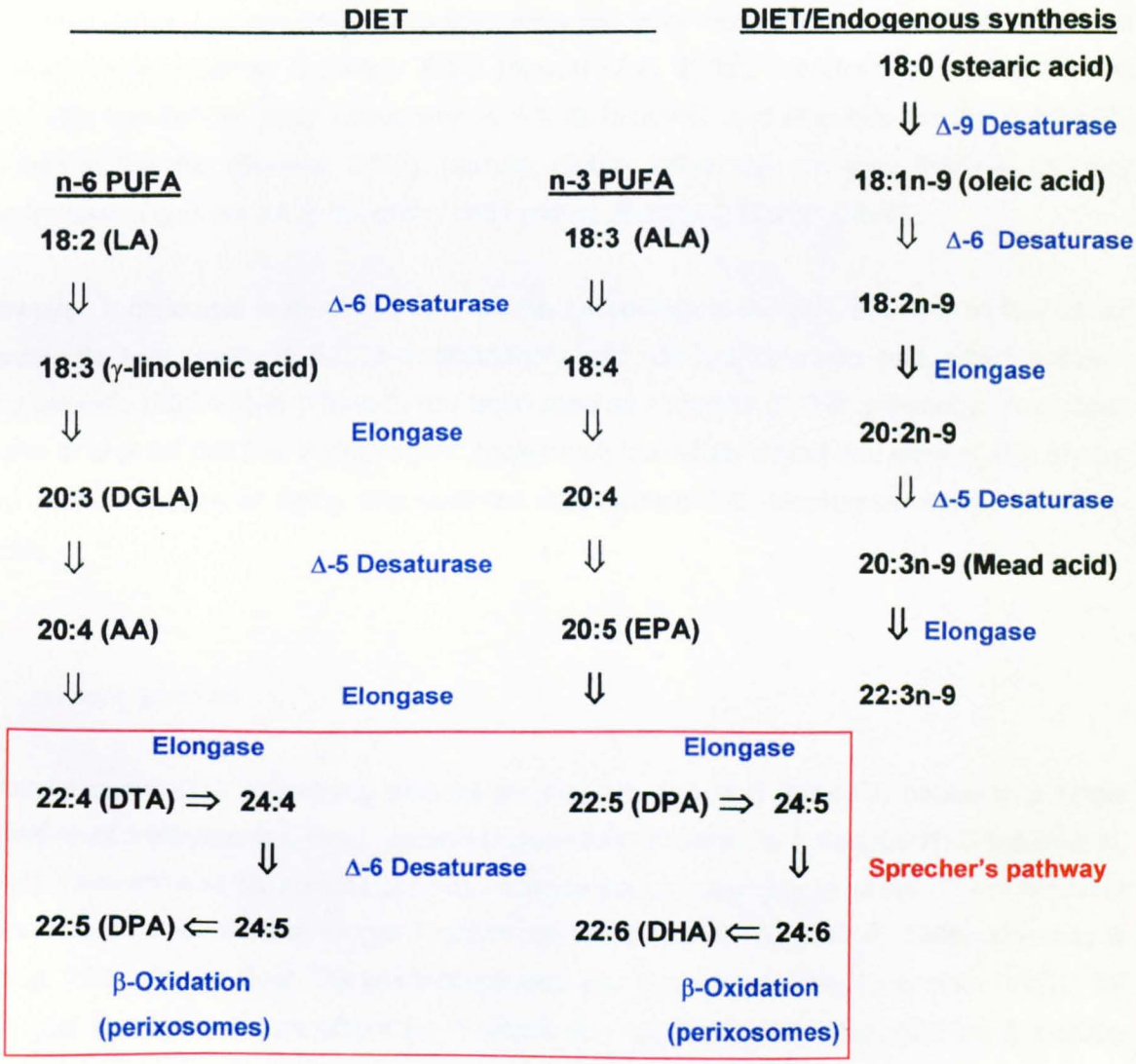


Figure 1.1. Fatty acids synthesis. PUFA, polyunsaturated fatty acids; LA, linoleic acid; ALA, α-linolenic acid; DGLA, di-homo-γ-linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DTA, docosatetraenoic acid, DPA, docosapentaenoic acid of the n-6 or n-3 pathway; DHA, docosahexaenoic acid.

Synthesis of DHA (22:6n-3) from EPA (20:5n-3) is an indirect conversion, including chain elongation to 24: 5n-3, followed by desaturation to tetracosahexaenoic acid (24:6n-3) by the Δ-6 desaturase and perixosomal shortening, the so called the Sprecher's pathway, and similarly for the end product of the n-6 pathway (Sprecher, 1992, 1993; Innis, 1992, 2000; Decsi et al, 1995; Ozanne et al, 1998; Lauritzen et al, 2001; Burdge & Calder, 2005). However, the exact regulation of the translocation process of DTA (22:4n-6) from the ER to the peroxisome and the limited β-oxidation pathway are not known (Burdge & Calder, 2005).

Δ-6 desaturase is a rate limiting enzyme in the EFA synthesis pathway and it has greater affinity for ALA than LA than oleic acid (18:1n-9). Thus, the substrates of the different pathways compete each other for desaturases and will influence the proportions of the end product formed (Garrow & James, 1993; Plourde et al, 2006). Conversion of ALA to DHA is generally low (whole body conversion < 5% in humans) and depends on the n-6 PUFA content in the diet (Brenna, 2002). Usually, dietary influences are such that n-6 pathway predominates and the AA is the major end product (Burdge & Calder, 2005).

However, if oleic acid is much higher than the LA content in the diet, then the oleic acid will desaturate excessively to 5,8,11-eicosatrienoic acid (20:3n9) or mead acid, which explains why the ratio (20:3n-9)-to-(20:4n-6) has been used as a marker of EFA deficiency. Prolonged intake of high fat diet (i.e. Western diet, cholesterol, trans-fatty acids) and alcohol, low energy and protein intakes or aging and diabetes may reduce Δ-6 desaturase activity (Brenner, 1989).

1.3. Triacylglycerols

Triacylglycerols (TG; chemically defined as fats) are esters of three FA bound to a single molecule of a trihydroxy alcohol glycerol (Figure 1.2) (Thabrew & Ayling, 2001; Cordain et al, 2005). They are also called neutral lipids because the carboxyl groups of the FA are bound in ester linkage and can not longer function as acids (McKee & McKee, 1996; Thabrew & Ayling, 2001). In the liver, TG are incorporated into Very-low-density lipoprotein (VLDL) for transport to adipose tissue where fat is stored and serve as energy fuel (McKee & McKee, 1996; Thabrew & Ayling, 2001). Mobilisation of stored fat initiates by removal of a FA from sn-1 and sn-3 position of a TG molecule by the action of a local hormone-sensitive lipase to produce two free fatty acids (FFA) and 2-monoacylglycerol, which is controlled by a broad spectrum of hormonal paracrine and/or autocrine signals (Minich et al, 1997; Stich & Berlan, 2004). TG undergo continues lipolysis and re-esterification and the balance between these

two processes determines the rate of release of FFA from adipose tissue (Thabrew & Ayling, 2001). Alternatively, the rate of glycerol release has been used as an index of the rate of lipolysis (Stich & Berlan, 2004).

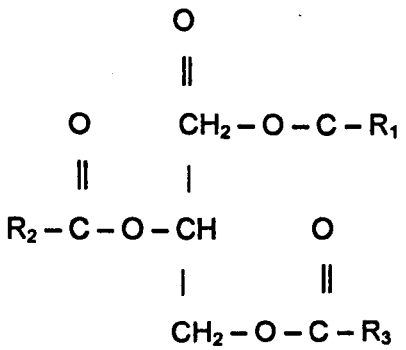
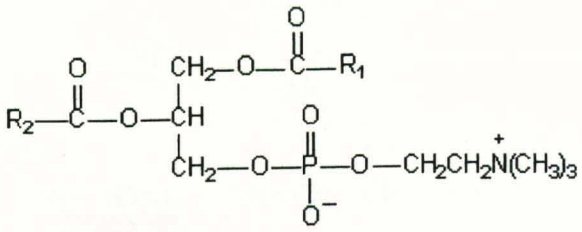


Figure 1.2. Triacylglycerol.

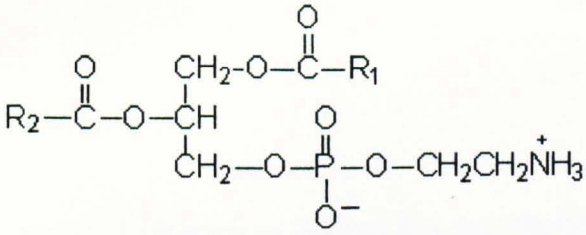
About 95% of the lipids in diet are composed of TG. After intestinal absorption, ingested EFA in the form of TG undergo hydrolysis by the action of pancreatic or gastric lipases, with pancreatic being the most predominant (Minich et al, 1997). Lipolysis of the long chain (>20C) is at a lower rate than shorter-chain n-3 PUFA. These observations may indicate a resistance towards enzymatic hydrolysis of the sn-1 and sn-3 acyl chains. It is evident that in whale oil TG, EPA and DHA are predominantly esterified at the sn-1 and sn-3 position and have been characterized as being lipase resistance (Minich et al, 1997). The reason is not known, but it is conceivable that the stereospecific conformation of EFA and their LCPUFA may prevent efficient binding of lipase to the TG molecule.

1.4. Phospholipids

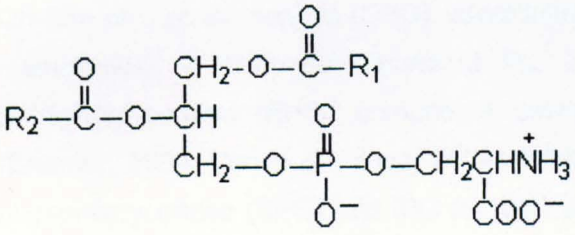
Membrane lipids are mainly composed by phospholipids (PL) and smaller amounts of cholesterol, glycolipids or cardiolipin (in mitochondrial membrane) (Richardson, 1973; Thabrew & Ayling, 2001). PL encompass phosphoglycerides and sphingomyelin (SPM) (Figure 1.3). Phosphoglycerides like TG, are phosphatidic acid derivatives (McKee & McKee, 1996; Dow et al, 1997; Thabrew & Ayling, 2001; Vance & Vance, 2004). With the exception of inositol phosphoglycerides (IPG), all PL in general are formed by the esterification of an amine to the phosphatidic backbone (Dow et al, 1997; Thabrew & Ayling, 2001). Whilst, SPM is synthesised by joining either choline or ethanolamine phosphate to ceramides.



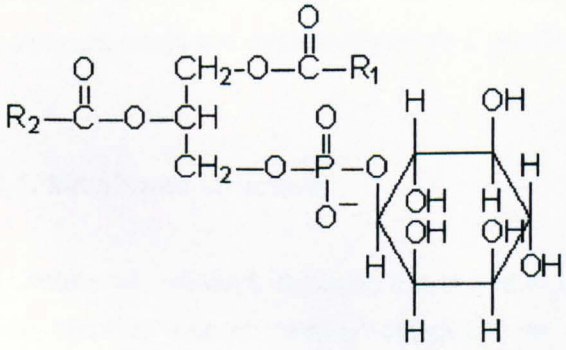
Choline phosphoglycerides



Ethanolamine phosphoglycerides



Serine phosphoglycerides



Inositol phosphoglycerides

Figure 1.3. (Adopted by http://themedicalbiochemistrypage.org/lipid-synthesis.html) Phospholipids.

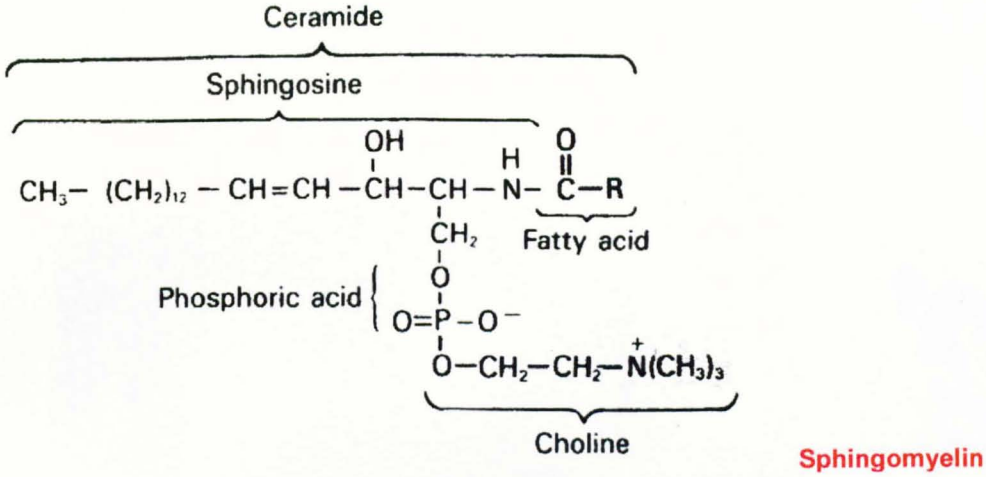


Figure 1.3. (Adopted by Thabrew & Ayling, 2001) Phospholipids (continues).

Choline phosphoglycerides (CPG), constituting up to 55% of the lipid content of the biological membranes, is the most abundant PL. The second most abundant is ethanolamine phosphoglycerides (EPG) comprising between 20-40% of the membrane lipid content (Brenner, 1984; Vance & Vance, 2004). Whilst, the remaining 10-15% is made up of serine phosphoglycerides (SPG) and IPG (5-10%) and shingolipids (4-7% is SPM) (Brenner, 1984). Additionally, PL can vary in their backbone sn-1 and sn-2 positions of FA chains. Usually, the sn-1 position of a PL contains a saturated (most abundant) or mono-unsaturated fatty acid while sn-2 position holds a PUFA (Budowski & Crawford, 1985). Indeed, C20 and C22 polyenoic acids are exclusively at sn-2 position.

1.5. Membrane structure

Membranes are fluid, dynamic structures in which both lipid and proteins (Figure 1.4) exhibit considerably lateral motility important in many aspects of cellular function, such as cytoplasmic streaming or formation of gap junctions with neighbouring cells (Dow et al, 1997; Maxfield, 2002). The ratio of protein-to-lipids together with the proportions of the membrane lipid components vary greatly between different types of membrane. However, within a specific type of membrane the proportions of the different lipid classes are remarkably constant (Thabrew & Ayling, 2001). A membrane with many functions is high in protein (protein/lipid ratio is 3:2 in inner mitochondrial membrane) while a membrane with fewer

function contain much less protein (protein: lipid ratio is 1:4 in plasma and 0:3 in myelin) (Dow et al, 1997; Thabrew & Ayling, 2001).

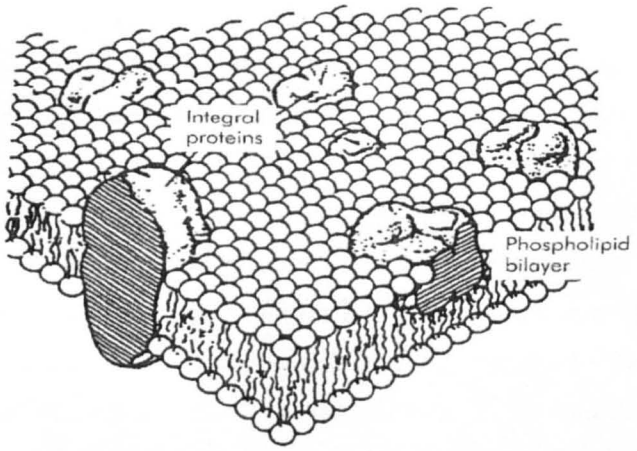


Figure 1.4. (Adopted by Thabrew & Ayling, 2001). The Singer-Nicolson fluid mosaic model of membrane structure in which globular integral proteins with stripple surfaces are randomly distributed in the plane of PL bilayer.

The lipids in biological membranes are organised as a continuous bilayer (Figure 1.2) (Lee, 2000; Przestalski et al, 2000). In this model, the amphipathic structure of the polar membrane lipids directly determines the bilayer structure by providing a hydrophobic environment in the middle of the bilayer for the hydrocarbon chain, with the lipid polar head groups encountering the aqueous phase (Yeagle, 1989; Lee, 2000). There are now more than 1,000 distinct molecular species of lipids recognised in membranes, suggesting a more complex lipid structure (Merrill & Schroeder, 1993), and the way that lipids pack together side by side in a bilayer depends on their shape (Lee, 2000).

Membrane fluidity or order, vital for function of proteins located within lipid molecules (Dow et al, 1997), will depend on the balance between the individual phosphoglycerides and on the nature of the fatty acid acyl chains incorporated into the phospholipids (Budowski & Crawford, 1985; Garg & Sabine, 1988; Garrow & James, 1993; Bausch, 1993). Hence, the effect of dietary fat will be predominantly on the acyl groups, and diatery PUFA will be incorporated into membrane lipids on the basis of the degree of preference of the acyl-transferases for the different PUFA and the efficiency of the conversion of the parent PUFA to its LCPUFA derivatives (Budowski & Crawford, 1985). This further suggests that the dietary balance between (n-6)-to-(n-3) PUFA is a determinant of membrane fatty acid composition. NMR

studies have been focused on the physical properties of DHA on membrane lipids due to its role in the function of rhodopsin in the rod outer segment disc membrane and its potential role to promote the formation of domains within the membrane containing significantly high concentrations of cholesterol (Everts & Davis, 2000).

The Singer-Nicolson fluid mosaic concept is a valid model illustrating how membrane is organised, although a small fraction of the lipid may interact specifically with the membrane proteins (Vereb et al, 2003). The last 10 years biophysicists have suggested that the inhomogeneities in lipid distribution in the membranes may form various types of specialised lipid domains (floating among the bulk phsopholipids bilayer), termed as lipid rafts and more recently as caveolae, important in endocytosis and other biological processes, cell signalling and trafficking (Brown & London, 2000; Simons & Toomre, 2000; Kenworthy, 2002; Maxfield, 2002; Razani & Lisanti, 2002; Nichols, 2003; Magnani et al, 2004). In polarised cells, lipid rafts are found in the apical plasma membrane, whilst they are abundant in the axonal membrane of neurons (Simons & Toomre, 2000; Min & Crawford, 2004). On the other hand, caveolae "cave-like" are a subset of lipid rafts found in cell surface as flask-shaped plasma membrane inavigations containing caveolin (Kenworthy, 2002; Razani & Lisanti, 2002).

Moreover, caveolae have a primary role in regulating the lipid balance by stimulating long chain fatty acid uptake via the action of fatty acid translocase protein (FAT/CD36), which is preferably located in the caveolae (Pohl et al, 2004). However, the native structure and composition of lipid rafts and cavaeolae have been difficult to assess and their structure and function are still controversial (Kenworthy, 2002; Razani & Lisanti, 2002). Nonetheless, there is novel evidence that lipid rafts and caveolae clustering proteins on the inner leaflet (Kenworthy, 2002).

1.5.1. Specific functions of membrane phospholipids

Choline phosphoglycerides (CPG), the outer leaflet of cell membrane has important metabolic and structural functions in membranes, and influences surface tensions in lung alveoli (McKee & McKee, 1996; Thabrew & Ayling, 2001). For example, in respiratory distress syndrome, a common disorder of the premature infant is caused by the lack of dipalmitoyl lecithin, a surfactant of the inner surfaces in the lung that acts against adherence due to surface tension (McKee & McKee, 1996; Thabrew & Ayling, 2001). As a result, these preterm infants with respiratory distress syndrome are likely to die of suffocation (McKee & McKee, 1996). Furthermore, once CPG is cleaved by phospholipases it generates diacylglycerol,

lysophosphatidylcholine, phosphatidic acid and AA, all of which have a role in lipid second messenger roles (Vance & Vance, 2004).

Ethanolamine phosphoglycerides (EPG), the inner leaflet of cell membrane has a role in stimulating the calcium pump protein (Yeagle, 1989). EPG and serine phosphoglycerides (SPG) have also structural function in membranes, such as the synaptosomal and rod outer segment membranes, and both are enriched in DHA (Lauritzen et al, 2001). In addition, SPG markedly increases the activity of the membrane protein Na+ K+ ATPase (Richardson, 1973) and is a marker for apoptotic cells in different systems (Kingdom et al, 2000a; Vance & Vance, 2004).

On the other hand, inositol phosphoglycerides have important structural function, especially in the function of nervous tissue (Thabrew & Ayling, 2001). On hormonal stimulation, gives rise to diacylglycerol (DAG) and inositol triphosphate which can act as second messengers (Thabrew & Ayling, 2001; Hurley, 2003; Vance & Vance, 2004). Phosphoinositides are the main sources of DAG in cell signalling via the action of protein kinase C (PKC), and cytoskeletal regulation and membrane trafficking (Merrill & Schroeder, 1993; Hindenes et al, 2000; Hurley, 2003). The phosphoinositide cycle controls process in cell division and dietary PUFA and the proportions of n-6 and n-3 PUFA appear to affect the cycle. Hence, it is expected that the current western diet, which is high in SFA, and unsaturated FA (i.e. n-6 PUFA), but deficient in n-3 PUFA would alter considerably the phosphoinositide cycle and the related regulated processes.

Shingomyelin (SPM) is an important structural PL and the ratio of CPG- to-SPM is used as a marker for respiratory distress syndrome (Wijnberger et al, 2003; Poggi et al, 2003).

1.6. Polyunsaturated fatty acids and eicosanoid synthesis

Eicosanoids are autocoids, generally active within the organ they produced and hence fundamental autocrine and paracrine regulators (McKee & McKee, 1996; Uauy & Hoffman, 2000). They serve as catalysts in many physiological functions; inflammation, fever, vasoconstriction and vasodilation, regulation of blood pressure, blood clotting, bronchial constriction, immune system modulation, control of reproductive process and tissue growth and sleep/wake cycle (Benirschke & Kaufmann, 1995; Kelley et al, 1997; O'Banion, 1999; Uauy & Hoffman, 2000).

Eicosanoids are a large family of subsets of bioactive compounds, which include prostaglandins (PG), prostacyclins, thromboxanes, leukotrienes, hydroxy/hydroperoxy and epoxyeicosatrienoic acids. Of these subsets, for example PG, first discovered in late 1930s in the seminal fluid, are produced in the cells by the a) n-6 pathway; series -1 and -2 PG are synthesized from DGLA and AA, respectively and b) n-3 pathway; EPA is metabolized to series-3 PG. Oppose to series-1 (DGLA derivatives), series-2 PG (AA derivatives) are involved in swelling, inflammation, clotting and dilation. Series-3 PG protects against heart attack and stroke and functions to attenuate excessive series-2 production. However, utilization of EPA, a PUFA that has anti-thrombotic effects, for synthesis of the series-3 eicosanoids is very low (Garg et al, 1990). Since AA is the major PUFA in cells, the series-2 of the eicosanoids is the most abundant and generally the most active (Figure 1.5) (McKee & McKee, 1996; Bogatcheva et al, 2005).

1.6.1. The action of arachidonic acid in the cell

Generally, AA is considered an inflammatory PUFA due to its capacity to metabolize to highly active inflammatory compounds (Malasanos & Stacpoole, 1991; Huang et al, 1997). AA has been demonstrated to produce coronary constriction followed by a longer lasting dilation (Talesnik, 1986), to increase permeability in microvessels and to induced edema in the rat brain (Beck et al, 1998). Thus, it is considered an atherogenic n-6 PUFA (Raheja et al, 1997). In line with the above, the high consumption of saturated fatty acids and n-6 PUFA in the Western countries are considered to predispose humans to the so called 'diseases of Western Civilisation', notably cardiovascular diseases (Simopoulos, 1991; Raheja et al, 1997; Scollan et al, 2001; Cordain et al, 2005). These diets have a high n-6:n-3 ratio in dietary fats and such fat intake deranges the EFA metabolism leading to unbalanced overproduction of AA metabolites (Raheja et al, 1997).

Crawford (2000) argued on the need to reduce LA (precursor of AA) content in the diet; *"This is necessary to reduce adverse effects of excesses of AA and its eicosanoid products".*

The average amount of AA in Western diets has been estimated to be approximately between 50-300mg/day (Nelson et al, 1997b). Also, the n-6:n-3 ratio in the current diet is 10:1 compared to the primitive human 1:1 (Scollan et al, 2001) or even in the area of 8-16:1 (Budowski & Crawford, 1985; Lauritzen et al, 2001). Consequently, recommendations have

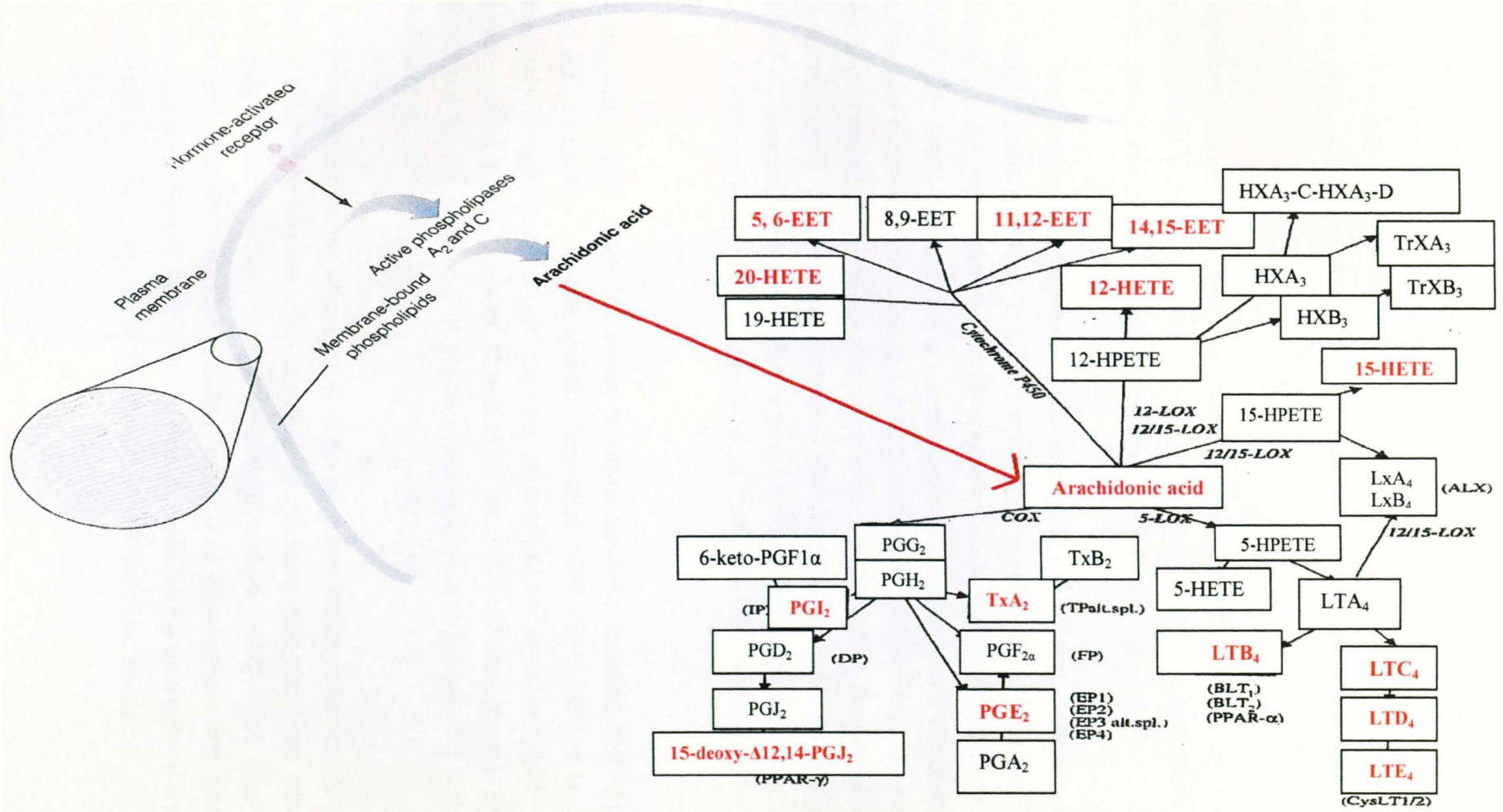


Figure1.5. (Modified from Bogatcheva et al, 2005) Arachidonic acid cascade. Hydrolysis of phospholipids primarily by phospholipase A_2 leads to arachidonic acid (AA) formation in the cell. AA conversion products are shown in diagram with their receptors in parentheses and names of primary enzymes under the appropriate arrow. PG, prostaglandin; LT, leukotriene; H(P)ETE, hydro(pero)xyeicosatetraenoic acid; EET, epoxyeicosatrienoic acid; HX, hepoxilin; TrX, trioxilin; Lx, lipoxin; COX, cyclooxygenase; LOX, lipoxygenase; Cytochrome P450, cytochrome P450 epoxygenase. The most important substances are in red.

been made to the Western populations to increase dietary n-3 intakes, EPA and particularly DHA due to their antiatherogenic effects of the n-3 PUFA, ability to decrease tissue level AA (Garg et al, 1990; Malasanos & Stacpoole, 1991; Sinclair & O'Dea, 1993; Crawford, 2000) and to inhibit AA prostanoid metabolism (Talesnik, 1986; Garg et al, 1990; Lauritzen et al, 2001).

Conversely, Nelson et al (1997a,c) showed that dietary AA in a level eightfold above that found in a typical Western diet has little or no effect on blood lipid, lipoprotein or apoprotein levels, blood coagulation, platelet function and platelet fatty acids.

AA is also involved with inflammation and swelling at site of injury. This was also evident in cell cultures, where trauma and mechanical manipulation may stimulate PG production (products of AA metabolism) and cells may release PG during adherence to culture plates (Rose et al, 1990). However, this is a rather protective mechanism to immobilize the infected site in order to prevent further injury and facilitate healing. Additionally, AA is a physiological regulator of the adipocyte glucose transport system by regulating the expression of glucose transporter type 4, GLUT4 (Long & Pekala, 1996) and has a critical role as a participant in a variety of receptor/agonist-mediated signal cascades (Hindenes et al, 2000) and gene expression (Rizzo et al, 1995; Stuhlmeier et al, 1997) via the activation of PKC (Rizzo et al, 1995).

Furthermore, PG participate in a variety of cellular functions, including modulation of vascular tone, cell proliferation and differentiation (Lim et al, 1997, 1999; Paria et al, 2000). On the other hand, insufficiency of AA may be responsible for vascular fragility and cerebral hemorrhage in preterm infants (Crawford et al, 1997) or low plasma AA could have contributed to the weakness of neural blood vessels and subsequent rupture in elderly (Golfeto et al, 2001).

In pregnancy, the placenta among other PUFA increases the proportion of AA about two-folds and reduces the proportion of LA delivered to the fetus during its brain growth spurt and particularly in the umbilical plasma at term (Crawford, 2000). This may supports the importance of AA for fetal and placental membranes development and postnatal growth. Hence, membrane lipid analysis of the human placenta will be reported in a later section and the possible role of EFA in placental development will be further discussed.

1.7. The physiology of the human placenta

The principal objective of human placentation is to bring the fetal and maternal circulations into close proximity to each other for the continuous supply of nutrients and survival of the fetal allograft (Graham & Lala, 1992; Page, 1993; Benirschke & Kaufmann, 1995; Harding & Bocking, 2001).

Placental structure involves three main elements; growth and expansion of the cytotrophoblast shell/basal plate into the uterine stroma to tap the spiral arteries, continued branching of the fetal villi or chorionic plate, and formation of partial septa arising from the basal plate and projecting into the intervillous space (placental villous tissue) (Dewbury et al,1993; Harding & Bocking, 2001). Its complex villous structure greatly increases the surface area of contact between the fetal circulation in the placenta and the maternal circulation to meet the high demands of the growing fetus (Kliman, 2000). If the blood flow to the placental bed through the uterine spiral arteries is compromised, it could result in preterm birth and fetal loss (Kliman, 2000).

1.7.1. First trimester placentation

The process of placentation by definition prerequisites implantation and involves very precise vascular interaction for placental and fetal membranes, derived from the trophoblast cells (Damsky et al, 1994; Berischke & Kaufmann, 1995; Lockwood et al, 1999; Loke & King, 2000). The trophoblast is a fetally-derived placental tissue that is interimposed between the fetus and the mother in direct contact with her blood in the hemochorial placentae (Zuchermann & Head, 1986). During early pregnancy fetal cells circulate in maternal blood (Gosseye & Fox, 1984; Van Wijk et al, 2001) and the placenta tissue displays a high degree of angiogenesis (Dantzer et al, 2000). The endothelium has a key role in this process and it is highly variable depending on species –specificity, location in the organ or vascular tree and physiological conditions. Hence, endothelium 'malfunctioning' is the major cause for placental vascular disorders (Dantzer et al, 2000).

1.7.1.1. The prelacunar stage before placentation

The blastocyst is formed at the 4th d after fertilization with inner cell mass (ICM, embryoblast cells) and outer ring of trophoblast cells (≈50-60 cells) (Kurjak et al, 1997; Morrish et al, 1998;

Rossant, 2001). The placenta is formed as soon as the blastocyst implants or the fetal membranes have establish close and stable contacts with the uterine mucosa (Berischke & Kaufmann, 1995; Burrows et al, 1996; Kurjak et al, 1997). However, there is a limited period, known as the receptive phase (implantation window) that the endometrium will be exposed to implantation and this particular "window" receptivity seems to be a property of the endometrial epithelial lining. Experimental removal of the uterine epithelium allows the blasocyst to implant completely (Bischof et al, 1998).

The fist step of implantation is called apposition (Graham & Lala, 1992; Berischke & Kaufmann, 1995; Enders, 2000) and is initiated after about the 6^{th}-7^{th} d following fertilization with differentiation of the trophoblast cells into the invasive syncytiotrophoblast (Page, 1993). It involves an initial attachment or adhesive phase in which the trophoblast epithelium adheres to the uterine epithelium, usually adjacent to the ICM, followed by considerable changes in the uterine stroma (Graham & Lala,1992; Burrows et al, 1996; Lockwood et al, 1999; Enders, 2000; Harding & Bocking, 2001). The trophoblast is the site of the developing fetal membranes, including the placenta (Jaffe, 1998; Rossant, 2001) while ICM forms the embryoblast, which will give rise to the embryo, cord and amnion (Jaffe, 1998; Rossant et al, 2001). The area where the ICM is located is called the embryonic pole (Page, 1993; Berischke & Kaufmann, 1995; Morrish et al, 1998; Kingdom et al, 2000a)

At the 7^{th} d after fertilization, as implantation proceeds, the trophoblast that will give rise to the future placenta, proliferate rapidly into two layers, cytotrophoblast (inner) and syncytiotrophoblast (outer layer) (Graham & Lala, 1992; Kurjak et al, 1997; Kingdom et al, 2000a; Kliman, 2000). Since the embryo does not show definite chorionic villi, nurture of the embryo is by diffusion and erosion of maternal tissues, capillaries, glands and stroma (7d). At the prelacunar stage (7-8 days after fertilization) the implanting blastocyst is a flattened vesicle (0.1x0.3x0.3mm) and composed of 107-256 cells most of which make up the outer walls of the blastocystic cavity, the trophoblast (Berischke & Kaufmann, 1995). At about 8d the embryo is still only partially embedded in the endometrium (Page, 1993; kurjak et al, 1997; Kingdom et al, 2000a), but at 10d (interstitial implantation) it is almost completely surrounded by endometrial tissue (Page, 1993; Kingdom et al, 2000a). This invasive nature of the blastocyst, interstitial implantation, reduces the number of anatomical layers separating maternal and fetal blood to three layres: villous trophoblast, stroma and fetoplacental vascular endothelium (Kingdom et al, 2000a).

Among the various key molecules for successful implantation, prostaglandins may also participate in this process (Lim et al, 1997, 1999; Paria et al, 2000). In mice, it is evident that

COX-2 (an cyclooxygenase isoenzyme) is essential for blastocyst implantation and decidualisation, suggesting a key role of the AA mediators in embryo-uterine interactions during implantation (Lim et al, 1997, 1999; Paria et al, 2000).

1.7.1.2. The uniqueness of human implantation

The way that the apical plasma membranes of the trophoblast of the blastocyst (embryo) attached to the apical membranes of uterine endothelium is common in all species (Berischke & Kaufmann, 1995; Kimber & Spanswick, 2000; Bowen & Burghardt, 2000).

However, there is a considerable variation in the manner that implantation is accomplished by different species (Cross, 1998; Enders, 2000). This is demonstrated by the fact that the 'window receptivity' of mice implantation is less than 24h at 4d of pregnancy, whereas in human the adhesion cascade occurs over about 5-6 d (Cross et al, 1994; Aplin, 1996; Kimber & Spanswick, 2000; Rossant, 2001; Parast et al, 2001). Also in other primates (rhesus monkeys) implantation occurs almost immediately to the uterine epithelium (Bowen & Burghardt, 2000) while in domestic animals (pigs, cattles, goat, sheep and horses) there is no displacement or invasion of the maternal tissue by fetal tissue and the conceptus remains within the uterine lumen throughout gestation (epitheliochorial implantation) (Cross et al, 1994; Bowen & Burghardt, 2000).

In contrast, human implantation is particularly invasive as placental trophoblast infiltrate deep into the myometrium (Fisher, 1989; Aplin, 1991; Librach et al, 1991; Loke & King, 2000; Rossant, 2001). Human tropholbast invasion is extensive reaching the first third of the myometrium (Fisher et al, 1989; Tarrade et al, 2001), whereas invasion in even closely related primates is limited to the endometrium, suggesting that certain of the mechanisms used by human trophoblast to penetrate the uterus are unique (Fisher et al, 1989). A possible explanation to the uniqueness of human implantation is that extra-uterine pregnancies are common in human mammals (Bischof & Campana, 1997).

The haemochorial placenta, found in primates and rodents exhibits the greatest extent of uterine tissue destruction. As a result, the uterine epithelium underlying basement membrane and maternal endothelium all are being lost. Hence, maternal blood in the placenta is in direct contact with the trophoblast (Graham & Lala, 1992; Harding & Bocking, 2001). Nonetheless, in humans, because there is only one layer of trophoblast between the maternal blood and fetal epithelial the human placenta has been classified as haemonochorial placenta (Page,

1993). However, survival of the implanting blastocyst requires that trophoblast gain access to the maternal circulation (Lockwood et al, 1999; Tarrade et al, 2001), which takes us to the lacunar stage.

1.7.1.3. First trimester uteroplacental circulation

The extravillous cytotrophoblasts penetrate the uterine spiral arteries to initiate morphological changes that increase greatly intervillous blood flow (Jaffe, 1998; Lockwood et al, 1999; Loke & King, 2000). These morphological changes required decidualization of the endometrial stromal cell, which occur at risk of decidual hemorrhage that could lead to spontaneous abortion, placental abruption and preterm birth (Lockwood et al, 1999).

The chorion is the highly specialized outermost extraembryonic membrane and develops from tropholbast and the associated mesodermal layer beneath the tropholbast. Numerous, small finger-like extention, villi, develop from the chorion and penetrate deeply in the uterine tissue, forming the villous chorion. The villi on the surface of the uterine cavity produce the smooth chorion. The villous chorion becomes highly vascular and as the embryonic heart begins to function, blood is pumped close to the uterine wall. Eventually, it becomes the principal embryonic part of the placenta. The amnion also surrounds the embryo and the fetus and eventually fuses to the inner layer of the chorion.

The embryonic part of the placenta is termed the chorion frondosum, whereas the maternal side is called the deciduas basalis into which the villi penetrate. The first step in the formation of a functional placenta is the appearance of blood vessels in the chorion. The developing chorionic villi are in contact with maternal tissues by the 3rd wk of gestation. These villi continue to enlarge and branch, forming a network that grows into the deciduas basalis of the endometrium. The villi will contain fetal blood vessels of the allantois. Maternal blood slowly percolates through lacunae lined by syncytiotrophoblast. The lacunae fuse into increasingly larger lacunar networks, the primordial of the intervillous space of the placenta. Endometrial capillaries dilate into maternal sinusoids. The syncytiotrophoblast erodes some of capilllaries, causing maternal blood to seep into the lacunar networks to establish a primitive unteroplacental circulation. The chorion continues to enlarge within the endometrium and by the 4th wk, yolk sac, embryo and chorion are suspended within an expansive, fluid-filled chamber. The connection between the embryo and the chorion is the body stalk contains the distal portions of the allantois and blood vessels that convey blood to and from the placenta. The yolk stalk is the narrow connection between the endoderm of the embryo and the yolk

sac. At 6-8 wk of gestation the fetal portion of the placenta consists of floating and achoring villi and cell islands (Genbacev & Miller, 2000). However, till 8 weeks of gestation, the maternal spiral arteries do not open directly into intervillou space (Burton et al, 1999). This is because aggregates of trophoblastic cells derived form trophoblastic shell plug their distal segments, hence, the maternal blood percolates through intercellular spaces in the trophoblastic shell (Burton et al, 1999).

The developing placenta can be observed by transabdominal ultrasound between 6-8 wk of gestation and appears as a thickening of a portion of the gestational sac (Dewbury et al, 1993). By the end of the second month the net wt of the chorionic sac is 6-10g, the thickness of the chorion at the implantation pole is 6mm and at the antiimplantation pole about 3mm. (Berischke & Kaufmann, 1995). The thickness of the villous trophoblast varies 10-30μm and near the end of this period the mesenchymal villi show increased numbers of macrophage and the first signs of the development of the immature intermediate villi (IIV) (Berischke & Kaufmann, 1995). Blood vessels development within the developing IIV is characterized by branching angiogenesis such that the placenta expands to produce 10-16 generations of stem villi (Kingdom et al, 2000a&b). IIV are the precursors of the stem villi and as early as 8 wk, the central vessels of IIV near the chorionic plate start developing a compact adventitia, thus these vessels slowly transform into arteries and veins (Demir et al, 1997). The villous stems are almost completely occupied by connective tissue. The mean maternofetal diffusion distance is reduced from 50 to 100μm during the second month and between 4-5 μm at term (Berischke & Kaufmann, 1995).

During much of the first trimester (≤10-12 wk of gestation), in anchoring villi cytotrophoblast invasion is confined to the uterine parenchyma (interstitial invasion) (Genbacev et al, 1995; Fisher, 2000; Enders, 2000; Kliman, 2000), and the growing embryo is separated from the maternal circulation by the trophoblastic shell (Genbacev & Miller, 2000). Hence, up to 12 wk of gestation, maternal plasma is found into the intravillous space (Berinischke & Kaufmann, 1995; Burton et al, 1999) with relatively few erythrocytes present (Burton et al, 1999) which is filtered by the uteroplacentla vessels, but at 13 wk maternal blood flows into the intervillous space that increases to about 100-200ml at term (Benirschke & Kaufmann, 1995). At the end of the first trimester a subpopulation of cytotrophoblast also invades the resident uterine arteries (spiral artioles) and veins, endovascular invasion (Genbacev et al, 1995; Fisher, 2000; Kliman, 2000). This process diverts arterial blood to the intervillous space, where the floating villi, covered with multinucleated syncytiotrophoblast, reside (Fisher, 2000) or cytotrophoblast invasion anchors the fetus to the mothers and creates the large-diameter, low-resistance vessels that carry blood to the floating villi at the maternal-fetal interface

(Genbacev et al, 1995; Kurjak et al, 1997; Kliman, 2000). Hence, establishment of an effective intervillous circulation occurrs late in the first trimester of human pregnancy (Jaffe, 1998). Between 12 -16 wk there is a further retrograde migration of vascular trophoblast into the intramyometrial segments of the spiral arteries where the same process of endothelial replacement and medial destruction occurs (Gosseye & Fox, 1984). These physiological vascular changes result in the muscular spiral arteries being converted into thin-walled, flaccid uteroplacental arteries which can readily accommodate the greatly augmented blood flow required in pregnancy (Gosseye & Fox, 1984).

1.7.2. Second trimester

During the second trimester of pregnancy there is a reduction in the fetoplacental vascular impedance (Kingdom et al, 2000a). Histologically, this period is characterised by a transformation of the first primitive generation of the tertiary villi (mesenchymal villi) into IIV, characterised by an expanded loose stroma and an increase in capillary density due to branching angiogenesis (Kingdom et al, 2000a). Trophoblast cells from the surface of IIV continue to proliferate, they transform into mesenchymal cells, which in turn differentiate into stem villi (Demir et al, 1997). Histologically, this process is characterised by condensation of the loose stroma, regression of the peripheral capillary network and differentiation of central capillaries into artioles and venules, hence 10-16 generations of stem villi are formed in the mature placenta (Kingdom et al, 2000a).

1.7.3. Third trimester

During the third trimester, fetal viability is attained between 24-26 wk of gestation, at a time when a significant switch occurs in development of the placental villous tree (Kingdom et al, 2000a&b). The IIV continue to transform into stem cells whilst their precursors, the mesenchymal villi switch to produce mature intermediate villi, MIV (kingdom et al, 2000a). These structures are characterised by elongated unbranched capillaries, within which the longitudinal growth of capillaries exceeds that of the villi themselves, hence, capillary loops "prolapse" laterally in the overlying villous trophoblast, bringing the fetal blood close to the intervillous space, to form terminal villi (Kingdom et al, 2000a&b). By term the surface area available for gas exchange in terminal villi reaches 13 square metres and their capillaries contain some 80ml (25%) of total fetoplacental blood volume (Kingdom et al, 2000a).

At term, the placenta is like a disc thickening of the membranous sac that is formed by splitting the membranes into the chorionic (top) and the basal (bottom) plates (Benirschke & Kaufmann, 1995). Both plates confine the intervillous space within which the maternal blood is perfused and circulates directly around the trophoblastic surfaces of the placental villi (Benirschke & Kaufmann, 1995). The villi are projected inside the intervillous space and contain the fetal vessels, which are connected to the fetal circulatory system by the chorionic plate and the umbilical cord. At the margins of the placenta the chorionic plate covering the intervillous space fuses with the basal (bottom) plate forming the chorionic leave (Benirschke & Kaufmann, 1995). The syncytiotrophoblast cell layers of the chorionic villi which form the functional unit of the term placenta is a polarized structure in that the plasma membrane is divided into an apical, mother facing brush border (BBM or MVM) and a basal fetal-facing plasma membrane called the basal membrane (BM) (Eaton & Oakey, 1994; Anand et al, 1996; Powell et al, 1999). The syncytiotrophoblast is a higly polarized epithelial layer responsible for regulating materno-fetal exchange (Eaton & Oakey, 1994).

1.7.4. Placental epidemiology

Epidemiological studies suggest that long-term consequences for health may derive from stimuli or insults sustained by the fetus during intrauterine development (Page, 1993; Loke & King, 2000). Advanced studies in stereology and molecular biology on the invasive nature of trophoblasts to form these complex placental and fetal villi structures have assisted the epidemiological studies to reconsider the role of the placenta in disease processes, proposing that placental weight (wt) is an indicator of placental size and the development of cardiovascular diseased later in life (Kingdom et al, 2000a). Consequently, the evidence that postnatal blood pressure is inversely related to birthweight, BWT (Page, 1993; Kingdom et al, 2000a; Blake et al, 2001) and positively to placental wt suggest that such discordance between placental and fetal size may evoke circulatory adaptation in the fetus, altering structures in the child and leading to the development of hypertension in the adult (Page, 1993; Kingdom et al, 2000a). Hence, it is conceivable that the development of ischaemic heart disease and stroke as well as non-insulin dependent Diabetes Mellitus (NIDDM) may be attributed to in utero impaired fetal growth and development during fetal life.

In early pregnancy placental exceeds fetal growth, whereas in the second half of gestation placental wt gain is so slow that does not parallel the steep rise in fetal wt (Harding & Bocking, 2001). With the correlation between placental wt, BWT and head circumference (HC) and the fact that the placenta grows before the fetus, it is conceivable that fetal growth retardation is in part due to impaired placental development (Crawford et al, 1989). The

placenta weighs about 100g by the forth month of pregnancy and has assumed its definitive form (Page, 1993). The slow placental wt gain is compensated for by the major changes in the microstructure and transport characteristics of the placenta (Harding & Bocking, 2001).

1.7.5. Fetal essential fatty acid supply

The fetus has unique requirements for lipids and essential fatty acids (EFA). During intrauterine life, the placenta selectively transfers arachidonic (AA) and docosahexaenoic (DHA) acids from the maternal circulation to the fetus, a process known as biomagnification (Crawford et al, 1976; Crawford, 2000; Lauritzen et al, 2001). The fact that the placenta lacks desaturase activity (Chambaz et al, 1985; Dutta-Roy, 2000a) and fetal fatty acid (FA) synthesis is considered to be very low (Carnielli et al, 1996; Salem et al, 1996; Campbell et al, 1997), supports the fact that the supply of linoleic (LA), α-linolenic (ALA) acids and their long chain polyunsaturated (LCPUFA), in particular AA and DHA depends on maternal diet and their concentrations in the maternal circulation (Campbell et al, 1996). In fact, the considerable amounts of n-6 and n-3 PUFA being stored in fetal brain and adipose tissue during the third trimester, to meet the high fetal demand for these FA, it further suggests that the mother mobilizes AA and DHA from her own stores (Benassayag et al, 1997).

1.7.5.1. Placental lipid transport and metabolism

Both the placenta and the fetus utilize FA from the maternal circulation throughout gestation (Smith et al, 1992; Page, 1993). Once reaching the placenta, lipids and other nutrients or solutes must cross the syncytiotrophoblasts. The human placental syncytiotrophoblast is the site of exchange of nutrients, lipids and minerals between the mother and the fetus (Lafond et al, 1994; Campbell & Dutta-Roy, 1995; Anand et al, 1996; Kingdom et al, 2000a&b).

Triacylglycerols (TG) cannot cross the placental membranes intact (Campbell & Dutta-Roy, 1995; Campbell et al, 1996; Haggarty, 1999). However, specific binding sites for very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) receptors are expressed in the human placental trophoblast (Haggarty, 1999). Essentially, lipoprotein lipase (LPL) on the maternal surface of the syncytiotrophoblast hydrolyses TG carried by the maternal VLDL (Smith et al, 1992; Bonet et al, 1992; Page, 1993; Handing & Bocking, 2001). Whilst, there is no LPL on the fetal border of the placenta (Page, 1993). Additionally to LPL, a unique triacylglycerol hydrolase has been identified in brush border

membrane that it is possible to be involved in the release of free fatty acids (FFA) from intracellular TG stores of the placenta (Waterman et al, 1998). The former is not altered in clinical conditions, like diabetes, whereas the latter is increased (Waterman et al, 1998).

During the third trimester there is a rapid increase in plasma FFA concentrations (Campbell et al, 1998a&b; Dutta-Roy, 2000a) and in FFA transport capacity attributed to the increased lipolysis in the adipose tissue, LPL activity and hydrolysis of the circulating TG, at a time that fetal deposition is increased (Bonet et al, 1992; Handling & Bocking, 2001). The placenta can selectively transfer LCPUFA and EFA to the fetal circulation in preference to the non-EFA, in the order of AA>>LA>>ALA>>oleic acid (Dutta-Roy, 2000a; Campbell et al, 1996). However, an earlier report suggested that the affinity of the placenta to transfer AA was lower than oleic acid and also that the order of binding affinity of the two EFA was reversed (Haggarty et al, 1997). Hence, the human placenta preferentially transfer the important n-3 PUFA, DHA from the maternal to fetal circulation and that the overall selectively for transfer is DHA>ALA>LA>AA (Haggarty et al, 1997, 1999).

1.7.5.2. Placental fatty acid uptake

A variety of mechanisms of FA uptake have been proposed including passive diffusion and specific fatty acid binding proteins, such as ubiquitous plasma membrane fatty acid-binding protein (FABPpm, 43 kDa), cytosolic binding proteins (FABP), fatty acid translocase (FAT/CD36, 88 kDa) and fatty acid transport protein (FATP, 63 kDa) (Campbell et al, 1998a&b; Dutta-Roy, 2000a&b).

On the other hand, Lafond et al (1994) showed that the uptake of LA by brush border (maternal side) was higher than that of the basal membrane (fetal side). This is in disagreement with the concept that FA can penetrate whole cells by passive diffusion (Lafond et al, 1994). In contrast, FABP first discovered in early 70s (Storch & Thumser; 2000), has a greater *in vitro* affinity for particular FA in the plasma pool FFA of the women during the last trimester compared to humans serum albumin (Dutta-Roy, 2000a). It is generally accepted that serum albumin acts as a reservoir of fatty acids for replenishment of those taken up by cells (Campbell et al, 1996; Dutta-Roy, 2000a), but FABppm may be involved in the sequestration of FFA bound to albumin from the maternal plasma for delivery to the fetus (Campbell & Dutta-Roy, 1995; van Nieuwenhoven et al, 1996). Additionally, the placenta selectively transfer DHA, known to be important for visual maturation and the fetal brain, and

FABPpm may also be involved in this process, which similar to AA, has high affinity for DHA (Campbell et al, 1998a).

Location of FAT/CD36 and FATP on both sides of the bipolar placental cells may allow bidirectional flow of FA, EFA and non-EFA and LCPUFA, across the placenta, whereas the exclusive location of FABpm on the maternal side may favor the undirectional flow of maternal LCPUFA to the fetus (Campbell & Dutta-Roy, 1995; Campbell et al, 1998a&b; Dutta-Roy, 2000a). Additionally, a special alpha-fetoprotein (AFP) conformation exists in the intervillous space with a high affinity to bind to PUFA, especially AA and DHA, protecting these FFA against catabolism or esterification and could contribute to the uptake and distribution of EFA to fetal tissues (Benassayag et al, 1997, 1999). Moreover, Ruyle et al (1990) suggested that a marked proportion of DHA is transported by the placenta and subsequently is incorporated into the red blood cells (RBC) of the fetus which further implies that erythrocytes may supply DHA to the developing fetal tissues.

Cytoplasmic heart-and liver- type FABP have also been observed in the placenta (Das et al, 1993; Campbell & Dutta-Roy, 1995; Dutta-Roy, 2000b). Heart-FABP binds only LCPUFA while liver-FABP binds heterogeneous ligands such as, bile salts, heme, peroxisomal proliferators, selenium, lysophosphatidic acid and eicosanoids (Storch, 1993; Dutta-Roy, 2000b). Liver-FABP may also be involved in the synthesis of eicosanoids in the fetoplacental unit and furthermore, there is a speculation that these FABP may interact with several fatty acid-mediated cellular processes (cell growth, cell signaling and regulation of gene expression) (Campbell et al, 1996, 1998a&b; Dutta-Roy, 2000b; Storch & Thumser, 2000), which consequently offers new insights in the role of the placenta in the supply of EFA and LCPUFA in physiological and clinical conditions, such as intrauterine growth retardation, low birthweight for gestational age, gestational Diabetes Mellitus (GDM) and preterm birth (Campbell et al, 1996, 1998a&b; Dutta-Roy, 2000b). Herein, FA analysis of the preterm and term-GDM placentae is reported in another section. Also, in diabetic rats, insulin deficiency downregulate FABP expression levels in adipocytes and upregualte in heart and muscle cells (van Nieuwenhoven et al, 1996).

1.7.6. Arachidonic acid metabolites in the placenta and parturition

Uterine contractility and parturition can be divided into the following phases; i) phase 0 of parturition corresponds to pregnancy, a time of relative uterine quiescence, ii) phase 1 of parturition is associated with activation of uterine function leading to upregulation of genes

required for contractions, iii) phase 2 of parturition involves stimulation of the uterus by uterotonins, including prostaglandins (PG), oxytocin and cornicotropin-releasing hormones (CRH) and iv) phase 3 of parturition includes expulsion of the placenta and the involution process and has been attributed primarily to the effects of oxytocin (Challis & Smith, 2001; Challis et al, 2001). The increased mobilization of AA in the preparatory phase 1 of parturition, however, it still remains to be established (Zakar & Hertelendy, 2001).

A major pathway of AA release is via the action of phospholipase A_2, PLA_2 (Lopez Bernal et al, 1992; Benassayag et al, 1997; Zakar & Hertelendy, 2001) and PLA_2 activity increases at term via a process involving FABPpm (Benassayag et al, 1997). The AA metabolites of the cyclooxygenase (COX) pathway are involved in parturition, uteroplacental hemodynamics, and preparation of the birth canal and separation of fetal membranes (Boone et al, 1993). The placental prostanoids may arise from trophoblast, stromal cells and fetal vessels endothelium (Sorem & Siler-khodr, 1995). Alternatively, increased PG synthesis may be a consequence of fetal hypothalamic-pituitary-adrenal-axis (HPA) activation and/or uterine mechanical stretch (Challis & Smith, 2001; Challis et al, 2001).

An augmentation of intrauterine PG production is also part of the pathomechanism of preterm birth (Zakar & Hertelendy, 2001). Preterm labour is one of the leading causes of perinatal morbidity and mortality (Buhl et al, 1995). Indeed, amniotic fluid levels of PG increased in women in preterm labour than in non-laboring women at the same gestational age (GA), but the difference was more pronounced in cases complicated by preterm premature rapture of membranes (PROM) or intrauterine infection (Zakar & Hertelendy, 2001). Additionally, a dysregulation of the vasocistrictor eicosanoids may lead to preterm labour. However, compared with the healthy term placenta, all IUGR (term and preterm) human placentae showed no aberrant thromboxane A_2 - to prostacyclin ratio (TXA_2/PGI_2) favouring vasoconstriction (Sorem & Siler-Khodr, 1995). Another objective in one of the studies described in the latter sections is to test if the AA content of the placental membranes increased towards term consistent with increased vascularisation and reached a critical level that could initiate parturition through PG derived from AA.

1.8. Prematurity

Premature birth is defined as those infant born before 37 completed gestational wk (<259d) and term as those born 37-41 wk (259-293d) or later (international definition) (Jewell et al, 2001). Preterm involves more complex and much different adaptive events than the term infants, since cessation of pregnancy occurs up to 4 months earlier accompanied by rapid tissue accretion and nutrients turnover (Decsi et al, 1995).

Consequently, extrauterine development of preterm and low birthweight, LBW (<2,500 g) infants involves an altered physiological development, posing the newborn at risk for major diseases of prematurity, because intravenous nutrition via placenta and maternal controls over fetal metabolism has come to an end owing to preterm birth. LBW infants can also be categorised as small for gestational age (SGA) preterm infants. These are LBW infant in developing countries and they show altered growth potential and nutrients deficits (Thureen &Hay, 1993).

1.8.1. Epidemiology of Preterm birth

Preterm birth occurs in approximately 5-10% of all pregnancies. Preterm birth is a major clinical problem associated with 70% perinatal mortality and 75% neonatal morbidity and its prevalence is increasing (Challis & Smith, 2001; Challis et al, 2001). Although some preterm births may be elective, approximately 30% occur in association with an underlying infectious process and about 50% are idiopathic preterm births of unknown cause (Challis & Smith, 2001; Challis et al, 2001).

Infants that have born premature have an increased incidence of cerebral palsy, neurological handicap and pulmonary disorders. Swedish and British data showed that the incidence of cerebral palsy in LBW and premature infants has raised nearly three fold since mid 1960s (Crawford et al, 1992). Additionally, the incidence of LBW, perinatal mortality and handicap are greater in lower socioeconomical populations (Crawford et al, 1989). Hence, inadequate nutrition, including arachidonic (AA) and docosahexaenoic (DHA) acids during pregnancy may be an important cause of LBW.

1.8.2. Diseases of prematurity

Pathophysiological and medical complications in premature infants involve several diseases in preterm babies, such as bonchopulmonary dysplasia, respiratory distress

syndrome (RDS), hypoxic pulmonary vasoconstriction (HPV), retinopathy of prematurity (ROP), central nervous system (CNS) deficits, intraventricular haemorrhage (IVH) and cerebral pulsy (Bell et al, 1980; Saugstad, 1990; Blayney et al, 1991; Crawford et al, 1992; King & Cronin, 1993; Thureen &Hay, 1993; Yamaguchi et al, 1996).

The IBCHN data showed that the preterm and LBW infants were born with reduced cord blood AA and DHA (Leaf et al, 1992a&b; Bitsanis et al, 1999; Crawford et al, 2003) and of low antioxidant defence mechanisms (Phylactos et al, 1994). We have also reported reduced cord blood AA and DHA in relation to BWT, placental weight (wt) and head circumference, HC (Crawford et al, 1989, 1997; Leaf et al, 1992b; Crawford, 2000). A parallel study of preterm plasma long chain polyunsaturated fatty acids (LCPUFA) showed that AA was associated with BWT, whereas DHA with gestational age, GA (Leaf et al, 1992b). Furthermore, the investigation on the umbilical arteries over a wide BWT range demonstrated abnormal essential fatty acid (EFA) deficiency (20:3n-9/AA) and DHA insufficiency (docosatetraenoic-to-docosapentaenoic acid n-6 ratio; DTA/DPA n-6) indexes (Crawford et al, 1989).

Hence, nutritional deficits during fetal and early postnatal life would impair blood vessels development with long term consequences (Crawford et al, 1997). In addition, neonatal erythrocytes have reduced membrane fluidity and associated with shortened life and susceptibility to oxidative stress (Jain, 1989). Therefore, EFA and antioxidant deficits, important for cell membranes, particularly the endothelium and neural tissues could provide the biochemical prerequisite for the membrane disorders to which preterm babies are at high risk.

1.8.2.1. Bronchopulmonary dysplasia and respiratory distress syndrome

Bronchopulmonary dysplasia is a frequent sequela of acute lung injury in the preterm infants (Blayney et al, 1991), characterised by the development of a hyaline membrane (Thureen &Hay, 1993). Bronchopulmonary dysplasia can be developed by exposure to high concentrations of oxygen and decreased availability of antioxidant substrates due to fasten ventilation (Spahr et al, 1980). Studies in rabbits suggested that the development of the hyaline membrane in the lung was due to exposure to oxygen resulting in increased permeability of the blood capillaries in the lungs (Saugstad, 1996). Once the endothelium is infected, the injured lung becomes stiff and fibrotic with reduced lung compliance. Consequently cells become smaller in size and number resulting in apoptosis (Saugstad, 1990).

Bronchopulmonary dysplasia is a multifactorial disease causing lung damage in the premature infants (Spitzer et al, 1981). In general, it is associated with increased energy expenditure, chronic hypoxia, metabolic disturbances after admission of therapeutic drags, decreased gastrointestinal absorption of nutrients, and congestive heart failure (Thureen &Hay, 1993). The disease involves peroxidation of AA to prostaglandins (PG), leading to inflammation and oedema (Frank, 1982; Saugstad, 1990; Sanders et al, 1994; Crawford et al, 1997). The cause of leakiness is considered to be a deficiency of surfactant, which is dipalmityl membrane phosphoglyceride (Crawford et al, 1997).

Nonetheless, the complexity of the pathogenesis of bronchopulmonary dysplasia may also suggest a relationship between fluid administration and increased risk of respiratory distress syndrome (RDS) in the premature infants (Brown et al, 1978) in association with other edemagenic factors like oxygen toxicity, mechanical trauma to the lung, and congestive heart failure (Brown et al, 1978; Bell et al, 1980). Abnormal water metabolism may be associated with the pathogenesis of pulmonary dysfunction in infants with respiratory distress syndrome, RDS (Green et al, 1983). Renal, cardiac, vascular and pulmonary causes are a possible explanation for such relationship (Green et al, 1983; Costarino et al, 1985).

The association of EFA deficiency with lung disease and high risk of respiratory problems it is well-established. EFA deficiency is associated with lung dysfunction and further impairment of Bronchopulmonary dysplasia in infants with RDS, including abnormalities in platelets function and bleeding (Thureen &Hay, 1993).

1.8.2.2. Hypoxic pulmonary vasoconstriction

Studies in rats demonstrated that there is an increased and prolonged reactivity of rat pulmonary vasculature to hypoxia in the presence of red blood cells, RBC (Yamaguchi et al, 1996). Hypoxic pulmonary vasoconstriction (HPV) may act as a regulator of the distribution of pulmonary blood flow maintaining the levels between ventilation and blood flow in the lung. HPV is regulated by reactive oxygen species (ROS). In the presence of RBC, HPV can be restored after exposure to high ROS, involving antioxidant mechanisms operating within RBC (Yamaguchi et al, 1996). RBC are rich in enzymes against oxidation including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). These enzymes increase blood viscosity and vascular resistance but only SOD protects against hypoxia regardless the status of oxidative stress (Yamaguchi et al, 1996).

1.8.2.3. Retinopathy of prematurity

Retinopathy of prematurity (ROP) has been described as the first disease in the entity of the neonatal diseases of prematurity (Saugstad, 1990). ROP was first discovered in 1940s and early 1950s and identified as retrolental fibroplasia as a result of high concentrations of O_2 used for treatment (Ng et al, 1988). The pathogenesis of ROP involves two phases: a) the vaso-obliteration phase that distracts the growth of retinal capillaries and b) the vasoproliferative phase characterised by an irregular sponge-like vascular pattern (Gobel & Richard, 1993).

The most significant factors involved in the pathogenesis of ROP are the ventilation time, xanthine administration, BWT, GA, and maternal bleeding (Charles et al, 1991). Sepsis was significant only in severe ROP in association with IVH, RDS, blood transfusion, and longer ventilation time (Charles et al, 1991). The strong association between ROP, bronchopulmonary dysplasia, CNS deficits, LBW, and intraventricular haemorrhage (IVH) suggest that the key component among the variables may be the high O_2 administration during ventilation (Brown et al, 1990). It is now evident that ROP is a common disorder of the preterm and LBW infants, resulting in blindness due to peroxidation of DHA (Ng et al, 1988; Gobel & Richard, 1993). DHA is known to have a specific function in the photoreceptor neuromembranes of the retina (Neuringer et al, 1984).

1.8.2.4. CNS deficits

The risk of developing CNS deficits rises sharply as BWT falls below 1.5Kg (Crawford, 1992; Crawford, 2000). Brain lipids contain PUFA which play an important role in modulating the structure, fluidity, and brain membranes. More than 60% of the brain fatty acids (FA) are polyunsaturated fatty acids (PUFA) with prevalence of AA and DHA. LBW is strongly associated with CNS in relation to n-3 and n-6 PUFA deficiency (Leaf et al, 1993). EFA deficiency in the rat model during rapid brain cell division and growth resulted in reduced brain wt and cell number in association with impaired learning ability (Neuringer et al, 1986).

1.8.2.5. Intraventricular haemorrhage

The incidence of major neurodevelopmental disability in infants born premature is between 11-35%. The most common complication of prematurity in relation to neurodevelopmental disorders is the outcome of either intra-ventricular or peri-ventricular haemorrhage (Leaf et al, 1993; Crawford, 1992). Intraventricular haemorrhage (IVH) is a

severe complication of premature infants in association with significant increased in the incidence of eye disease, leading to up to 38% visual loss (King & Cronin, 1993). Cerebral perfusion is greatly decreased in severe IVH due to optic atrophy which may be caused by an increase in the pressure effect on the optic nerve (King & Cronin, 1993).

AA and DHA are key components of neural and vascular membranes and, hence, deficits of AA and DHA would be expected to lead to loss of the membrane integrity manifested by haemolysis and haemorrhage (Crawford, 1992). Preliminary data also suggested that vitamin E supplementation can reduce the incidence of IVH in preterm babies (Thureen &Hay, 1993). Whilst, deficits in vitamin E along with deficits in membrane lipids n-3 PUFA induce haemorrhage in the chick brain (Crawford et al, 1997).

1.8.2.6. Cerebral palsy

Studies on British and Sweden babies showed a severe increase in the incidence of cerebral palsy in preterm infants (Pharoah et al, 1990). In Western Australia, the rate of cerebral palsy in infants under 1500g rose significantly from 12.1 in 1968 to 64.9 in 1985 (Stanley & Watson, 1992). Birth asphyxia was not the main cause which implies that the increased survival of LBW infants resulted in more cerebral palsy due to either postnatal complications of immaturity or prenatal damage to the fetal brain (Stanley & Watson, 1992).

The integrity and function of the brain depends mainly on the profile of membrane lipids and their FA (Crawford et al, 1992). Animal studies demonstrated that depletion of EFA utilised by the brain result in the loss of membrane integrity, cell DNA, and cell function, suggesting similar effects on the preterm babies (Pharoah et al, 1990; Crawford et al, 1992). The functional effects include neurodevelopmental deficits in visual and cognitive development as well as in life span (Pharoah et al, 1990). It is also conceivable that inadequate provision or imbalance between AA and DHA (such as the Western diet) during brain development to affect visual development and may lead to haemorrhage with inflammation, ischemia and tissue destruction (Crawford et al, 1997). The ischemic lesion periventricular leucomalacia, a antecedent of cerebral palsy, results in large-scale release of AA with exacerbating vasoconstrictive consequences (Crawford et al, 1997).

1.8.3. Placental essential fatty acid transfer and prematurity

The placenta selectively transfers AA and DHA from maternal circulation to the fetal circulation. In human mid-pregnancy the proportions of long chain polyenoic derivatives of

the parent EFA gradually increase with the phospholipid content of the fetus (Uauy et al, 1989). The n-3 and n-6 long chain polyunsaturated fatty acids (LCPUFA) are required in relatively large amounts during foetal and early postnatal development for the synthesis of prostaglandins and other eicosanoids and for disposition in membrane lipids of the fast growing brain and other tissues (Koletzko, 1992). Brain growth is associated with increased incorporation of LCPUFA into the phospholipid primary located in the cerebral cortex, indicating that no blood - brain barriers exist to FA transfer (Farquharson et al, 1992).

Both AA and DHA are used for neural membrane structure and function. A good example is the photoreceptor that requires EFA for functioning and antioxidant enzymes (Cu/Zn-superoxide dismutase) for antioxidant protection of membrane integrity (Phylactos et al, 1994). In addition, DHA provide a specific structural environment within the phospholipid bilayer that influences important membrane functions such as ion or solute transport receptor activity and enzyme activity (Uauy-Dagach & Mena, 1995). The n-3 PUFA are of special interest because they recognised as antiatherogenic and essential nutrients (Anderson et al, 1990).

In preterm infants the availability of DHA is related to development of visual function (Desci et al, 1995) and that of AA to body growth (Desci et al, 1995; Ghebremeskel et all, 1995). The lack of desaturase activity in human placenta microsomes at the 18-22 wk of gestation suggests that the fetal AA is either transferred directly from the mother or synthesised in fetal liver by transferred linoleic acid, LA (Coleman, 1989; Salem et al, 1996). However, postnatally, AA drops to a third of the intrauterine level despite that its precursor LA rises more than 3-4 folds, suggesting that the rate of conversion of LA and AA is too slow to meet the high demands for membrane growth or LA is adversely replacing AA (Leaf et al, 1992b). In addition, deficiencies of n-3 PUFA lead to disturbances in behaviour, impaired vision, and abnormal electroretinograms (Anderson et al, 1990). There is evidence that the LBW infants and preterm infants are exposed to deficits of unsaturated FA which may imply that maternal malnutrition could result in placenta malfunction and, consequently, intrauterine malnutrition (Crawford et al, 1997).

1.9. Diabetes Mellitus

The vast majority of diabetic cases fall into two broad etiopathogenic categories, type 1 and type 2 Diabetes Mellitus (DM). Type 1 or insulin-dependent Diabetes Mellitus (IDDM) or juvenile-onset diabetes, results for a cellular mediated autoimmune destruction of the β-cells of the pancreas leading to absolute insulin deficiency (Committee report, 1997). Type 2 or non insulin dependent Diabetes Mellitus (NIDDM) or adult onset diabetes is a term used for individuals who have insulin resistance (IR) and usually have relative insulin deficiency (Committee report, 1997). The risk of developing type 2 diabetes increases with age, obesity and physical inactivity (Mazzone, 2000). NIDDM occurs in women with prior gestational Diabetes Mellitus (GDM) and in individuals with hypertension or dislipidemia and its frequency varies in different racial/ethnic groups (Cordero & Landon, 1993; Dahri et al, 1995; Committee report, 1997).

GDM is defined as any degree of glucose intolerance with onset first recognised during pregnancy (Committee report, 1997; Wijendran et al, 1999; Butte, 2000). GDM has also been defined by the Third Workshop Conference on GDM as a carbohydrate intolerance of varying severity with onset or first recognition during pregnancy, irrespective of the glycemic status after delivery (Kuhl, 1998). The definition applies regardless of whether insulin or only diet treatment was used or if the condition persists after pregnancy (Committee report, 1997; Kuhl, 1998). GDM is a heterogeneous disorder in which age, obesity, and genetic background contribute to the severity of the disease (Kuhl, 1998; Butte, 2000). GDM bears close resemblance to NIDDM and hence, GDM could be considered as an "early onset" NIDDM (Kuhl, 1998).

GDM is a disease of autoimmune origin and there is evidence to suggest that it occurs as a result of a combination of insulin resistance and a diminished insulin secretion (Kuhl, 1998). On the other hand, Bartha et al (2000) reported that impairment of insulin secretion and action may act conjointly or separately and it is not know yet, which of the two is the main cause of GDM. Additionally, the pathogenesis of GDM differs between lean and obese diabetic, suggesting that lean GDM have a relative insulin deficiency in contrast to obese GDM, who manifest insulin resistance and hyperinsulinemia (Bartha et al, 2000).

1.9.1. Epidemiology

The prevalence of DM may be 3-5% in Western population, but may get to 50% in specific populations (i.e. Nauruan or the Pima Indians) and 15-20% in Hispanics and Afro-Americans (Dahri et al, 1995). Impaired glucose tolerance is strikingly more common in women than men. Between 21-39 years of age, 11-20% of many populations world wide may be afflicted by impaired glucose tolerance. In some populations such as female Muslim Asian Indians it may reach 32% (Dahri et al, 1995).

In pregnancy, DM is one of the most common maternal illnesses that result in anomalous offspring. It has been estimated that 0.2-0.3% of all pregnancies are complicated by pre-existing diabetes (Cordero & Landon, 1993) and 1-5% by GDM (Cordero & Landon, 1993; Wijendran et al, 1999). Pregnancy-induced IR and GDM are generally reversible after pregnancy, however, approximately 30-50% of women with history of GDM may develop Type 2 DM later in life, particularly the obese women (Wijendran et al, 1999; Shao et al, 2000).

The frequency of major congenital anomalies has been estimated 6-10%, which represents 2-3-fold increase over the frequency in the general population (Reece et al, 1993). Infants born to IDDM have a 2-8 times higher risk of having major congenital malformations (Baker & Piddington, 1993; Cordero & Landon, 1993). Disorders of fetal growth (40%), hypoglycaemia (20%), prematurity (15%), intrapartum asphyxia and respiratory distress (15%) are some of the clinical problems affecting Type 1 DM or IDDM (Cordero & Landon, 1993). Congenital malformation in the offspring accounts for approximately 40% of perinatal deaths (Reece et al, 1993). The offspring of GDM women have grater incidence of perinatal complications and a higher prevalence of obesity and diabetes (Shao et al, 2000). Women who were diabetic, obese and postmature had Increased risk for macrosomia (Cordero & Landon, 1993). Intrauterine growth retardation (IUGR) has been seen in 3-7% to normal pregnancies and up to 20% of diabetic pregnancies. Similar rates for SGA infants have been observed among offspring of GDM and IDDM (Cordero & Landon, 1993).

The most predominant is Type 2, affecting more that 90% of all diabetics, because of IR having the primary role in the development of NIDDM (Phillips, 1998). Insulin is known to enhance lipogenesis and both NIDDM and obesity are characterised by hyperinsulinemia (Nutrition Reviews, 1991). Obese people are also at a greater risk of developing cardiovascular disease, CVD (Butte, 2000).

1.9. 2. Clinical manifestations

DM is a group of metabolic diseases characterized by hyperglycaemia, resulting from deficits in insulin secretion, insulin action or both (Committee report, 1997; Coulston, 2000). DM is a common metabolic disorder that affects carbohydrate, protein and fat metabolism (Committee report, 1997; Coulston, 2000). Patients with diabetes may experience a variety of serious complications, such as microvascular (retina or kidneys), neuropathic (sensory, motor, or autonomic) and macrovascular complications (Labrouche et al, 1996; Coulston, 2000; Mazzone, 2000). Diabetics have an increased incidence of peripheral vascular, cerebrovascular, and cardiovascular diseases including hypertension and myocardial infarction (Dahri et al, 1995; Committee report, 1997).

Glucose tolerance deteriorates in both normal and GDM pregnancy despite of the increase in insulin secretion, indicating that pregnancy is an insulin-resistance state (Kuhl, 1998; Butte, 2000). Still, maternal complications related to GDM also include an increased rate of caesarean delivery and chronic hypertension (Committee report, 1997).

1.9.3. Insulin resistance

Impaired insulin action is central to a cluster of prevalent diseases including NIDDM, obesity, hypertension, dyslipidemia and CVD (Pan et al, 1995; Mazzone, 2000). Insulin resistance (IR) is also associated with altered function of vessel wall cells. Increased adherence to endothelial cells was demonstrated from mononuclear cells in IDDM subjects (Mazzone, 2000). Moreover, GDM associated with hypertension is characterised by high IR (Bartha et al, 2000). However, considering the reciprocal changes in insulin sensitivity and β-cell function that normally accompany late pregnancy, mild gestational diabetes during the third trimester is characterised by impairment of pancreatic β-cell function rather than an exaggeration of the normal IR of late pregnancy (Buchanan et al, 1990).

To some extend IR is regulated by the perixosome proliferators-activated receptors (PPARs), which regulate adipocyte differentiation and gene expression, and FA (particularly n-3 PUFA) are regulators of these nuclear receptors (Ryan et al, 2000). In addition, muscle membrane bound fatty-acid binding proteins increase with obesity (Bloomgarden, 2000).

1.9.3.1. Obesity-associated insulin resistance and chronic diseases

Obesity is associated with two important cardiovascular risk factors, IR and hyperinsulinaemia (Boden, 1998). Free fatty acids (FFA) are elevated in most diabetic subjects and induce IR in many organs (Grill & Ovigstad, 2000). Peripheral IR was evident in obese healthy and diabetic subjects due to elevated plasma FFA levels and hepatic IR in diabetics and possibly in obese healthy (Boden, 1998; Patti, 1999). Peripheral IR is defined as inhibition of normal insulin stimulation of whole body glucose uptake and hepatic IR as inhibition of normal insulin suppression of hepatic glucose production (Boden, 1998). Because, peripheral insulin sensitivity is mainly determined by the degree of insulin-stimulated glucose uptake in the skeletal muscles (Vessby, 2000), it suggests that the skeletal muscle is the main determinant of IR (Pan et al, 1995; Vessby, 2000).

Randle's observation that fatty acids (FA) compete with glucose for oxidation led to the hypothesis that increased fat oxidation can cause IR in skeletal and cardiac muscle (Patti, 1999). In the muscle, there is a marked decrease in glycogen formation with high FFA levels and a lesser decline in glucose metabolism (Bloomgarden, 2000). However, the muscle oxidative capacity increases with increasing insulin sensitivity and decreases with increasing muscle adiposity. In diabetes co-existing with obesity, basal fat oxidation is decreased resulting in hyperglycaermia-induced muscle glucose oxidation (Bloomgarden, 2000). On the other hand, that the FFA inhibit insulin suppression of hepatic glucose production is controversial (Boden, 1998). Hepatocytes from obese subjects synthesise more FA than those from lean subjects, but there were no differences in de novo synthesis due to NIDDM (Nutrition Reviews, 1991). Insulin stimulated lipogenesis by 78% in cells from lean subjects, 33% in cells from obese and 17% in cells from obese subjects with NIDDM (Nutrition Reviews, 1991).

In contrast, in the human pancreatic cells, glucose-induced insulin excretion is coupled to fatty acid oxidation in β-cells, with ensuing reduction in glucose oxidation, in parallel with decreased pyruvate dehydrogenase enzyme (Grill & Ovigstad, 2000). Whilst, acute elevation of FFA cause 50% greater insulin secretion with increased insulin levels, hence, chronic increase of FFA "disables β-cell compensation" (Bloomgarden, 2000).

1.9.3.2. High fat diet (Western type of diet)

High-fat diet is associated with impaired insulin sensitivity and increased risk of developing diabetes, independent of obesity and body fat localisation. In addition, this risk may be influenced by the type of fatty acids (FA) in the diet (Vessby, 2000). High proportion of saturated fatty acids (SFA) in the membrane may impair insulin action by altered insulin receptor binding/affinity and ability to translocate/insert glucose transporters, changes of phospholipids FA-interaction with function of second messenger and leaky membranes (Pan et al, 1995; Kuhl, 1998; Boden, 1998; Ryan et al, 2000; Vessby, 2000). Increased saturation of the membrane FA and reduced Δ-5 desaturase activity have been associated with IR (Vessby, 2000). Similarly, Pan et al (1995) showed that both impaired insulin action and obesity are independently associated with reduced Δ-5 desaturase activity, but increased adiposity was found to be associated with reduced elongase activity and higher Δ-9 desaturase activity.

Compelling data in support of environmental influences have arisen from studies of Japanese, Pacific Islanders and Pima, populations in which the incidence of IR and DM is markedly higher among populations adopting a "Westerrn" lifestyle and diet, despite similar genetic backgrounds (Patti, 1999). These data support that excessive nutrient intakes and a positive energy balance may play a role in the maintenance of IR in these genetic predispose individuals.

Moreover, insulin sensitivity is potentially enhanced by a range of diet-related changes including a reduction of visceral adiposity (the most important determinant of IR) and SFA, and a redistribution of the proportions of n-3/n-6 PUFA (Mann, 2000). Indeed, long-chain SFA, predominantly palmitic acid appear to stimulate insulin release more potently than unsaturated fatty acids (Grill & Ovigstad, 2000). Also, the n-6/n-3 ratio might be important in determining insulin sensitivity (Vessby, 2000). This is in accordance with the Lyon Heart study that demonstrated that over a period of 5 years it is possible to significantly reduce stearic and increase oleic acid, ALA, n-3/n-6 ratio and EPA to achieve long-term changes in the nature of dietary fat to achieve weight (wt) reduction and, consequently, would enhance insulin sensitivity (Mann, 2000).

Furthermore, experimental diabetes may manifest itself in a defect in liver microsomal FA desaturation and increased activity of glucose-6-phosphatase, however, dietary fat manipulation has the potential to change at least some of the abnormalities in the microsomal membrane (Venkatraman et al, 1991). Feeding rats on fish oil diet reduced cholesterol and

triacylglycerol (TG) levels (Venkatraman et al, 1991). Additionally, feeding diabetic rats a high PUFA/SFA improve insulin binding to adipocytes (Field et al, 1990). The beneficial effect of dietary fish oil on insulinemia, plasma lipids and insulin-stimulated glucose metabolism in IR slightly diabetic rats has been demonstrated (Luo et al, 1996).

1.9.3.3. Fatty acids and gene expression in diabetes

In NIDDM, chronic exposure to insulin represses glucose transporter type 4 (GLUT4) mRNA and protein abundance (Tebbey et al, 1994). In the muscle, FFA may cause IR by inhibiting CLUT 4 gene expression (Boden, 1998). In addition, a high-fat diet down-regulates the expression of the insulin-sensitive CLUT4 in both white and brown adipose tissue (Holness & Sugden, 1999). Clarke (2000) also reported that the impaired glucose metabolism in association with ingestion of fat appear to be the cumulative product of impaired translocation of GLUT4 activity, suppressed expression of GLUT4, inhibited expression of hepatic glycolytic and lipogenic enzymes and impaired insulin signalling.

In contrast, LCPUFA decrease mRNA levels of the insulin responsiveness glucose transport GLUT4 in fully differentiated 3T3-L1 cells by decreasing GLUT4 gene transcription and by destabilising the GLUT4 message (Boden, 1998). PUFA exert their effects on lipid metabolism and thermogenesis by upregulating the transcription of the mitochondrial uncoupling protein-3 an inducing genes encoding proteins involved in fatty acid oxidation, such as carnitine palmitoyltransferase and acyl-CoA oxidase while simultaneously down-regulating the transcription of genes encoding proteins involved in lipid synthesis, such as fatty acid synthase, FAS (Clarke, 2000).

On the other hand, the *in vivo* studies demonstrated that dietary n-6 PUFA could provide a nutritionally based regulation of transporter gene expression (Tebbey et al, 1994). In addition, many cells possess a high affinity arachidonyl-CoA synthase which facilitates selective accumulation of AA even when other FA are in excess (Tebbey et al, 1994). The AA-induced near total loss of GLUT4 protein coupled with the decreased translocation potential, markedly diminishes the ability of the cell to respond to insulin through utilisation of GLUT4, indicating that AA partially mimics tumor necrosis factor-α and insulin action after chronic supply to 3T3L1 adipocytes (Tebbey et al,1994).

In contrast, the depressed activity of the regulatory enzymes Δ-6 and Δ-5 desaturases by streptozotocin (STZ)-induced diabetes altered the FA composition of various tissues with a

decrease in AA (Rimoldi et al, 2001), which may account as much as 25% of total phospholipid fatty acids. In experimental diabetes, the defect in rat liver microsomal Δ-6 desaturation activity is accompanied by a sevenfold reduction in Δ-6 desaturase mRNA abundance (Rimoldi et al, 2001). Similarly, in human diabetes, suppression of Δ-6 has been reported (Shin et al, 1995). In IDDM it was suggested that two factors are involved in the hepatic Δ-6 desaturase mRNA decrease; the shortage of insulin and the increase in glucagon (Rimoldi et al, 2001). Insulin administration to diabetic rats induces Δ-6 desaturase mRNA within 24h in a fashion similar to Δ-9 desaturase (stearoyl-CoA-desaturase), whereas glucocorticoids, testosterone and estradiol and other hormones depress Δ-6 and enhance Δ-9 desaturase activity (Rimoldi et al, 2001). Conversely, insulin treatment restores FA composition earlier in the liver than red blood cell membranes in STZ- rat (Shin et al, 1995).

1.9.4. Endothelium-mediated vascular dysfunction in diabetes

Endothelium-mediated vascular dysfunction in diabetes is a result of diminished nitric oxide (NO) formation, however, it is important to mention the eicosanoid production by diabetic endothelial cells (Wardle, 1995). Vascular diseases in diabetes has been linked to enhanced production of eicosanoids and the AA metabolites of the lipoxygenase (LO) pathway possibly participate in the diabetes-related complications. Increased 12-hydroxyeicosatetraenoic acid (12-HETE), a known proinflammatory eicosanoid, was reported in diabetic rats and in aortae of atherosclerotic rabbits (Sasson et al, 1999).

Atherosclerosis begins with injury to the vascular endothelium, and the resultant exposure of collagen induces platelet adhesion. As platelet aggregation progresses, thromboxane A_2 (TXA_2), platelet-derived growth factors, and leukotrienes are released (Malasanos & Stacpoole, 1991). TXA_2 induces further platelet aggregation and vasoconstriction, whereas leukotrienes attract neutrophiles and monocytes. Moreover, the correlation between circulating insulin and systolic blood pressure may be mediated by the inhibitory effect of insulin on the production of vasodilatory prostaglandins (PG) products of AA by adipose tissue (Chatzipanteli et al, 1996). A reduction in AA and other n-6 PUFA in membrane PL have been reported in the non-pregnant models of IR (Wijendran et al, 1999). Platelets may have a specific defect of prostaglandin E_1 (PGE_1) synthesis quite distinct from the Δ-5 and Δ-6 desaturase defects known to be associated with experimental diabetes and this defect may contribute to platelet hyper-agreeability in diabetes (Mikhailidis et al, 1986).

1.9.5. Diabetic pregnancy and vasculopathy

1.9.5.1. Maternal vasculopathy

The vascular endothelium maintains blood fluidity under the control of selective permeability of substances through the vascular wall and physiological chemical reactions on the cell membrane (Takeda & Kitagawa, 1992). Vascular wall AA metabolism may contribute to the significant decrease in myometrial blood flow (Takeda & Kitagawa, 1992). As uterine blood flow influences fetal growth, the significant decrease in fetal wt follows but with higher fetal-to-placental wt ratio in the pregnant STZ- rats at 20d (Takeda & Kitagawa, 1992). In general morphological changes may be due to cell membrane damage or mitotic abnormalities, however, such morphological changes are related to changes in cell membrane ion permeability and fatty acid metabolism (Takeda & Kitagawa, 1992). On 20d of gestation, in the diabetic rat the endothelial cell surface show many small holes and microvilli appeared with unclear intracellular junctions, suggesting possible transport disturbances in this area (Takeda & Kitagawa, 1992). The appearance of vascular change in pregnant IDDM may be caused at least by alterations in the AA metabolism of the vascular wall (Takeda & Kitagawa, 1992).

1.9.5.2. Embryopathy

In vivo and in *vitro* animal studies showed that hyperglycaemia has a teratogenic effect during organogenesis (Reece et al, 1993). Congenital malformations result form maternal metabolic abnormalities during the first 6-7 wk of gestation (Goldman & Goto, 1991; Buchanan & Kitzmiller, 1994). This effect is related to defect in yolk sac endodermal cells characterised by reduced rough endoplasmic reticulum, ribosomes, and mitochondria suggesting that hyperglycaemia during organogenesis has a primary deleterious effect on yolk sac function and embryopathy (Reece et al, 1993; Eriksson, 1995).

Although hyperglycaemia during the first trimester can lead to congenital malformation of different organs, the development of the heart and neural tube appears to be the most sensitive (Dhanasekaran et al, 1999). The precise mechanism responsible for abnormal fetal organogenesis is unclear, but fuels such as sugars (glucose, galactose, mannose), ketones, fuel-related principles such as somatomedin inhibitors, insulin, trace elements, and lately, myoinositol, AA, PG and free oxygen radicals have all been implicated (Baker & Piddington, 1993; Goto & Goldman, 1994; Eriksson, 1995; Hod, 1996; Wiznitzer et al, 1999).

The deficiency of AA produced by *in vivo* and *in vitro* hyperglycaemia appears to be either due to an altered intracellular embryonic AA metabolism or decreased extracellular AA availability (Engstrom et al, 1991; Goldman & Goto, 1991; Goto & Goldman, 1994). Similarly, a deficient level of tissue and embryo myoinositol due to high glucose and/or polyol levels, apparently results in a deficient phospholipid (PL) turnover with an ensuing inhibition of protein kinase C, PKC (Goldman & Goto, 1991). Myoinositol is a vital precursor of cell signalling effectors, leading to formation of phosphoinositides and diacylglyceros (DG) through the partially hydrolysis of phospholipids (Baker & Piddington, 1993; Hod, 1996). DG is involved in the maintenance of Na/K-ATPase activity, as mediated by the enzyme PKC, which is thought to convert ATPase from the inactive to the active form (Hod, 1996).

The hypothesis is that normal development of various structures including the palate, neural tube, genitalia and heart, requires signal trasnduction-stimulating inositol phosphoglycerides (IPG) turnover coupled to the AA cascade leading to PG production (Goldman & Goto, 1991; Baker & Piddington, 1993). Several evidence support the "myoinositol deletion hypothesis" as well as the implication of the AA cascade in the mechanisms of the diabetic embryopathy (Baker & Piddington, 1993). Firstly, supplementation with myo-inositol can restore the membrane phospholipids integrity that is depleted by hyperglycaemia (Wiznitzer et al, 1999). Also, the significant protective effects of myoinositol supplementation against the teratogenic effects of a high glucose concentration on neural tube fusion can be reversed by indomethacin, an inhibitor of AA metabolism (Baker & Piddington, 1993).

Secondly, AA supplementation protected against hyperglycaemia-induced teratogenesis, both *in vitro* and *in vivo* (Baker & Piddington, 1993). Similarly, supplementatin with AA prevented the teratogenic effects of hyperglycaemia on cultured mouse embryos and decreased the incidence of anomalies in the offspring of STZ-treated diabetic rats (Pinter et al, 1988; Goto & Goldman, 1994). Also, in experimental animals, administration of AA, PG, or Vitamin C or Vitamin E reduced the incidence of diabetic embryopathy (Dhanasekaran et al, 1999). Thirdly supplementation of prostaglandin E_2 (PGE_2), to high glucose culture medium protected against embryonic malformations. Lastly PGE_2 levels in mouse embryo are high around the time of neural tube fusion and then decrease, whereas in the embryos of diabetic mice, these developmental changes are blunted (Baker & Piddington, 1993).

1.9.5.3. Placental nutrient transfer in Diabetes Mellitus

Several of the physiological hypotheses describing the specific development of the mother and offspring in diabetic pregnancy, such as the "hyperglycaemia-hyperinsulinaemia" theory and the "fuel-mediated teratogenesis" concept, postulated major alterations of the maternal fetal transport of nutrients (Eriksson, 1995). Indeed, it is generally assumed that the fetus receives increased amounts of glucose, but also alterations in the transfer of lipids and amino acids from the diabetic mother have been postulated. A fundamental functional parameter for nutrient transport is the establishment of a proper uteroplacental blood flow, which is reduced in diabetic pregnancies in association with increase maternal glycaemia (Honda et al, 1990; Eriksson, 1995).

In the severely diabetic animals the profound reduction in free fatty acids (FFA) transfer, secondary to the reduction in the uteroplacental blood flow, may lead to inadequate supplies of essential fatty acids (EFA) for fetal growth (Honda et al, 1990). This EFA profile is in contrast to the transfer of glucose across the placenta, which is not decreased in concert with the decreased uteroplacental blood flow (Honda et al, 1990; Thomas & Lowy, 1992). Hence the fetus of the diabetic mother may be exposed to a considerable imbalance of nutrients. In contrast, Goldstein et al (1985) reported an increase specifically in linoleic acid (LA), which suggests that the rat may have developed a selective mechanism for the placental of EFA to ensure fetal growth. On the other hand, induction of diabetes at 13-14d of gestation in Goldstein's study may be insufficient to induce significant changes in the placental function. Moreover, the reduced body wt in the STZ-pregnant rats coincides with the reduction in fetal plasma insulin levels, and the overall picture contrasts with the macrosomia and hyperinsulinemia normally found in infants of diabetic women (Herrera et al, 1985). However, prevention of fetal macrosomia and hyperinsulinism in diabetic rats may be a consequence of the incapacity of rat fetuses to store fat before birth even after insulin treatment, as well as of the shorter period of gestation compared with humans (Herrera et al, 1985).

In contrast, hypertriglycaemia, a feature of GDM, a good example of *in utero* programming (Phillips et al, 1998) and Type 2 DM (Knopp et al, 1986; Butte, 2000), occurring in late gestation in both human and rat pregnancy, may increased FA transfer across the placentas, since tracylglycerols (TG) transport may still be possible though the permissive, and sustained activity of placental lipoprotein lipase, LPL (Knopp et al, 1986). Pregnancy in IDDM is associated with a greater incidence of fetal abnormality and animal studies suggest that increased free radical production and antioxidant depletion may contribute to this risk (Bates et al, 1997). Whilst, GDM, known to affect fetal development in both animals and humans,

does not modify the mechanisms of placental transfer of metabolic fuels to the fetus and that the actual transfer is mainly dependent on the concentrations of these fuels in the maternal circulation (Herrera et al, 2002).

The metabolic programming may arise from defects in the materno-placental support of the fetus. A key factor may be the control exerted by the placenta on the influence of the maternal glucocorticoid environment on the fetus (Holness et al, 2000). The fetal adrenal gland becomes active in late gestation, and separation from maternal adrenal influences is particularly important to enable independent function of the fetal hypothalamic-pituitary-adrenal-axis, HPA (Holness et al, 2000). Intracellular glucocorticoids receptors are expressed in most fetal tissues form mid- gestation onwards, including liver and adipose tissue, whose structure and function in adult life is affected by early life programming events (Holness et al, 2000).

1.10. Fetal programming

The concept of the "fetal origin of adult disease" derived from the first epidemiological studies indicating that adverse environmental factors in early life could disrupt normal growth and development, leading to a more susceptible phenotype prone to cardiovascular disease, CVD (Barker et al, 1990, 1993), However, poor nutrition in fetal and early infant life is detrimental to the mechanisms maintaining carbohydrate tolerance (Phillips, 1998). Additionally, low birthweight (LBW) is associated with higher prevalence of the metabolic syndrome (the coexistance of raised blood pressure, glucose intolerance and dyslipidemia), which in turn is associated with insulin resistance, IR (Holness et al, 2000). The diabetic state alters the expression of key growth vascular growth factors contributing to the development of macrovascular and microvascular complications (Mazzone, 2000). Hence, the "thrifty phenotype hypothesis" may be a more satisfactory explanation of the epidemiology of NIDDM (Phillips, 1998).

The "thrifty phenotype hypothesis" specifically proposed that those individuals of LBW are characterised by early adaptations to a potentially adverse intra-uterine environment that optimise the use of restricted nutrients supply to ensure survival, but by favouring the development of certain organs over that of others that would lead to persistent alterations in the physiology and metabolism of the developing tissues (Holness et al, 2000). Thus, abnormal early growth, as indicated by thinness at birth has been shown to predict impaired

insulin action in adult men and women with the greatest IR being observed in the individuals who were thin at birth but obese as adults (Holness et al, 2000).

1.10.1. Fetal Insulin Hypothesis

The fetal insulin hypothesis offers an alternative explanation to intrauterine programming in response to maternal malnutrition for the consistent association between impaired fetal growth and IR during life and the link with hypertension and vascular disease (Hattersley & Tooke, 1999). This hypothesis suggests that LBW and measures of IR and, ultimately, glucose intolerance, diabetes and hypertension could all be phenotypes of the same insulin-resistant genotype (Hattersley & Tooke, 1999). Hence the predisposition to NIDDM and vascular disease is likely to be the result of both genetic and fetal environmental factors. (Hattersley & Tooke, 1999).

1.10.2. Fetal programming of low-protein-normal energy diet during pregnancy

Supportive evidence from epidemiological studies on the "Fetal origins of adult diseases" (Barker et al, 1990) implied that fetal nutritional deprivation is a strong programming stimulus (Phillips, 1998). This prompted to the development of various experimental animal models to understand the mechanisms underlying this developmental programming. The rat studies indicated that maternal undernutrition during pregnancy reduces fetal growth and is associated with the development of diseases, like type 2 diabetes, hypertension and CVD later in life (Snoeck et al, 1990; Woodall et al, 1996; Desai et al,1996; Ozanne et al, 1998; Sherman & Langley-Evans, 1998; Garofano et al, 1999; Fernandez-Twinn et al, 2003). The low protein dams had an excess of circulating FFA, suggesting that the excess FFA in maternal circulation could programme the previously reported reduction in Δ-5 desaturase activity in the offspring (Ozanne et al, 1998; Fernandez-Twinn et al, 2003). Hence, this could be a possible mechanism linking fetal growth retardation to insulin resistance (Ozanne et al, 1998).

Furthermore, it was demonstrated that LBW due to maternal nutritional deprivation is associated with raised blood pressure in adulthood (Woodall et al, 1998). Whilst, early administration of an angiotensin-converting enzyme inhibitor, captopril in rats with raised blood pressure, associated with fetal exposure to maternal low protein restriction, could prevent the development of fully hypertensive state (Sherman & Langely-Evans, 1998).

Moreover, maternal protein restriction imposed changes in maternal levels of glucose and circulating hormones in rat pregnancy, such as an increase in glucose, insulin, prolactin, and oestradiol and a reduction in progesterone and leptin, and these changes could influence the programming of eventual adult disease in the developing fetus (Fernandez-Twinn et al, 2003). The low protein neonates showed a reduction in β-cell proliferation and a decreased pancreatic islet vascularization (Snoeck et al, 1990; Garofano et al, 1999) which often precedes glucose intolerance or impairment of insulin secretion in adult life (Garofano et al, 1999). However, the concept that early malnutrition can impair β-cell growth and function and its association with glucose intolerance would reflect malnourished children from the developing countries (Desai et al, 1996) rather matching the features of the current epidemic of metabolic syndrome.

1.10.3. High-fat diet and CVD in animal models of fetal programming

Our collaborators have used extensively the Sprague-Dawley rat, ideal for dietary manipulation studies, to investigate how exposure to an adverse *in utero* lipid environment may "program" for disease in later life. Koukkou et al (1998) showed that a high-fat diet in pregnancy led to vascular dysfunction in rats weanling and young adult offspring and the vascular function was further deteriorated in weanlings if the maternal rat was diabetic (Koukkou et al,1998). Moreover, Gerber et al (1999) demonstrated fetal programming of blood pressure in adulthood as a cause of maternal high-fat intakes. Abnormal function of the vascular endothelium is now identified with a growing number of conditions associated with arterial disease including diabetes (Taylor et al, 1994; Taylor & Poston, 1994; Koukkou et al,1998).

The low plasma cholesterol in the high-fat fed dams is characteristic of the rodent response to a saturated-fat diet, hence FFA could also play a role to this response as they interfere with FA metabolism and certain FFA are precursors of vasoactive prostanoids (Koukkou et al,1998). Therefore, the effects of high-fat intakes in diabetic and non-diabetic pregnancies on the EFA composition of various vascular tissues of the offspring will be reported in later chapters in this thesis.

1.11. Hypothesis and aims of this investigation

Aims

The aims are to:

- Elucidate the effect of diabetic and preterm pregnancy on placental phospholipid fatty acid composition

- Investigate whether maternal diabetes and a high in fat diet in pregnancy adverse effect on tissue essential fatty acids of the offspring in laboratory animal model

Hypotheses

The hypotheses are that:

1. Early (obtained from elective abortion) and term placentae have comparable arachidonic acid composition

2. Gestational diabetes mellitus (GDM) and preterm delivery (<36 gestational wk and 6d) do not compromise placental arachidonic (AA, 20:4n-6) and docosahexaenoic (DHA, 22:6n-3) content

3. Maternal diabetes and high in fat intakes independently or in synergy do not have adverse effect on tissue essential fatty acid, and particularly AA and DHA, composition of offspring of experimental rat

Objectives

The objectives are to:

- Assess the fatty acid composition of the placental choline (CPG), ethanolamine (EPG), serine (SPG) and inositol (IPG) phosphoglycerides, shpingomyelin (SPM), cholesterol esters (CE), thriacylglycerols (TG) and free fatty acids (FFA) at deliveries from healthy women, occurring early in gestation (8-14 wk) and term (37-41 wk).

- Compare the fatty acid composition of the human placental CPG, EPG, SPG, IPG, SPM, CE, TG and FFA from term GDM and preterm with healthy term pregnancies.

- Analyse liver CPG, EPG, TG and FFA and heart CPG and EPG fatty acid composition of newborn (1d) and suckling pups (15d) born to either diabetic and control dams or diabetic and control dams fed on a 32.7%fat diet (substituted with animal lard) throughout pregnancy and lactation, including liver lipids of these dams at 16d or aorta CPG and EPG fatty acids of the 160d offspring born to dams fed on either a 20%fat-diet or a control diet during pregnancy.

CHAPTER 2. METHODS

2.1. Reagents and solvents

Chloroform HiPerSolv, Methanol HiPerSolv, Methanol dried AnalaR, Petroleum spirit 40-60°C AnalaR, Diethyl ether AnalaR, Formic acid 98/100% AnalaR, Sodium chloride AnalaR and Sodium sulphate anhydrous granular AnalaR were provided by Merck Ltd, Dorset, UK; 2,7-dichlorofluorecin, Heptane 99.9% HPLC grade, Water HPLC grade, Acetyl chloride, Potassium bicarbonate, Methylamine (40% aqueous solution) and Butylated hydroxytoluene (BHT) from Sigma-Aldrich Co Ltd, Dorset, UK; Nitrogen (oxygen free) from BOC Gases, Manchester, UK.

2.1.1. Lipid standards

Analytical standards for chromatography: L-A-phosphatidylcholine Type III-L crude extract from bovine liver; L-A-phosphatidyl-L-serine from bovine brain; L-A-phoshpatidylinositol from bovine brain; sphingomyelin from egg yolk and L-A- phosphatidylethanolamine from egg yolk, Neutral lipid synthetic standards (triacylglycerols and cholesteryl palmitoleate), Fatty acid methyl ester mixture (Figure 2.1), Saturated fatty acids (tetradecanoic, hexadecanoic, octadecanoic, eicosanoic, docosanoic, tetracosanoic acids) and cis-unsaturated fatty acids (-9-hexadecenoic, -9-octadecenoic, -9,12- octadecadienoic, -9,12,15-octadecatrienoic, -11-eicosaenoic,-8,11,14-eicosatrienoic, -11,14,17-eicosatrienoic, -5,8,11,14-eicosatetraenoic, -5,8,11,14,17-eicosapentaenoic, -7,10,13,16-docosatetraenoic, -7, 10, 13, 16, 19 -docosapentaenoic, -4, 7, 10, 13, 16, 19-docosahexaenoic and -15-tetracosaenoic acids) were provided by Sigma-Aldrich Co Ltd, Dorset, UK.

2.1.2. Other materials

Thin-layer chromatography plates, Silica gel 60A°, K6, 20x20cm, thickness 250µm, for sample applications up to 100µ, filter paper (Grade 1), TLC tank (rectangular glass tank with flat walls and lid for clean viewing), 75µl disposable microhaematocrit capillary tubes were supplied by Whatman Scientific Ltd, Kent, UK; Gas chromatography column, BP20 (30m x 0.32mm i.d., 0.25µm film); TCS Septa (PTFE coated triple layer silicone) by SGE Ltd, Milton Keynes, UK; Super-Clean moisture trap by Restek, UK; 16x160mm pyrex tube fitted with a

PTFE lined screw top; Hamilton 701N (fixed) microsyringe (capacity 10μl; 51mm needle length, pack of 6) by Fischer scientific, UK.

2.1.3. Instrumentation

BüCHI rotary evaporator by ORME scientific LTD, Middleton, Manchester, UK; GC 8000 series model 8560 (HRGC MEGA 2 Series), Fisons Instruments, Italy; EZChrom, Scientific Software Inc., San Ramon, CA.

All aspects of the sampling and all analytical procedures were recorded on pre-printed forms. All instruments, workbench and glassware were disinfected before use in 1% hycolin to avoid contamination from material left behind. Fatty acid analysis was performed under the guidelines set out in laboratory codes of practices.

Butyric acid methyl ester (C4:0) 4%
Caproic acid methyl ester (C6:0) 4%
Caprylic acid methyl ester (C8:0) 4%
Capric acid methyl ester (C10:0) 4%
Undecanoic acid methyl ester (C11:0) 2%
Lauric acid methyl ester (C12:0) 4%
Tridecanoic acid methyl ester (C13:0) 2%
Myristic acid methyl ester (C14:0) 4%
Myristoleic acid methyl ester (C14:1) 2%
Pentadecanoic acid methyl ester (C15:0) 2%
cis-10-Pentadecenoic acid methyl ester (15:1) (98%) 2%
Palmitic acid methyl ester (C16:0) 6%
Palmitoleic acid methyl ester (C16:1n-7) 2%
Heptadecanoic acid methyl ester (C17:0) 2%
cis-10-Heptadecenoic acid methyl ester (C17:1) 2%
Stearic acid methyl ester (C18:0) 4%
Oleic acid methyl ester (C18:1n-9) 4%
Elaidic acid methyl ester (C18:1, *trans*-9) 2%
Linoleic acid methyl ester (C18:2) 2%
Linolelaidic acid methyl ester (C18:2,*trans*-9,12) 2%
α-Linolenic acid methyl ester (C18:3n-3) 2%
γ-Linolenic acid methyl ester (C18:3n-6) 2%
Arachidic acid methyl ester (C20:0) 4%
cis-11-Eicosenoic acid methyl ester (C20:1) 2%
cis-11,14-Eicosadienoic acid methyl ester (C20:2) (98%) 2%
cis-11,14,17-Eicosatrienoic acid methyl ester (C20:3) (97-99%) 2%
cis-8,11,14-Eicosatrienoic acid methyl ester (C20:3) 2%
Arachidonic acid methyl ester (C20:4) 2%
cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester (C20:5) 2%
Heneicosanoic acid methyl ester (C21:0) 2%
Behenic acid methyl ester (C22:0) 4%
Erucic acid methyl ester (C22:1,*cis*-13) 2%
cis-13,16-Docosadienoic acid methyl ester (C22:2) 2%
cis-4,7,10,13,16,19-Docosahexaenoic acid methyl ester (C22:6) 2%
Tricosanoic acid methyl ester (C23:0) 2%
Lignoceric acid methyl ester (C24:0) 4%
Nervonic acid methyl ester (C24:1) 2%

Figure 2.1. Fatty acid methyl esters (FAME) mixture standard for chromatography: 100 mg ampule contains the above FAME with weight percentages of each component as indicated.

2.2. Tissue collection and processing

2.2.1. Human tissues processing

Ethical approval for the collection of the human placentae was granted by the East London Health Authority and a signed consent was obtained from the mothers.

2.2.1.1. Early placentae

Early (8-14 wk) in gestation placentae were collected by vacuum aspiration from legally termination pregnancies by the Physician in charge in sterile specimen tubes containing ice cold physiological saline, labeled with the type of sample, gestational age and study number, and transported to IBCHN for immediate analysis. Fresh placental samples were excised, washed several times in cold saline to remove all traces of blood. Blood clots within the placental villi were viewed with a magnifying glass and removed with sterile scissors. The tissue was blotted dry on filter paper, weighed and processed immediately for fatty acid (FA) analysis.

2.2.1.2. Placentae at delivery

Immediately after delivery, the proximal cord clamp was removed to allow blood drainage and the placenta free of fetal membranes was gently cleaned of traces of blood and meconium with a piece of gauze and weighed with the cord at the delivery room by the attending nurses. The length of the cord (where noted) was measured from the placental origin to the entrance of the fetal abdomen.

Representative samples were dissected free of vessels from the central region of the placenta (center of parenchyma to ensure sampling homogeneity) to eliminate variations in the membrane lipid composition and particularly neutral lipids, and transferred to the research laboratory in sterile specimen containers containing ice cold saline. Fresh samples were dissected and washed several times in cold saline to remove all traces of blood and processed immediately or prepared for storage at -70°C until analysis.

Early (≈ 6g) and term (≈ 4g) samples were weighed in dimmed light and extracted in duplicates and triplicates to accurately determine the total lipid in the placenta (see 2.3.3.1.).

2.2.2. Animal tissues

All experiments were performed in UK and approved by the British Home Office under the project license: PPL90/01970 (Chapter 5) and PPL90/765 (Chapter 6).

In the study described in Chapter 5 the offspring were killed at birth (1d) or at suckling period (15d) and the dams at 16d postnatally and in Chapter 6 the offspring were killed at 160d of life, all by CO_2 inhalation and cervical dislocation to eliminate stress related effects on vascular composition. Maternal liver and offspring's liver and heart were rapidly removed, planed in ice-cold physiological saline, blotted dry, weighed, catalogued and prepared for storage at -70°C. Similarly, thoracic aortas (comprising the section of the aorta from the end of the aortic arch to the diaphragm) were dissected free of adventitia (connective tissue and fat), snap frozen and stored to -70°C for subsequent FA analysis.

Fresh tissues were transported to IBCHN frozen on dry ice and stored in leak proof polyethylene sample bags with the study number, animal identification number, date of collection and organ type. Representative samples (≈2g) were taken from the central area of each liver tissue of the mothers to provide with a homogenous membrane lipid profile. Of the offspring's tissues, 2-3 samples were pulled together to provide with approximately 500mg of liver and 150mg of heart at 1d, 2g of liver and 500mg of heart at 15d, and 150mg of aorta at 160d of life. Length of storage at -70°C may be indefinite (Jensen & Clark, 1984) or T for prolonged storage should not rise above -40°C (Christie,1982, 1993), however, herein stored samples were extracted for total lipid and FA within 6 months of storage as reported by our collaborators at St. Thomas' Hospital (Khan et al, 2003).

The studies described in Chapters 5 & 6 were 'blind', meaning that throughout the experimental period, the dietary treatment (high-fat vs. Low-fat) or disease state (diabetic vs. non-diabetic) of the rats was unknown so that the produced FA data not to be biased.

2.2.3. Experimental diets

During the experimental periods, the experimental control (4.28% fat; corn oil in Chapters 5&6) and high-fat diets (final analysis: 32.9% fat in Chapter 5 or 20% fat in Chapter 6, mainly as animal lard) were transported on dry ice to the IBCHN laboratory to be analysed for FA.

Upon arrival, the experimental diets (≈6g of each), control and high fat diets, were extracted in triplicates to confirm validity of the FA composition. The mean %FA composition of the different lard% containing and the standard breeding diets are describe in the corresponding studies in Chapters 5&6, along with other nutritional information provided by our collaborators who bred these animals on the assigned experimental diets 10d prior to mating.

2.3. Total lipid and fatty acid analysis

All solvents and solvent systems described herein were prepared always fresh on the day of each step according to the experimental protocol. The known carcinogenic effects of CH_3Cl may set at risk the laboratory personnel/students, but under the health and safety procedures at London Metropolitan University extraction of tissues in CH_3Cl:MeOH took place in ventilated fume hoods or cupboard only. The homogenizer was also located in the fume hood and the samples were processed on ice. Alternatively, dichloromethane, a low risk for carcinogenesis solvent, having identical results to CH_3Cl is available at IBCHN.

2.3.1. Extraction of total lipids

Total lipid were extracted from human placentae or rat liver, heart and aorta by homogenization with high-speed tissue homogeniser in chloroform: methanol (CH_3Cl:MeOH premix) at a ratio of 2:1 (v:v), containing 0.1% butylated hydroxytoluene (BHT), based on a modification to the Folch et al (1957) method, previously reported at IBCHN (Ghebremeskel et al, 1995). The homogenizer drive to the grinders was from above to ensure that there is no contact of the solvent with any greased seals or bearing and BHT was added to prevent autoxidation of unsaturated fatty acids. The volume of premix, CH_3Cl:MeOH (2:1 v:v with 0.1% BHT) used was dependent on the weight (wt) of the tissues; Total lipid from 2g of placentae or liver of the dams (16d) and their pups (15d) were extracted in 90 ml of premix. Similarly, total lipid from 500mg of rat liver (1d) and heart (15d) or 150mg of aorta (160d) and heart (1d) was extracted in 45 or 25ml of the premix, respectively.

Each tissue (human or rat) was cut into pieces, homogenized in 20 ml of the premix and shaken. Consequently, the homogenizer was washed several times with premix and the recovered tissue was transferred into its corresponding extraction tube, topped up with premix to the final volume (i.e. 90 ml), sealed with ground stoppers, shaken, flashed under oxygen free nitrogen (OFN) to limit oxidation and stored at 4°C for 24h. All samples were

extracted in small batches of 10, including a blank sample per batch to check against solvents and reagents contaminants.

2.3.2. Partitioning-phase separation between lipid and non-lipid components

Once the tubes containing the extracted samples were taken out of the refrigerator, were let to cool down at room temperature (T) in the fume cupboard for 20-30 min in dimmed light to eliminate condensation and filtered into a separating funnel, through a metal conical funnel lined with filter paper (Grade 1). All extraction tubes were rinsed twice with 10 ml CH_3Cl:MeOH (2:1 v:v, with BHT) for any residual of the homogenate tissue to also pass through the metal funnel lined with filter paper to ensure complete transfer of all the crude lipid extract (lipids accompanied by non-lipid substances) into the separating funnels. All extracts in the separating funnels were washed with 0.85% sodium chloride solution, equivalent to 25% the total volume of the filtrate, flushed with OFN and left at 4°C for 24h to allow the organic phase (lipid in chloroform) to separate from the aqueous/methanol phase (non-lipid extracts).

For tissue wt 150mg: Each homogenate was filtered in a separating funnel, rinsed twice with 5ml premix (CH_3Cl:MeOH 2:1 v:v, 0.1% BHT) to carefully wash the filter paper as above, flushed under OFN and stored at 4°C. Then, the filter paper with filtrate was cut into pieces and re-extracted in 20ml of premix in the original extraction vessel and filtered, pooling the second extract with the first (stored at 4°C). The extraction vessel was rinsed as before, the total volume of the solvent system in the separating funnel was noted and 25% (v/v) of 0.85% saline was added.

2.3.3. Collection of lipids

Again, once the separating funnels were taken out of the refrigerator and equilibrated at room T in the fume cupboard under dimmed light, the lower organic layer containing the lipid was drained into a round bottom flask and the solvent was removed under reduced pressure at 37°C in a Buchi rotary evaporator. When dry, the flask was rinsed with 2 ml MeOH with BHT and the solvent was removed. This step was repeated once more to ensure that any residual water in the sample has been removed. A slight "modification" of the protocol was to add 1ml of CH_3Cl to the dried residue; if the diluted residue was clear (not cloudy), then it confirmed that any water residual was drawn off the lipid. This step is optional, but important

in order to determine the total lipid in the case of the placentae. After that, CH_3Cl was removed under reduced pressure as above and the dry residue from this was the total lipid extract.

Before last, the round bottom flask was rinsed with 2 ml CH_3Cl:MeOH (2:1 v:v, with BHT) to dissolve the lipid residue and the dissolved lipid was transferred into a 10ml labelled trident vial. This step was repeated twice to ensure full recovery of the lipid residue. Finally, the total lipid [dissolved in total 6ml CH_3Cl:MeOH (2:1 v:v, with BHT)] in the trident vial, was either reduced gently to a volume of about 1ml under OFN in a 37°C block heater, flushed and stored at 4°C overnight or in case of the placentae, the residue was dried down until it reached a constant weight (wt) in a pre-weighed trident vial to determine its total fat (see below).

2.3.3.1. Determination of the total lipid in the placental tissue

As stated earlier, placental samples at two times in gestation were extracted in duplicates or triplicates to accurately determine the total lipid in the placenta.

The total lipid extract of the placental was removed with chloroform:methanol to pre-weighed labeled trident vial and the solvent evaporated under a stream of OFN until the vial reached a constant wt. The difference between the final and starting wt of the vials containing the tissue lipid extract is the gross wt of the lipid and BHT. Corrections for the BHT content were made based on the wt of the blank extract containing BHT only.

Total lipid was expressed in mg/g tissue (blotted).

2.3.4. Thin-layer chromatography and separation of lipid classes

2.3. 4.1. Introduction

Thin-layer chromatography (TLC) is largely used for qualitative purposes for both organic and inorganic materials, and is especially useful to check purity (the absorbent determines the purity of a compound), to monitor reactions and the production processes. It is also permits optimization of the solvent system to improve resolution and compound separation within a mixture, and in comparison with column chromatography it only requires small quantities of

the sample. It is quick and inexpensive. The adsorbent is usually a very fine grade silica gel in a thin layer held on a glass surface (Christie, 1982, 1993; Fifield & Kealey, 1995). Silica gel functions primarily as a surface adsorbent if dried at 100°C or above, otherwise the adsorbed water acts as the stationary phase for a partition system. Its activity is due to the silanol (Si-OH) groups on the surface. In chromatography, silanol usually pertains to the hydroxyl groups attached to the surface of silica gel. These groups have very strong polar interactivity and are responsible for the strong polar character of silica gel.

TLC is the most widely used method for the separation of individual complex lipids classes (Christie, 1982; Wang & Gustafson, 1992; Fifield & Kealey, 1995). The lipid classes are adsorbed by a stationary phase according to the polarity of the functional groups of the individual lipid components in an appropriate solvent (mobile phase). The mixture is carried in the mobile phase (solvent system) across the plate and the components/solutes migrate at different rates due to differences in solubility, adsorption and size or charge (solvent selectively) which then can be identified, examined *in situ* (by scraping the lipid-containing areas from the plates into acid-washed tubes for determination of the amount of phosphate, i.e. plasmalogens) (Touchstone, 1995) or removed mechanically by scraping the adsorbent off the plate and extracting the substance from the absorbent.

Since TLC became a well established separating technique, commercial TLC plates are available and with the introduction of the gypsum binder and the use of silica particles a few micron in diameter, very high plate efficiencies became possible. AT IBCHN, for practical reasons the commercial pre-coated TLC plates, Silica gel 60A°, K6, 20x20cm, where K stands for kieselgel (silica gel in German), were employed. Silica gel it is the colloidal form of silica or silicon dioxide (SiO_2). Pure silica is colorless to white and silical gel usually resembles coarse white sand. Silical gel is appropriate as a drying agent, catalyst or catalyst carrier, and in purifying various substances. It offers fast separation with excellent resolution and the 60A° pores diameter plates have a preparative 250µm layer thickness and a fluorescence indicator.

2.3.4.2. Developing solvent systems for TLC analysis

Phospholipids (polar lipids): At IBCHN, the original solvent system for separation phospholipids (PL) was consisted of chloroform:methanol:water, 60:30:4 v:v:v in 0.1% BHT. BHT is added to the eluting solvent to protect the lipids during their ascending movement on the stationary phase. This developing system could efficiently separate by comparison to available commercial standards, the major membrane PL, ethanolamine (EPG) and choline

(CPG) phosphoglycerides followed by shingomyelin, SPM (lower relative mobility) (Figure 2.2).

However, separation of the minor PL fractions inositol (IPG) and serine (SPG) phosphoglycerides was poor and not reproducible by using the original solvent system. These could appear on the developed plate as a SPG/IPG complex or a dark non-fluorescent band. Absorbing compounds diminish (quench) the uniform layer fluorescence and detected as dark violet spots/zones on a bright green-yellow background under UV. The latter may also suggest that the eluant (the solvent system flowing up the plate by capillary action) did not show maximum selectivity in its ability to dissolve or desorb the substances being separated. If the eluting solvent was strongly adsorbed on the solid phase than IPG and SPG, then the mobile phase will greatly speed up their elution and replaced them on the absorbent without speeding the elution of SPM, CPG and EPG.

Because this thesis is the first to characterize the individual membrane PL components of the human placenta, to achieve maximum selectivity and elution of SPG and IPG, two different eluting systems were tested: (A) chloroform: methanol: acetic acid: water, 35:15:4:1 v:v:v:v in 0.1% BHT (Figure 2.3) and (B) chloroform: methanol: methylamine 40% aqueous solution (65:15:5 v:v:v) in 0.1% BHT (Figure 2.4).

Introducing acetic acid in solvent system (A), the eluting solvent flowing up the plate for 1h did not reach optimal separation. IPG and SPG appeared as a complex or the bands were closely eluted. Conversely, the presence of methylamine (or mono-methylamine; CH_3NH_2) in the solvent system (B), flowing up the plate for 1h and 40 min showed large elution differences among lipid subfractions, reaching optimal selectivity for IPG and SPG and SPM.

Overall, CPG and EPG of rat liver, heart and aorta were efficiently separated by using the original system, chloroform:methanol:water (60:30:4 v:v:v in 0.1% BHT) as a mobile phase. In contrast, for the human placenta PL a new solvent system, chloroform: methanol: methylamine 40% aqueous solution (65:15:5 v:v:v in 0.1% BHT) was developed to efficiently characterize along with the major, the minor PL components, IPG and SPG.

Neutral (non-polar) lipids and free fatty acids: Placental and liver neutral lipid (NL) classes and free fatty acids (FFA) were separated by using a standard solvent system, consisting of petroleum spirit:diethyl ether:formic acid:methanol (85:15:2.5:1 by volume with 0.1% BHT). Petroleum ether (involves highly volatile liquid hydrocarbon mixtures, including light

petroleum spirit, mainly consisting of pentanes) is more hydrophobic and more selective for more hydrophobic lipids than diethyl ether. Whilst, diethyl ether (bp 34.6°C) with petroleum ether improves the salvation ability of lipids components. Therefore, non polar solvents, such as diethyl ether and petrol spirit, are efficient for triacylglycerols (TG) analysis, the major component of dietary lipids.

Cholesterol esters (CE), TG and FFA were efficiently separated by comparison to synthetic standards (Figure 2. 5).

2.3.4.3. Development of chambers and TLC plates and fractionation of tissue lipids

Two developing chambers (TLC tanks) were used, chamber A for PL and chamber B for NL separation. The respective rectangular glass tanks had flat walls and a lid for clear viewing, beveled inside and out with no sharp edges. The median ridge of the tanks separated two 20x20 cm plates and the lead was heavy to tightly seal the tank. The chambers were preferably lined with a sheet of filter paper (Grade 1) soaked in the appropriate solvent mixture in order to saturate the atmosphere inside with solvent vapour, to speed up the analysis (especially with polar solvents) and in some cases to improve resolution.

The TLC plates were activated in a laboratory oven for at least 1h at 100°C to reduce the water content from the silica gel and were supported on a solid metal tray to ensure uniform heat distribution. The activated plates on the solid metal tray were stored in the sealed desiccators until use to avoid adsorbing atmospheric moisture. Before spotting, the activated TLC plates were scored approximately 2.5cm from the top by using a spatula to prevent the solvent over eluting and a line was drawn approximately 2 cm from the bottom of the plate with a soft pencil, as an 'application line' to guide sample application in the form of a refined streak. Usually 2-3 samples were applied per plate, including a blank to monitor purity of the solvents. The lipid extract was applied onto the plate by capillary action (75µl disposable microhaematocrit capillary tube) as a uniform line and as narrow as possible. Then, the vial of sample/aliquot was washed with 2-3 drops of chloroform to recover any lipid residual and applied also onto the plate.

Thereafter, the plates were placed vertically into the developed chamber A and left for approximately 90 min (chloroform:methanol:water; 60:30:4 v:v:v in 0.1% BHT) or 120 min (chloroform: methanol: methylamine 40% aqueous solution; 65:15:5 v:v:v in 0.1% BHT) and into the developed chamber B for 30 min (petroleum spirit:diethyl ether:formic acid:methanol;

85:15:2.5:1 by volume with 0.1% BHT). The plates were left for another 10 min, after the solvent front had reached the scored line on the top of the plate in order to improve definition of the bands. Exception was the new PL solvent system consisting of methylamine; improved PL fractionation and resolution was observed when removing the plate immediately from the tank once the solvent front had reached the scored line.

The plates were then removed to a darken fume cupboard, supported upright inside a cardboard spray box, air-dried and sprayed with a methanolic solution of 2,7 dichlorofluorescin (0.001% w/v) to render the lipids visible and left for 5-10 min. To avoid saturating application and diffusion of the zones, the spray was applied lightly from a 15cm distance with a uniform up-and-down and side –to-side motion until the layer was completely covered. Subsequently, the dried developed plates were removed into a dark room to visualize PL, NL and FFA bands. UV was used as a non-destructive visualisation method. The edge of each band was circled with a soft pencil and identified by visual comparison with commercially available standards. Each lipid band was scraped off with a spatula and transferred to a labelled pyrex tube (reaction tube) fitted with a PTFE lined screw top to be prepared for transmethylation.

2.3.5. Methylation- Preparation of fatty acid methyl esters

2.3.5.1. Transmethylation of fatty acids

Fatty acid methyl esters (FAME) were prepared by an acid-catalysed transesterification reaction. Transesterification involves the direct conversion of an organic acid ester into another ester of that same acid (ester exchange), hence, it avoids hydrolysis of lipids prior to preparation of esters. FFA were also esterified by heating them with a large excess of anhydrous methanol in the presence of acidic catalyst. The acids can catalyse the reaction by donating a proton to the carbonyl group, thus making it more reactive. However, in the presence of water the reaction is inhibited.

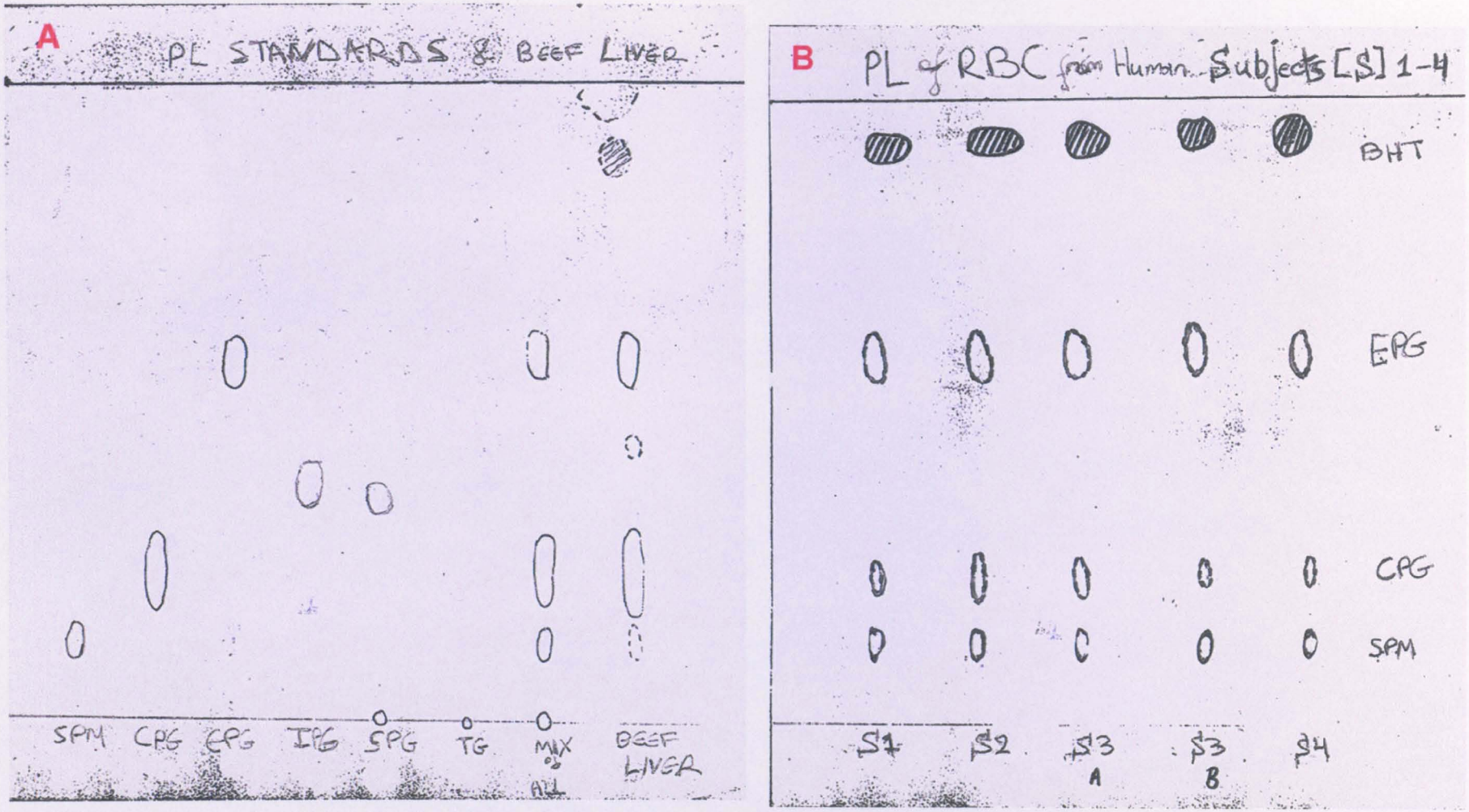


Figure.2.2. TLC separation of beef liver (plate A) and human RBC- phospholipids (PL), and PL standards; sphingomyelin (SPM), choline (CPG), ethanolamine (EPG), inositol (IPG) and serine (SPG) phosphoglycerides. Mobile phase: **chloroform:methanol:water** (60:30:4 v:v:v in 0.1% BHT).

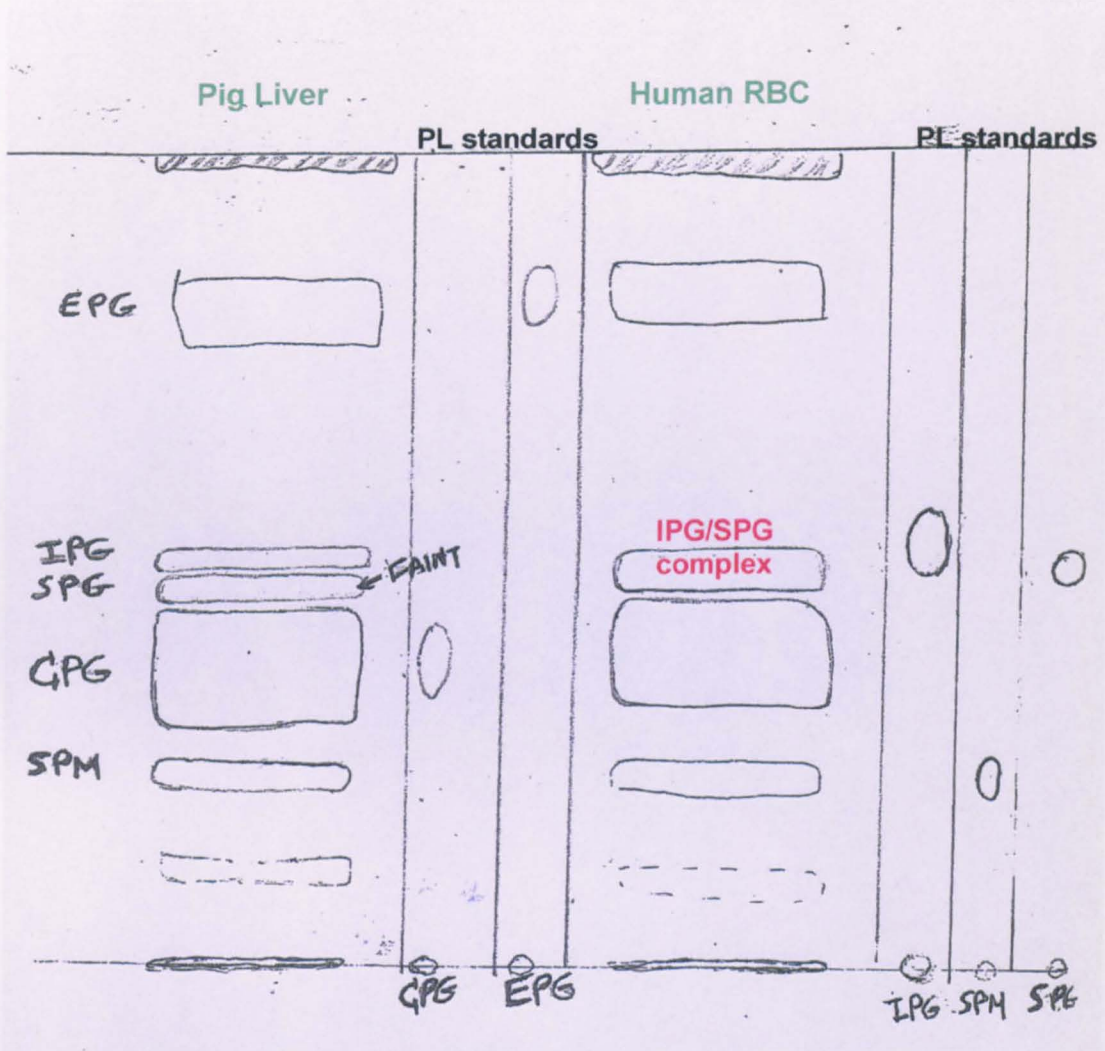


Figure 2.3. TLC separation of pig liver and human RBC-phospholipids (PL), and PL standards; sphingomyelin (SPM), choline (CPG), ethanolamine (EPG), inositol (IPG) and serine (SPG) phosphoglycerides. Testing PL eluting system (**A**): **chloroform: methanol: acetic acid: water** (35:15:4:1, v:v:v:v in 0.1% BHT).

TLC plate 1: placentae samples with PL standards

TLC plate 2: placentae samples

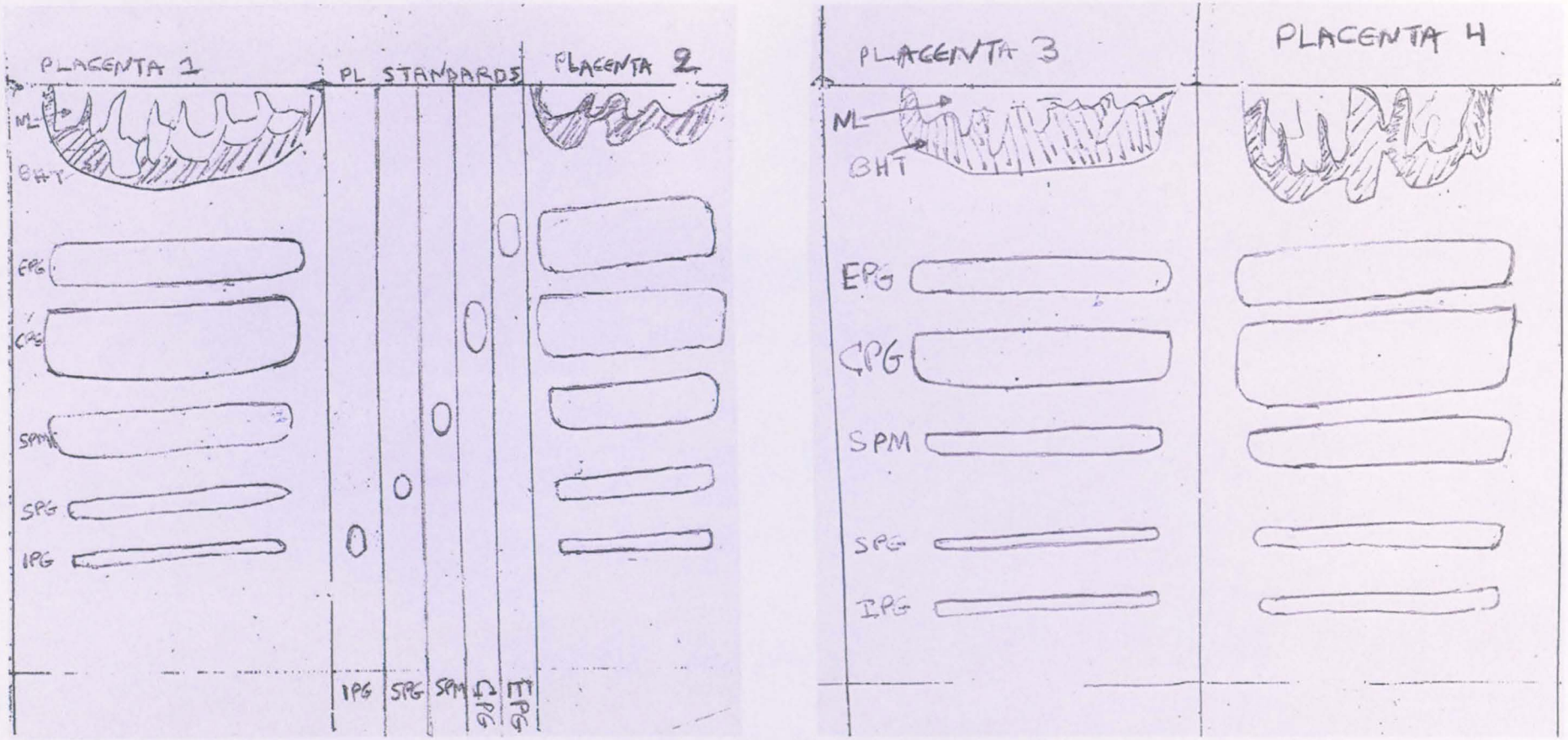


Figure 2.4. TLC separation of human placentae phospholipids (PL) and PL standards; sphingomyelin (SPM), choline (CPG), ethanolamine (EPG), inositol (IPG) and serine (SPG) phosphoglycerides. Testing PL eluting system (**B**): **chloroform: methanol: methylamine 40% aqueous solution** (65:15:5 v:v:v; in 0.1% BHT).

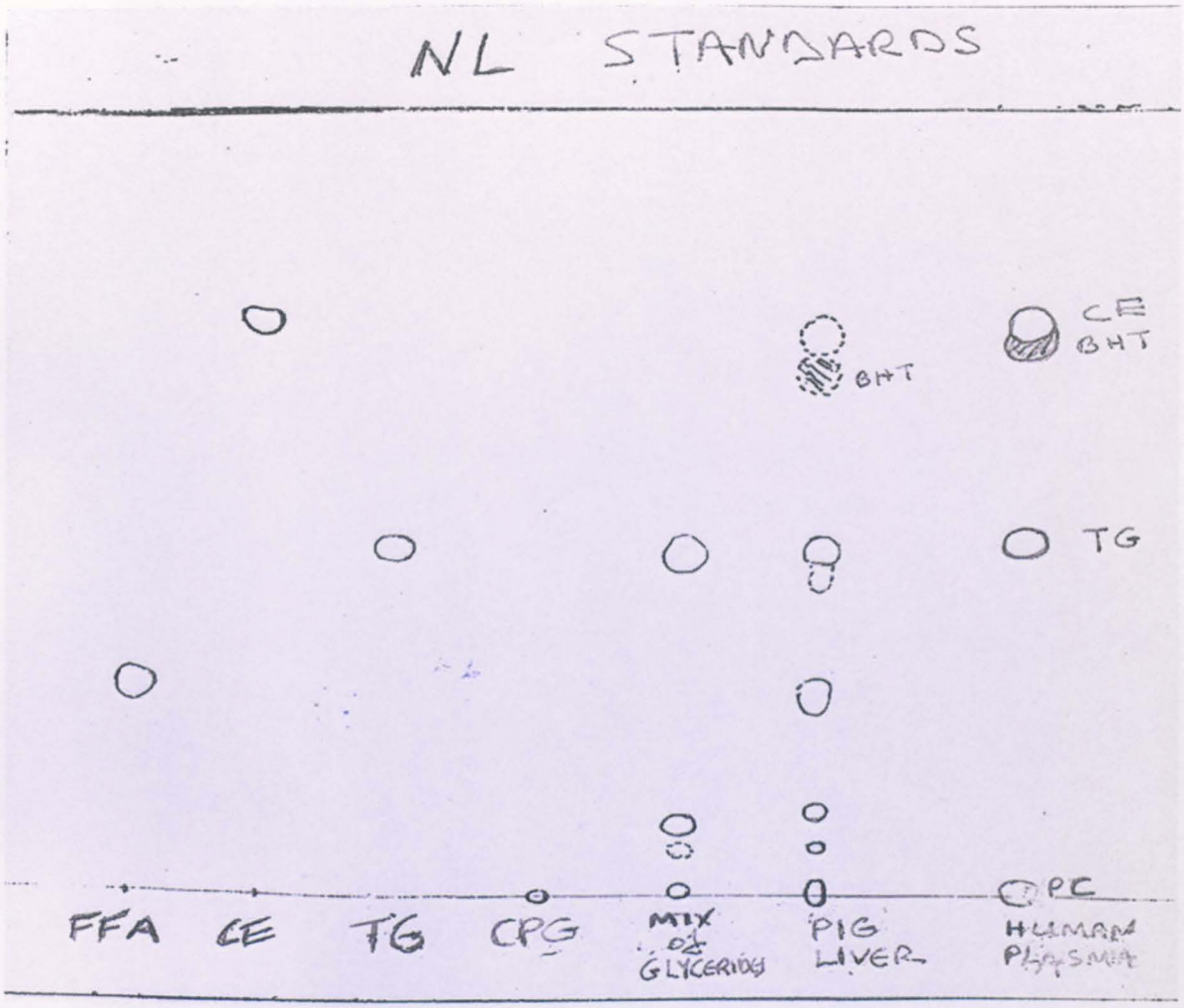


Figure 2.5. TLC separation of pig liver and human plasma free fatty acids (FFA) and neutral lipids (NL), and FFA and NL standards; cholesterol esters (CE), triacylglycerols (TG) and triacylglycerols mixture (mix). Eluting system: **petroleum spirit:diethyl ether:formic acid:methanol** (85:15:2.5:1 by volume in 0.1% BHT).

Methylating reagent (MR) was prepared as follows; 15g of acetyl chloride in 100ml of dry methanol: $CH_3OH + CH_3COCl \rightarrow CH_3CO_2CH_3 + HCl$. Methyl acetate may be formed as a by-product but does not interfere with the reaction at the concentrations suggested.The MR (acid reagent) was always prepared a) fresh because it has a limited shelf-time, unless refrigerated and b) carefully done by a simple procedure, adding acetyl chloride slowly (drop-wise) to cooled dry methanol while swirling the flask, to avoid some decomposition of PUFA, if it was to occur.

2.3.5.2. Extraction of fatty acid methyl esters

As stated earlier, each lipid band was scraped off the TLC plate, transferred carefully to a labeled pyrex tube and 4ml of the MR were added. Then, the reaction tubes were carefully mixed, flushed under OFN, vortexed and heated at 70°C for 3h in the absence of BHT. At 1 and 2h, the reaction tubes were removed from the oven, checked for leaks (if volume was reduced in some of the tubes, then they were allowed to cool at room T, topped up with MR and flushed again under OFN), all tubes were vortexed and placed back into the oven to continue the methylation process.

The extraction tubes, supported by a 3-row stand of 12 tubes each row (3 rows x12) were cooled in the fume capboard under dimmed light. An amount of 5% saline equivalent to the volume of the MR (4ml) in the cooled reaction tubes (with the silica and the lipid fraction) and 2ml petroleum spirit (40-60°C) were added, shaken vigorously and left to stand for 5 min to allow phase separation. Sodium chloride (5% w/v) stops the methylation reaction, completely saturates the aqueous solution, and improves the recovery of fatty acid methyl esters (FAME), particularly of the short-chain esters, in association with an optimum amount of extracting solvent (petroleum spirit). A disadvantage of extracting the methyl esters by commercially available TLC plates is that an emulsion is usually formed in the upper petroleum spirit (adsorbent surface and FAME) layer after the tubes (1st row) were stand for 5min to allow phase separation, however, the emulsion can be easily cleared by adding gently a few drops of pure methanol (no BHT).

The upper petroleum spirit layer with FAME from each reaction tube was removed to its corresponding test tube (2nd row) containing 2ml of potassium bicarbonate solution (2% w/v) to neutralize any acid transferred from the reaction tube, forming two layers, and vortexed. This step was repeated twice by adding 1ml petrol spirit each time into the reaction tube (1st row) and the petrol extract was pooled with the first extract in the bicarbonate wash (2nd row), vortexed to ensure that the extract was neutralised and the two layers were reformed. The top layer was then transferred to its corresponding test tube (3rd row) containing approximately 250mg of dry, granular sodium sulphate to remove water from the petrol. Thereafter, the petroleum extract with FAME-free of water was transferred to a 3ml trident vial and the solvent removed under OFN. The concentrated FAME were dissolved in 1ml heptane (containing BHT), flushed with OFN and stored at 4°C, awaiting gas chromatography analysis.

Collecting the FAME from tissue wt 150mg: As above, 4 ml of sodium chloride and 2 ml petroleum spirit were added to the cooled reaction tubes (1st row) and shaken, but centrifuged for 5 min at 4°C and 3000rpm. This step was repeated twice, by adding 1 ml petrol spirit each time to the reaction tubes, centrifuging, and removing the petrol extract to the same bicarbonate and dry sulphate tubes as the first extract. This method ensured that the FAME was transferred (recovered) from the cooled reaction tube, and the bicarbonate wash and the sulphate desiccant were done as efficiently as possible.

2.3.6. Gas chromatography

2.3.6.1. Introduction

In partition chromatography the stationary phase is conventionally a liquid and the chromatographic techniques were originally identified by the physical state of the mobile and stationary phase, as liquid-liquid partition chromatography or gas-liquid partition chromatography (Grant, 1996). This early terminology has been replaced by the less ambiguous terms, respectively, liquid chromatography (LC or HPLC) or gas chromatography (GC), because in GC, for instance, the stationary phase is immobilised by a polymerization process that can appear as an amorphous solid, but it behaves as a conventional liquid. The important feature of most of the modern techniques is that the solute distribution is linear and the symmetry of its peak shape is largely dependent in this linearity to separate and accurately quantify the solute (Willard et al, 1988; Harris, 1995; Scott, 1995; Grant, 1996).

In partition chromatography, linearity means that the partition coefficient (K) is constant: K=Cs/Cm; the concentration of solute in stationary phase (Cs) is proportional to its concentration in the mobile phase (Cm) and this ratio is constant under equilibrium conditions. The physical bases of chromatography is that the greater the ratio of partition coefficients between mobile and stationary phases, the greater the separation between two components of the mixture (Willard et al, 1988; Harris, 1995). More specifically, K determines the average velocity of each solute zone, the zone center as the mobile phase moves down the column (Willard et al, 1988).

GC or GLC is rapid, simple and capable to separate very complex mixtures (100 or more components). The mobile phase is the carrier gas and the stationary phase is the retentive part of the static medium which can either be a liquid or a solid material effectively distributed inside the column to maximize contact with the carrier gas (mobile phase). The overall

precision of quantitative analysis is 2-5%. Of all chromatographic techniques, capillary (open tubular columns) chromatography with flame ionization detector, FID (Weseler et al, 2008; van Eijsden et al, 2008, 2009) is the most powerful technique due to its ability to separate very complex mixtures. This open tubular column has many advantages over packed column. The diffusion rates in gasses are 4-5 orders of magnitude faster than in liquids, hence, the mobile phase (gas) in open tubular column can achieve fast equilibrium without being in such intimate contact with the stationary phase (Grant, 1996), providing higher resolution, shorter analysis time than the packed columns, and increased sensitivity to small quantities of analytes (Harris, 1995).

FID is a universal mass flow detector for GC applications such as fatty acid and carbohydrate analysis, flavor components and antioxidant. FID is mass sensitive and its response is irrelevant to changes in carrier gas flow rate (mobile phase). The detector is heated to avoid any condensation as FAME emerge at high concentration when they vacate the stationary phase and also to avoid water to be formed during combustion of hydrogen causing the flame to extinguish. FID is robust and easy to use. It has a very high sensitivity, excellent stability, the widest linear range (10 to the 7^{th}) and low noise, but it destroys the sample.

2.3.6.2. Analysis of fatty acid methyl ester

FAME are chemically the simplest ester derivatives with the lowest molecular wt, hence, they can be eluted from GC columns efficiently and at lower temperature (T) than other derivatives. Herein, FAME were separated by GC/FID with dual FID, split/splitless injector, amplifiers and 1 A200S autosampler, and fitted with a BPX20 polar capillary column (30m x 0.32mm i.d., 0.25μm film). BPX20 is equivalent to Carbowax, polyethylene glycol phase or WAX phase (Figure 2.6), a very polar stationary phase, suited to the analysis of alcohols, ketones and aldehydes, free acids, essential oils and aromatic compounds) (www.sge.com).

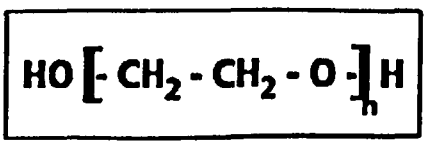


Figure 2.6. Polyethylene glycol.

The BPX20 characteristics also include: a polar bonded phase; fused silica type material; 0.43mm O.D, T range: 20°- 260/280°C (www.sge.com). The BPX20 0.25μm film is a standard

film of "medium" thickness for eluting high-boiling compounds. Bonded phases are immobilised and chemically bonded/crosslinked within the tubing. Bonded/crosslinked are two processes happen simultaneously by the manufacturer.

The HRGC MEGA 2 Series, 8560 is a more advanced GC instrument because it is has a built in electronic digital flow control (carrier gas pump of a dual mode). This is a non-manual gas setting by which the supply of the carrier gas can be provided under constant pressure or constant flow (dual mode). This electronic device allows to specify the type of carrier gas for FAME analysis (i.e. hydrogen or helium) so that to proceed with the characterisation of the column in order to optimise GC conditions and performance.

Hydrogen was preferred as a carrier gas or mobile phase at a constant flow rate. Hydrogen has a broad minimum van Deemter profile, which is vital for optimum performance, as compared to helium and nitrogen. In addition, it has a faster diffusion and lower viscosity than the two gases. Hence, it is the best carrier gas for capillary applications. Moreover, the problem of safety associated with the use of hydrogen as carrier gas has been successfully overcome by the availability of (a) hydrogen generators with low pressure output and low gas generation (max 300 ml/min) (b) modern GC that are designed with hydrogen in mind. Hydrogen is also cheaper than the next alternative helium. Whilst, N_2 gives the lowest detection limit (Harris, 1995). IBCHN uses hydrogen as carrier gas for fatty acid analysis with capillary GC/FID for over 20 years without any problem.

2.3.6.3. Identification and quantification of fatty acid methyl esters

An aliquot of the methylated sample (1μl) in heptane with BHTwas injected by the automatic sampler into a split injector. The samples were set to run in batches with a blank every 5 samples. The blank was pure heptane with BHT to correct for background effects (i.e. instrumental origin or added reagents) and to wash the column from sample residues left from a previous run. The run time per sample was 40 min under constant flow mode as set onto GC electronic digital flow controller, plus 5 min standby before the injection of the next sample (total 45 min). The flow rate of hydrogen to the column (carrier gas/mobile phase) and GC operational conditions are shown in Table 2.1.

Optimized GC conditions

Flow		
Flow rate (hydrogen/mobile phase)	1.5	ml/min
Split flow	29.8	ml/min
Split ratio	20:1	
Purge flow	4.06	ml/min
Hydrogen to FID (pressure)	45	KPa
Air to FID (pressure)	65	KPa
Velocity	26.6	cm/min
Temperature		
Oven	187°C	
Injector	235°C	
Detector	250°C	
Column conditioning	260°C	

Table 2.1. GC operational condition.

These were the GC optimised operational conditions to achieve the best resolution (baseline separation) based on a) selectivity (separation of peaks at the apex) and b) efficiency (the relationship between the retention time of the solute and the width of the solute peak upon elution). The separation of a mixture of standards is shown in Figure 2.7A-C. Efficiency is determined by the "effective number of theoretical plates", ideal sections of the column where the solute equilibrates between the stationary liquid phase and the mobile gas phase. The small ID of the column increased the number of plates during separation.

2.3. 6.3.1. Qualitative analysis

Methylated fatty acids extracted from a biological sample (a mixture of cod liver and vegetable oils, and pig liver total lipid extract) (Figure 2.8A-C) were tentatively identified by comparison of retention times (RT), relative retention times (RRT) and elongation chain length, ECL (semi-log plots, calibration curve; Figure 2.14) to authentic standards of known composition which were analysed under the same operational GC conditions (Figure 2.7A-C). The sample provided with an excellent means of standardizing the lipid procedures and

comparing the results to other samples. The optimized operation of the GC was demonstrated by the sharpness of the peaks and the lack of tailing during elution or a ghost peak within the chromatogram.

The RRT calculation and ECL equation (a series of standards of known composition were used to prepare the calibration curve in which FID response was plotted as function of mass) are given below:

$$RRT = \frac{\text{(RT of the unknown peak - RT of the Solvent front)}}{\text{[(RT of the reference FA (C18) - RT of the Solvent front)]}}$$

$$ECL = A \times (\log RRT) + B;$$

where A is the slope of the regression line and
B is the calculated chain length of the reference fatty acid, C18.

2.3.6.3.2. Relative quantitative analysis

Peak areas were integrated by the use of a computer Chromatography Data System, EZChrom. The identified FAME were expressed as %FAME (g/100g of total FAME) of all detected FA with a chain length of 8-24 carbon atoms.

The area normalization formula of each %FAME (area% is assumed to be equal to weight%) was calculates as shown below, based on the assumption that all FAME within a sample accounted for one chromatogram, meaning that the sum of total peak areas of a chromatogram represents 100% of the FAME, [Σ (total FAME)]:

$$\%FAME = [\text{peak area of the unknown FAME} / \Sigma \text{ (total FAME)}] \times 100$$

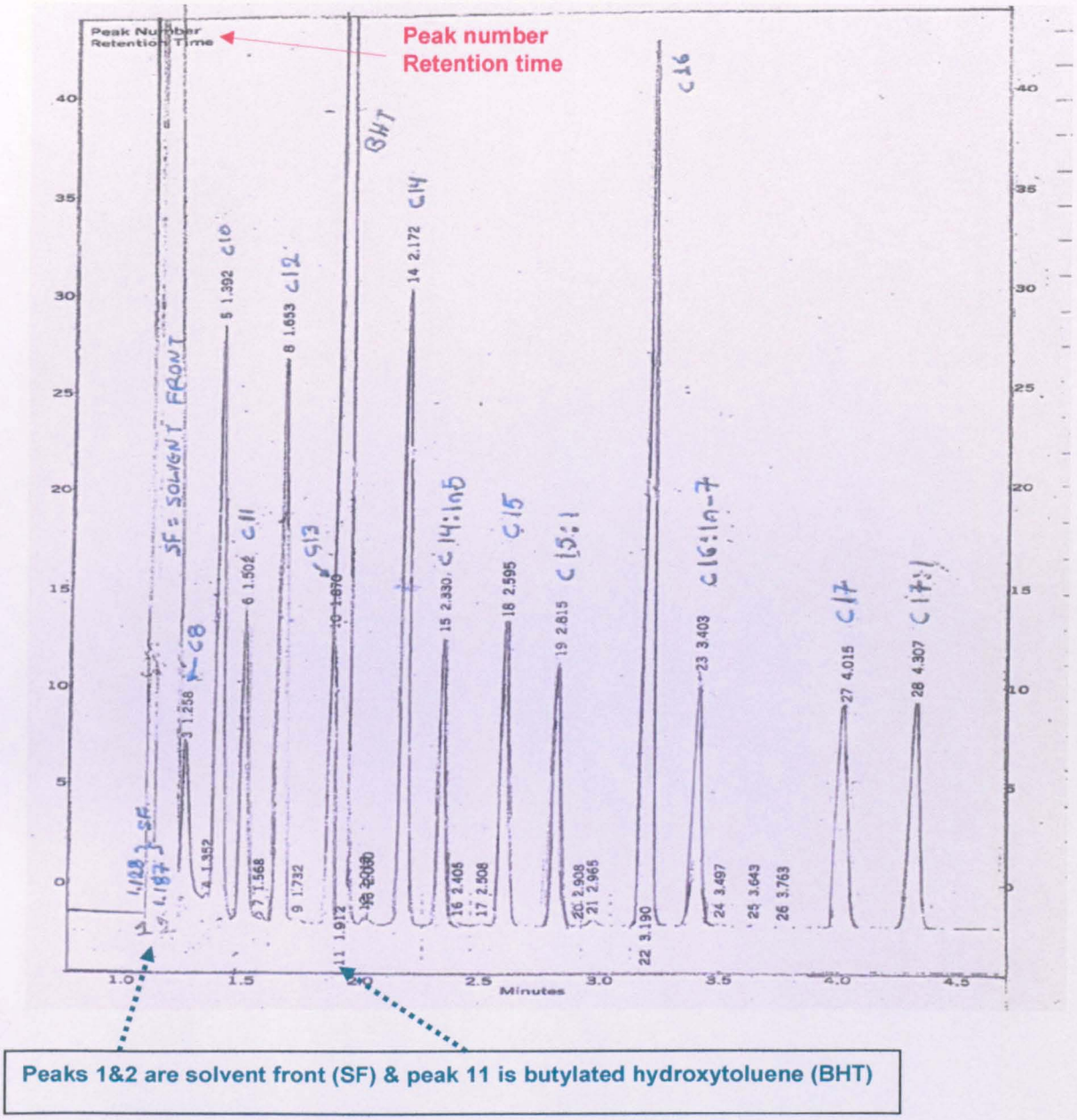


Figure 2.7A. Fatty acid methyl esters (FAME) mixture standard for chromatography (pp 67), eluting between 0-4.5 min. FAME were separated under the reported optimised GC/FID operational conditions (total run time 40 min).

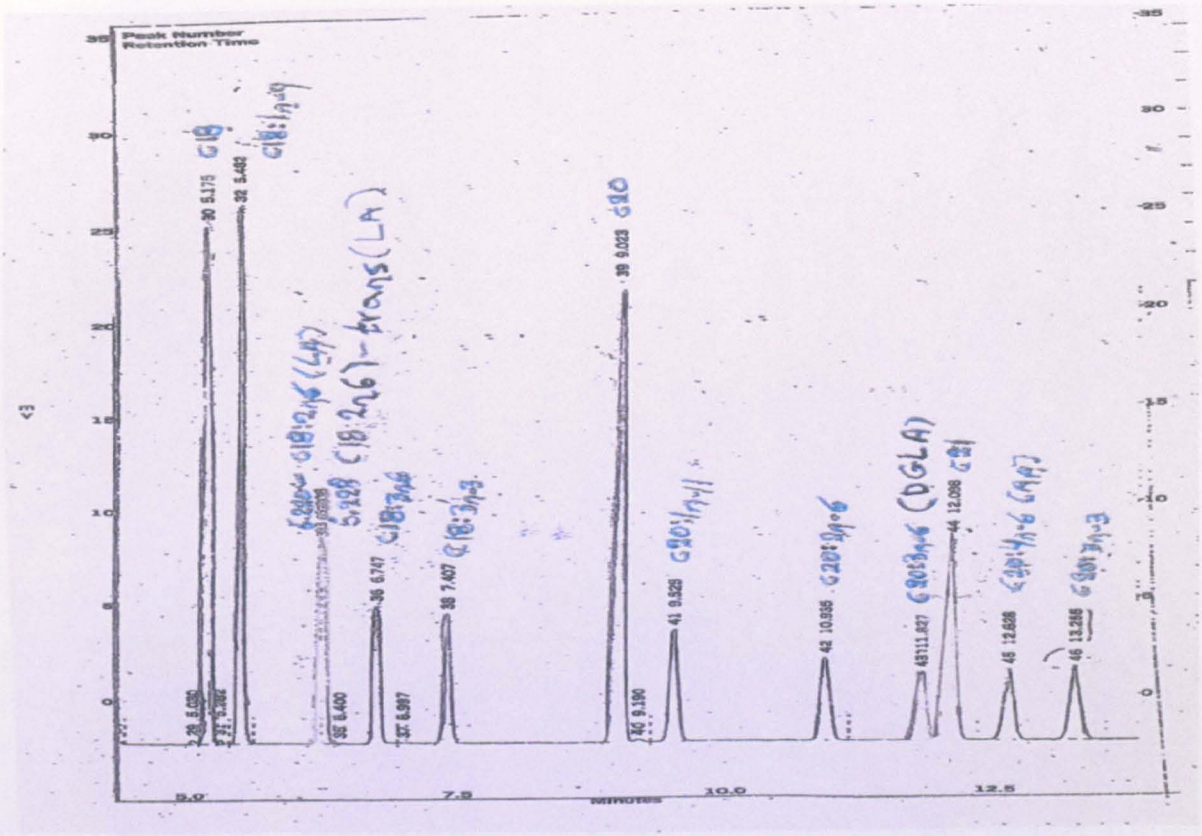


Figure 2.7B. FAME mixture standard for chromatography, eluting 4.5-14.5 min (total run time 40min).

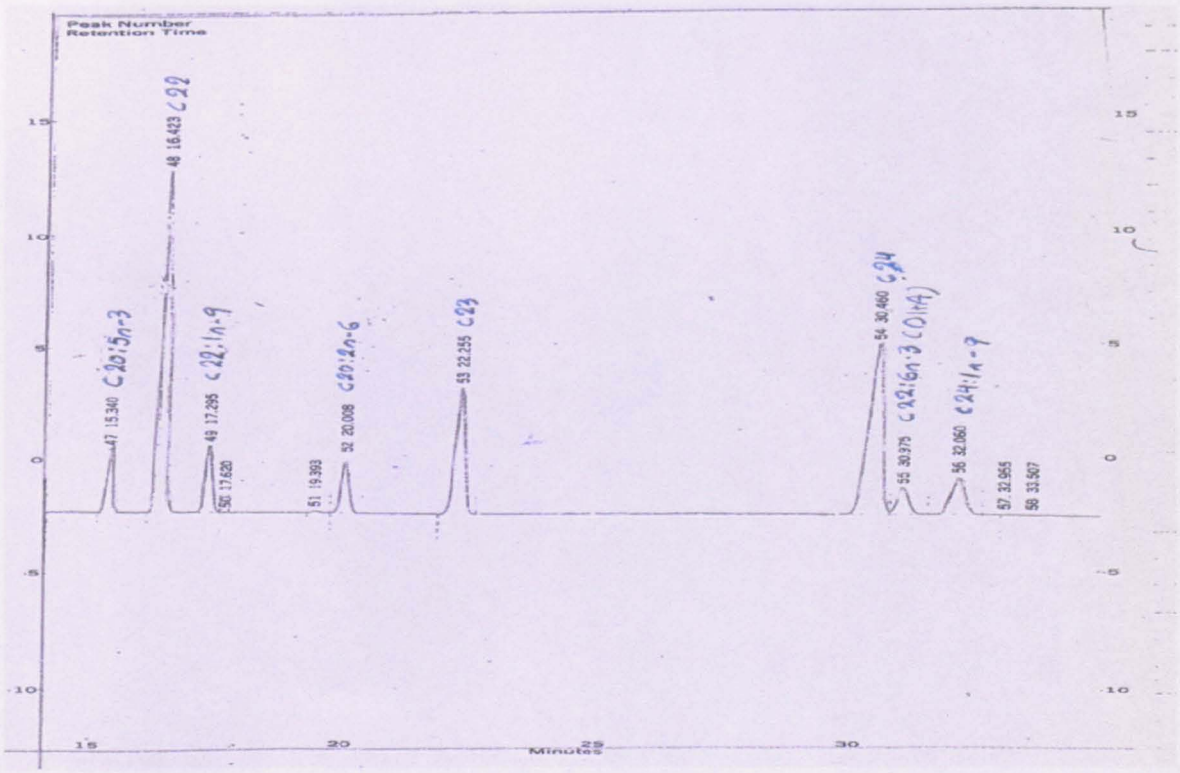


Figure 2.7C. FAME mixture standard for chromatography, eluting between 14.5 -40 min.

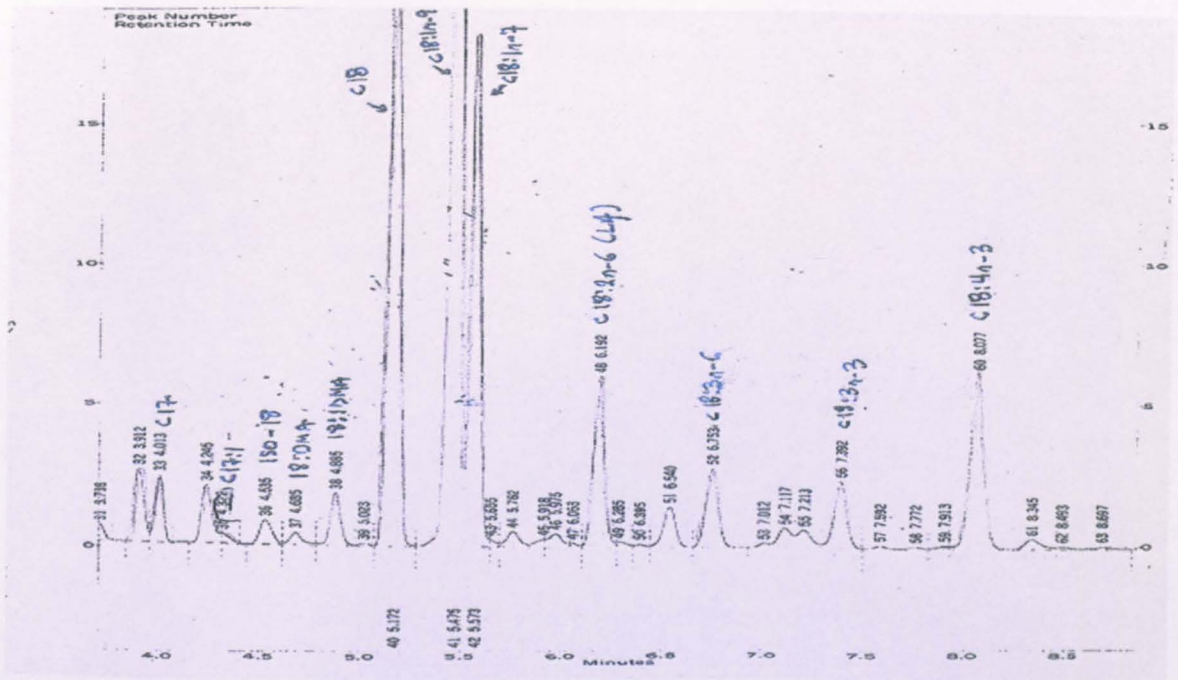


Figure 2.8A. Fatty acid methyl esters (FAME) extracted from a mixture of fish and vegetable oils and pig liver, eluting between 3.7-8.8 min. FAME were run under the same optimised GC/FID conditions to the FAME mixture standard for chromatography of known %weight composition (total run time 40 min).

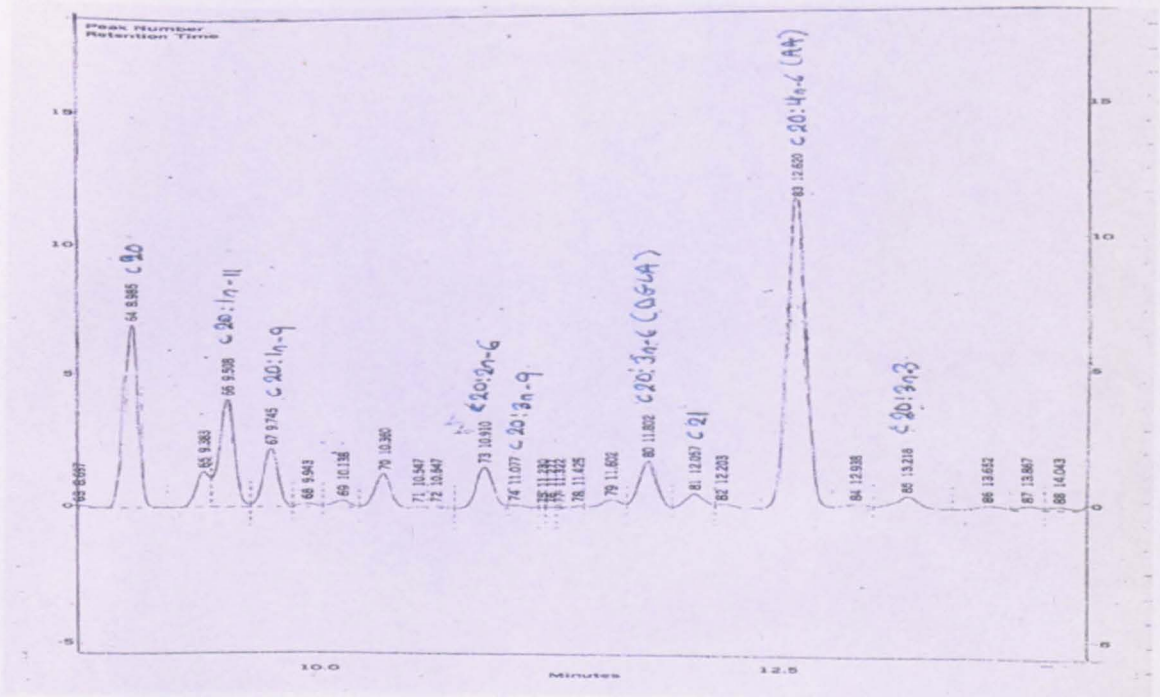


Figure 2.8B. FAME extracted from a mixture of fish and vegetable oils and pig liver, eluting between 8.7-14.1 min (total run time 40 min).

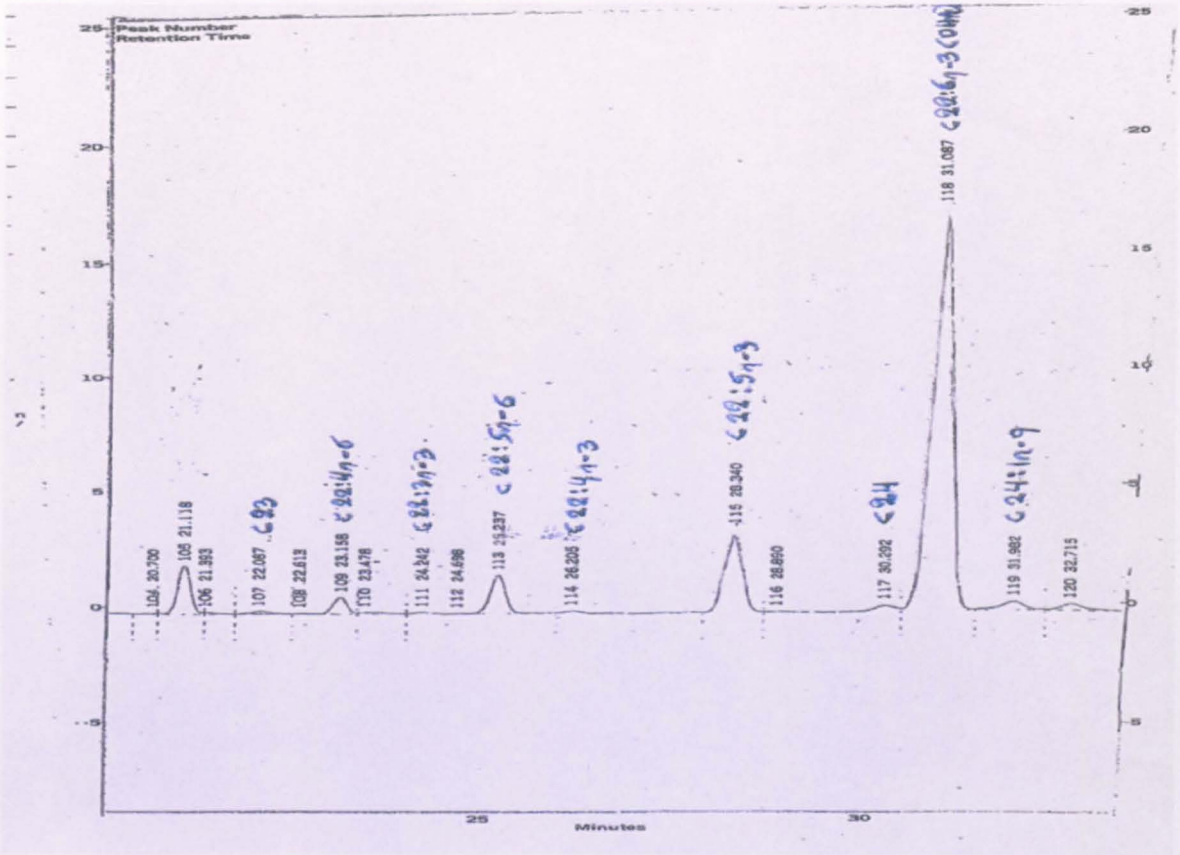


Figure 2.8C. FAME extracted from a mixture of fish and vegetable oils and pig liver, eluting between 20.5-40 min.

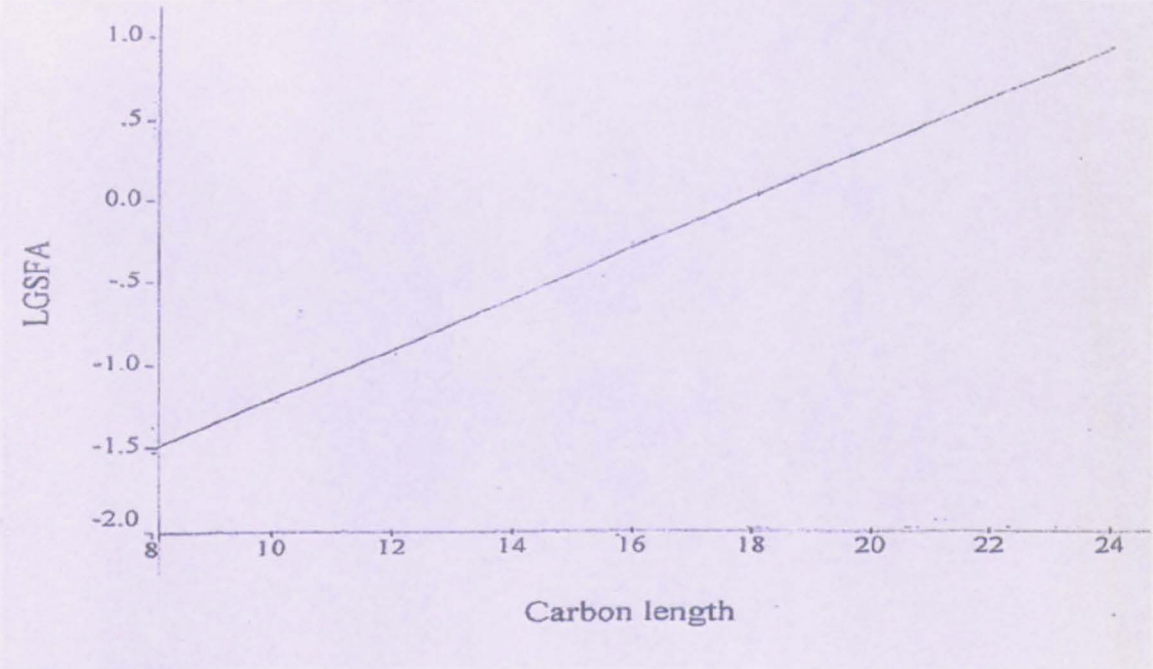


Figure 2.9. Calibration curve; where LGSFA=LogRRT of saturated fatty acids (C8-C24).

2.4. Power calculation

2.4.1. Statistics (sample size determination)

CHAPTER 3: Sixty-three (n=63) early placentae were analyzed to characterize primarily the major lipid components, choline (CPG) and ethanolamine (EPG) phosphoglycerides. Subsequent analysis for inositol (IPG) phosphoglycerides, important in cell signaling, serine (SPG) phosphoglycerides and sphingolyelin (SPM) was possible from forty-six (n=46) placentae due to limits of sample sizes. Analysis for triacylglycerols (TG), cholesterol esters (CE) and free fatty acids (FFA) was possible from fifty-four (n=54) placentae. Forty (n=40) term placentae were analyzed for comparative purposes (sample size at 95% power of detecting the difference at the 5% level for arachidonic acid (AA) based upon previous studies was estimated, n>11.7). The formula used to calculate the minimum sample size is outlined by Kirkwood (1992):

$$n= [(u+\upsilon)^2 (\sigma_1^2 + \sigma_2^2)] / (\mu_1-\mu_2)^2, \text{ where}$$

$\mu_1-\mu_2$, Difference between the two means

$\sigma_1 + \sigma_2$, Standard deviations

u, One-sided percentage point of normal distribution corresponding to 100%- the power (u.g. if power =90%, u=1.28)

υ, Percentage point of normal distribution corresponding to the (two-sided) significance level (e.g. if significance level=5%, υ= 1.96)

CHAPTER 4: Docosahexaenoic acid (DHA) is the most limiting fatty acid in pregnancy. Hence, the sample size was estimated based on previous DHA values in red blood cells (RBC) phospholipids (PL) of pregnant women with and without gestational diabetes (Min et al, 2005b). Eleven women in each group were required to detect a difference in placental CPG- DHA between the two groups with 80% power and a 5% significant level. Similarly, in preterm birth, the sample size was estimated based on previews studies on DHA in RBC-CPG of the preterm and term infant (Bitsanis et al, 2000) and other studies on DHA in umbilical artery phospholipids of the preterm and term infants (Crawford et al, 1990; Foreman-van Drongelen et al, 1995).

At least 11 samples were required to achieve 95% power to detect the difference at the 5% level for placental DHA. Similarly to Chapter 3, total of forty (n=40) preterm and GDM placentae were analyzed to characterize the major PL lipid components, CPG and EPG

together with IPG, SPG, SPM, TG, CE and FFA.

CHAPTER 5 & 6: On the basis of previously published studies from our collaborators at St Thomas' Hopsital for telemetric recording of blood pressure, at least 6 animals were required to achieve 90% power to detect a 10mmHg difference with a probability of $p<0.05$ (Anderson et al, 1999; Khan et al, 2003). For vascular studies, based on Khan's et al. data, an estimated of 8 animals were required to achieve 90% power to detect 20% difference in maximal relaxation with a probability of $p<0.05$. In one group of the dam's pups (suckling pups at 15d born to high-fat and diabetic dams, HFD) the number of pups at 15d was reduced to 4 due to the high mortality rates as a result of the combined effect of maternal diabetes and high-fat feeding.

Because this was an entirely novel study power calculations could also be dictated only by the number of animals available in each group, however n=4 is a quite small number of pups. Statisticians may agree to carry out a statistical test with n=4 samples, but this depends on the nature of the study. In medicinal statistics, this sample size can not provide with any information on the sample distributional normality and for the purpose of FAME, the data in that group (15d suckling pups from HFD) was presented without stating the significant differences (Chapter 5). For tissue PUFA, based on data from Chapter 5 at least 5 offspring were required in each group to achiever 80% power to detect the difference at the 5% for tissue DHA in further studies in this thesis.

2.4.2. Statistical analysis

Chapter 3: Data are expressed as means ± SD. Student's unpaired t-test was used to investigate differences in fatty acid composition between early and term placentae. To determine the effect of age, BMI, and ethnicity on LA, AA and DHA of the early placentae (8-14 wk of gestation), factorial analysis was performed with age, BMI and ethnicity as fixed factors and linoleic acid (LA), AA and DHA as dependent variable. Tukey's honesty significant difference post-hoc test was used if a significant difference was indicated.

At term, Mann-Whitney U non-parametric test were used to compare placental LA, AA and DHA in relation to age and BMI, and Kruskal-Wallis and Mann-Whitney U non-parametric tests were used to compare placental LA, AA and DHA between ethnic groups.

Chapter 4: Data are expressed as means ± SD. One-Way-ANOVA was used to compare placental lipid content and fatty acid composition between control, gestational diabetes mellitus (GDM) and preterm group. Likewise, age, placental weight (wt) and birthweight (BWT) and placental ratio and fetal/placental wt ratio between the three groups were compared by ANOVA and the Tukey's honesty significant difference post-hoc test was used if a significant difference was indicated ($P<0.05$).

Kruskal-Wallis and follow-up non-parametric tests using Mann-Whitney U test were conducted to compare a) gestational age, BMI and the foetal measurements of biometry between control, GDM and preterm groups and b) placental LA, AA and DHA in relation to ethnicity. Mann-Whitney U non-parametric test was used to compare fetal femur length between preterm and controls as well as to compare placental LA, AA and DHA between a) the appropriate (AGA) and large (LGA) for gestational age newborn of the control mothers and b) preterm and very preterm groups.

The strength of association (Pearson's Correlation Coefficient if data was normally distributed or Spearman's rank correlation coefficient, if $n<20$ or data was not normally distributed) was calculated between a) maternal, placental weight and foetal measurements of biometry and b) fatty acids and their ratios and maternal, placental and fetal dimensions. Where several related variables showed a significant correlation, the multiple linear stepwise regression model was used to determine relative significance. Age, BMI and ethnicity, gestational age (GA), head circumference (HC) and other fetal measurements, sex and mode of delivery were the main independent variables used in regression analysis while BWT, placental wt and placental ratio, fatty acids and their ratios were the dependent variables.

Chapter 5: Data are expressed as means ± SD and as medians with interquartile ranges (25-75% IQR). Kruskal-Wallis non-parametric ANOVA and follow-up non-parametric tests using Mann-Whitney U test were conducted to compare fatty acids between low-fat diabetic vs. control, high-fat diabetic vs. high-fat control, high-fat control vs. low-fat control, and high-fat diabetic vs. low-fat diabetic dams at 16d after delivery and their offspring (aged 1d and 15d).

Chapter 6: Data are expressed as means ± SD. Student's unpaired t-test was used to investigate differences in aortic fatty acid composition between high-fat and control groups.

In all studies (Chapter 3-6), differences were considered significant if $P<0.05$. All data were analyzed by the use of computer statistical software, SPSS for Windows (Release 11.5).

CHAPTER 3. FATTY ACID COMPOSITION OF EARLY AND TERM HUMAN PLACENTA OF HEALTHY PREGNANCY

3.1 Introduction

The principal objective of placentation in mammals with the haemochorial type of placenta is to bring the maternal and fetal circulations into close proximity to each other for the continuous supply of nutrients and optimal fetal growth (Graham & Lala, 1992; Page, 1993; Harding & Bocking, 2001; Pocock et al, 2004). Additionally, the placenta partially or completely functions in metabolism, hematopoiesis of the bone marrow and endocrine secretion to support proper function of the still immature embryonic and fetal organs (Benirschke & Kaufmann, 1995).

The process of placentation begins with implantation of blastocyst beneath the uterine epithelium and differentiation of trophoblast cell lineage into embryonic and extra-embryonic structures of conceptus (Cross, 1998; Kingdom et al, 2000a). This invasive behavior follows a precise chronology of vascular events during the first trimester of gestation (Kingdom et al, 2000b; Lunghi et al, 2007). These events involve placental tissue angiogenesis (a process responsible for tissue growth and placental morphology which is highly displayed in early gestation), organogenesis and progressive establishment of the two circulations within the placenta in preparation for the second phase of pregnancy, the fetal growth (Page, 1993; Jaffe, 1998; Kingdom et al, 2000b; Dantzer et al, 2000; Lunghi et al, 2007; Espinoza et al, 2007a&b). In addition, the endothelium has a primary role in the process of angiogenesis (Page, 1993; Jaffe, 1998; Kingdom et al, 2000b; Lunghi et al, 2007).

Human fetal growth and development has a unique requirement for the supply of dietary lipids because of the extensive involvement of cell membrane growth during early development. It is evident that during intrauterine life the human placenta selectively transfers arachidonic (AA, 20:4n-6) and docosahexaenoic (DHA, 22:6n-3) acids from the maternal circulation to the fetus (Crawford et al, 1976; Crawford, 2000). AA is the major essential fatty acid component of the inner cell membrane lipid in human vascular endothelium (Crawford et al, 1997). DHA has a primary role in the function of the retina and the brain (Anderson & Maude, 1972; Crawford & Sinclair, 1972; Uauy & Hoffman, 2000). The supply of AA and DHA to the fetus, by the placenta, depends on the maternal diet, circulating lipids and the length of gestation (van Houwelingen et al, 1992; Rum & Hornstra, 2002; Haggarty, 2002).

The BeWO cell line has been used as an *in vitro* model of human trophoblast to study fatty acid (FA) uptake and metabolism (Campbell et al, 1997, 1998a&b; Crabtree et al, 1998; Dutta-Roy, 2000a&b), because it can differentiate in culture in syncytiotrophoblast-like cells (Crabtree et al, 1998) and produce placenta specific proteins (most likely p-FABPpm) (Campbell et al, 1997; Crabtree et al, 1998). Additionally, research interest has been focused on the AA metabolites in pregnancy and parturition, as they act locally and participate in a variety of cellular functions, modulation of vascular tone, the response to injury, muscle contraction, cell proliferation and differentiation (Boone et al, 1993; Buhl et al, 1995; Lim et al, 1997, 1999; Paria et al, 2000; Kamata et al, 2006a&b). However, knowledge on the nature of the placental lipids is limited.

The endothelial cell lining of the vascular system has the highest membrane to cytoplasm ratio of any cell and constitutes the largest single organ mass of the adult. Consequently it will require a high proportion of membrane lipids and hence essential fatty acids (EFA). The human placenta, as a fast growing vascular network, will also be expected to exercise a high demand for the same EFA. To assess the lipid requirements in early development and test if the proportions of AA increase or are conserved in placental membranes throughout pregnancy, we determined the lipid content and FA composition of the human placental phospholipids (PL) and neutral lipids (NL), in early gestation (8-14 wk of age) as compared to term.

3.2. Methods

3.2.1. Subjects and selection criteria

Placentae from total one hundred and three (n=103) healthy pregnant women were obtained at two periods of gestation, early (8-14 wk) and term (38-41 wk).

The mothers were non-smokers and non-alcoholics, normotensive and free of medical and obstetric complications. The early termination of the pregnancy was due to socio/psychological reasons.

3.2.2. Sample collection

Placentae were collected (Ethical approval see section 2.2.1.) from legally terminated pregnancies by evacuation and term vaginal or caesarean delivery from St' Andrews Hospital and Newham General Hospital, East London.

3.2.2.1. Early placentae

Because this thesis is the first to report placental fatty acid (FA) data, sample collection and processing (section 2.2.1.1.) were based on the reported protocols on isolation of trophoblast cells from the human placentae (Kliman et al, 1986; Fisher et al, 1989; Genbacev et al, 1993). Collection of the early placentae (8-14 wk of gestation) by vacuum aspiration was preferred, because it captures a substantial mass (≈6g) of intact tissue without, for example, activating certain proteases to change tissue consistency and more importantly it eliminates contamination with maternal blood. Similarly, other protocols obtained early placenta by suction curettage (Pulkkinen et al, 1998) or suction aspiration (Lopez Bernal et al, 1992).

Gestational age (GA) was reported by the patient's GP and confirmed on the day of the termination of pregnancy by the doctor in charge. Unfortunately, detailed information on patient's background and characteristics was not always available from the clinic's antenatal records, because the subjects were healthy with no past history record or due to incomplete notes by the subject's GP or attending nurses.

3.2.2.2. Term placentae

GA was estimated taking in account the last menstrual period (LMP) and confirmed by ultrasound. The subject's clinical history was checked from the clinic's antenatal record and as above detailed information on patient's characteristics was not always available. Body mass index (BMI) was calculated as follows BMI = [weight (Kg)/ (height (m))^2] and a low pre-pregnancy BMI was defined as <18.5 (www.nhlbi.nih.gov).

Fresh samples (section 2.2.1.2) were cut into pieces with sterile scissors, washed repeatedly in cold saline by pressing gently against the walls of the beaker with a glass rod until the supernatant was free of visible blood, blotted dry on sterile filter paper and either processed immediately or stored at -70°C until analysis. On the other hand, Larque et al (2003) reported that subjecting placental samples to three washings with physiological saline was adequate.

3.2.3. Sample size and statistics

These are described at sections 2.4.1. & 2.4.2.

3.3. Results

3.3.1. Maternal characteristics

The early and term placentae were 12.0±1.62 and 39.8±1.19 wk of gestation respectively. The term placentae were complete with intact membranes and normal lobes. The mean weight (wt) where reported was 541.3 ±125.1 g (n=24).

The women, who terminated their pregnancy between 8 and 14wk of gestation, had a BMI (n=48) of 23.8±3.90 Kg/m^2 and they were 26.2±5.71 y old (n=62, range: 17-38 y of age). The mothers, who delivered at term, were 29.1±5.58 y old (n=26, range: 20-41 y of age) and they had a pre-pregnancy BMI (n=19) 24.4±4.59 Kg/m^2. The early gestation women were an extremely diverse population that differed also in religion, culture and ethnicity (Caucasian, n=24; African, n=22; Asian, n=13; Undeclared, n=4). To the contrary, the majority of the mothers, who delivered at term, were Asians (n=22) while a small number were from other ethnic groups (Africans, n=10; Caucasians, n=8).

In the factorial analysis conducted to determine the effect of age, BMI and ethnicity (Caucasians, Africans and Asians) on linoleic acid (LA), AA and DHA of the early placenta, there were no effects or factors interaction (P>0.05) (Table 3.1). Similarly, at term there were no differences in these fatty acids in relation to age and BMI (Table 3.2) and ethnicity (Table 3.3).

		AGE (y)	%FA	BMI (Kg/m^2)	%FA	Ethnicity (% FA) Caucasians	Africans	Asians	MANOVA	p-value
CPG	LA	<25	11.5±1.51	<25	11.6±1.40	11.8±1.46	12.4±1.51	11.3±1.68	Ethnicity	>0.05
		>25	12.0±1.66	>25	12.0±1.59				AGE	>0.05
									BMI	>0.05
									AGE x BMI	>0.05
									AGE x Ethnicity	>0.05
									BMI x Ethnicity	>0.05
	AA	<25	18.4±3.26	<25	18.8±3.41	18.4±3.36	19.7±2.75	18.4±3.47	Ethnicity	>0.05
		>25	19.3±2.96	>25	19.9±2.65				AGE	>0.05
									BMI	>0.05
									AGE x BMI	>0.05
									AGE x Ethnicity	>0.05
									BMI x Ethnicity	>0.05
	DHA	<25	1.81±0.59	<25	1.81±0.41	1.76±0.58	1.97±0.51	1.59±0.48	Ethnicity	>0.05
		>25	1.80±0.50	>25	1.84±0.59				AGE	>0.05
									BMI	>0.05
									AGE x BMI	>0.05
									AGE x Ethnicity	>0.05
									BMI x Ethnicity	>0.05

Table 3.1. Effects of maternal ethnicity, body mass index (BMI) and age on choline (CPG) phosphoglycerides- linoleic (LA), arachidonic (AA) and docosahexaenoic (DHA) acids of the placenta in early gestation (8-14 wk): %FA; % total fatty acids, Y; years, MANOVA; multivariate analysis of variance. Values are means ± SD,
Age: <25 (n=28); >25 (n=34),
BMI: <25 (n=29); >25 (n=19),
Ethnicity: Caucasians (n=24); Africans (n=22); Asians (n=13).

		AGE (y)	%FA	BMI (Kg/m^2)	%FA	Ethnicity (% FA) Caucasians	Africans	Asians	MANOVA	p-value
EPG	LA	<25	4.89±1.04	<25	4.82±1.12	4.70±1.11	4.93±1.10	4.69±1.08	Ethnicity	>0.05
		>25	4.63±1.11	>25	4.43±0.71				AGE	>0.05
									BMI	>0.05
									AGE x BMI	>0.05
									AGE x Ethnicity	>0.05
									BMI x Ethnicity	>0.05
	AA	<25	28.4±2.13	<25	28.7±2.19	27.9±2.03	29.0±1.91	28.7±2.65	Ethnicity	>0.05
		>25	28.6±2.15	>25	28.4±1.98				AGE	>0.05
									BMI	>0.05
									AGE x BMI	>0.05
									AGE x Ethnicity	>0.05
									BMI x Ethnicity	>0.05
	DHA	<25	8.13±1.52	<25	8.11±1.24	8.17±1.28	8.43±1.54	7.87±2.95	Ethnicity	>0.05
		>25	8.32±2.05	>25	8.66±2.55				AGE	>0.05
									BMI	>0.05
									AGE x BMI	>0.05
									AGE x Ethnicity	>0.05
									BMI x Ethnicity	>0.05

Table 3.1. (Continues) Effects of maternal ethnicity, body mass index (BMI) and age on ethanolamine (EPG) phosphoglycerides- linoleic (LA), arachidonic (AA) and docosahexaenoic (DHA) acids of the placenta in early gestation (8-14 wks): %FA; % total fatty acids, Y; years, MANOVA; multivariate analysis of variance.
Values are means ± SD,
Age: <25 (n=28); >25 (n=34),
BMI: <25 (n=29); >25 (n=19),
Ethnicity: Caucasians (n=24); Africans (n=22); Asians (n=13).

Lipid classes		AGE (Y)	%FA	BMI (Kg/m^2)	%FA
CPG	LA	<25	12.4±2.16	<25	12.6±2.28
		>25	13.8±3.70	>25	15.5±3.90
	AA	<25	17.5±2.73	<25	17.1±2.23
		>25	17.2±2.40	>25	16.6±3.12
	DHA	<25	1.72±0.53	<25	1.72±0.48
		>25	1.62±0.59	>25	1.59±0.67
EPG	LA	<25	5.63±1.46	<25	6.58±2.03
		>25	6.91±2.05	>25	6.72±2.13
	AA	<25	24.6±2.06	<25	24.8±1.25
		>25	24.9±1.47	>25	24.5±2.06
	DHA	<25	9.78±1.67	<25	9.54±1.54
		>25	8.44±2.04	>25	8.09±2.44

Table 3.2. Effects of maternal body mass index (BMI) and age on choline (CPG) and ethanolamine (EPG) phosphoglycerides- linoleic (LA), arachidonic (AA) and docosahexaenoic (DHA) acids of the placenta at delivery (38-41wk of gestation): %FA; % total fatty acids, Y; years.
Values are means ± SD,
Age: <25 (n=7); >25 (n=19),
BMI: <25 (n=12); >25 (n=7) (Mann-Whitney U non-parametric test, $P>0.05$),

	CPG (% total fatty acids)			EPG (% total fatty acids)		
	Caucasians	Africans	Asians	Caucasians	Africans	Asians
LA	12.0±2.40	13.4±2.76	14.2±3.00	5.83±1.49	6.50±2.32	6.41±1.49
AA	16.2±3.09	17.4±2.57	17.5±2.22	25.5±1.64	24.3±1.51	24.6±2.00
DHA	1.30±0.32	1.79±0.48	1.69±0.59	8.45±0.78	9.08±0.72	8.90±2.24

Table 3.3. Effects of ethnicity on choline (CPG) and ethanolamine (EPG) phosphoglycerides- linoleic (LA), arachidonic (AA) and docosahexaenoic (DHA) acids of the placenta at delivery (38-41wk of gestation). Values are means ± SD, n=8 (Caucasians); n=10 (Africans); n=22 (Asians) (Kruskal-Wallis and Mann-Whitney U non-parametric tests, $P>0.05$).

3.3.2. Lipid content and fatty acid composition of the placenta

Total lipid determination was described in section 2.3.3.1. Total lipid (mg/ g tissue, blotted) was significantly elevated at term (13.8±2.51, n=40) compared with the early placenta (8.49±1.26, n=63) (P<0.0001).

Fatty acid composition (% total fatty acids) of the individual placental lipid components at the two time points in gestation are presented in Table 3.4-3.11, including the following fatty acid (FA) ratios: docosapentaenoic -to- docosatetraenoic n-6 (DPA/DTA n-6) and DPA n-6/DHA as markers of DHA insufficiency, AA/LA as a marker of the elongation and desaturation of the n-6 polyunsaturated fatty acids (n-6 PUFA) as well as total AA precursors-to-AA [∑(AA precursors)/AA], AA/DHA as an index of the balance between these PUFA, total metabolites n-6 PUFA-to- total metabolites n-3 PUFA [∑met n6/∑met n-3] as an index of the balance between the total metabolites of both parent essential fatty acids (EFA), LA and α-linolenic acid (ALA), and total n-6 PUFA-to-total n-3 PUFA (∑n-6/∑n-3) as an index of the balance between the n-6 and n-3 PUFA.

3.3.2.1. Phospholipids

3.3.2.1.1. Choline phosphoglycerides- fatty acids

In choline phosphoglycerides (CPG) the proportions of stearate (C18), C20 (P<0.005), palmitoleate (C16:1), oleate (C18:1), C20:1 and total monounsaturated fatty acids (∑mono), P<0.0001 were lower at term compared with earlier in gestation (Table 3.4). By contrast, palmitate (C16) and total saturated fatty acids (∑SFA), P<0.0001 were elevated at term in placental CPG.

Of the n-6 PUFA, the percentage of 18:3n-6 (P<0.01), 20:2n-6 (P<0.05) and AA (P<0.005) was lower, whereas LA (P<0.005) was higher at term compared with the early gestation (Table 3.4). These differences could explain the decreased AA/LA at term (P<0.0001) in placental CPG. Of the AA metabolites (Table 3.4), DTA (22:4n-6) and DPA n-6 (22:5n-6) were lower (P<0.0001) and as a result, the ratio DPA/DTA n-6 was elevated at term compared with earlier in gestation (P<0.005). Of the n-3 PUFA, docosapentaenoic acid (DPA n-3, 22:5n-3), P<0.05 and DPA n-6/DHA (P<0.0001) were reduced, but ∑n-6/∑n-3 (P<0.05) was elevated at term.

3.3.2.1.2. Ethanolamine phosphoglycerides – fatty acids

In ethanolamine phosphoglycerides (EPG), the percentage of palmitate, C24 ($P<0.05$), stearate ($P<0.005$) and ∑SFA ($P<0.0001$) was increased at term compared with earlier in gestation (Table 3.5). At term, the proportions of palmitoleate, oleate and ∑mono ($P<0.0001$) were elevated and C22 ($P<0.005$) and C20 ($P<0.01$) were reduced in EPG (Table 3.5).

The term placenta compared with earlier in gestation had higher LA ($P<0.0001$), 18:3n-6 ($P<0.01$), DPA n-3 ($P<0.005$) and ∑n-3 ($P<0.05$) and lower AA, DTA, DPAn-6 and ∑n-6 ($P<0.0001$). Consequently, AA/LA, DPAn-6/DHA, ∑met n-6/∑met n-3 ($P<0.0001$), AA/DHA ($P<0.005$) and ∑n-6/∑n-3 ($P<0.05$) were reduced at term in placental EPG (Table 3.5).

3.3.2.1.3. Inositol phosphoglycerides- fatty acids

In inositol phosphoglycerides (IPG), the proportions of stearate, C20:1 and oleate ($P<0.0001$) were reduced at term compared with earlier in gestation (Table 3.6). At term, however, C22 ($P<0.005$), C22:1 ($P<0.05$), palmitate, C24 and C24:1 ($P<0.0001$) were elevated in IPG.

In placental IPG, AA, DTA ($P<0.0001$), 18:3n-6 and ∑n-6 ($P<0.05$), the parent EFA, ALA (18:3n-3, $P<0.005$) and DHA ($P<0.05$) were lower, whereas DPA n-3 and DPA/DTAn-6 ($P<0.0001$) were higher at term compared with earlier in gestation (Table 3.6).

3.3.2.1.4. Serine phosphoglycerides- fatty acids

Regardless the increased proportions of stearate ($P<0.005$), the major SFA, palmitate, C20 ($P<0.0001$), C22 ($P<0.005$) and C24 ($P<0.05$), all monounsaturated fatty acids and ∑mono ($P<0.0001$) in serine phosphoglycerides (SPG)were lower at term compared with earlier in gestation (Table 3.7).

Of the n-6 PUFA, DTA, DPA n-6 and 20:2n-6 were reduced ($P<0.01$, $P<0.05$ and $P<0.005$ respectively) while ∑n-6 were elevated ($P<0.0001$) at term compared with earlier in gestation in placental SPG. The enhanced proportions of LA contributed to the lower AA/LA ratio at term ($P<0.005$). Of the n-3 PUFA, the proportions of eicosapentaenoic acid (EPA, $P<0.05$) were lower, whereas DPA n-3 ($P<0.0001$), DHA and ∑n-3 ($P<0.005$) were

higher at term compared with earlier in gestation (Table 3.7). As a result, DPAn-6/DHA and AA/DHA were reduced at term ($P<0.0001$ and $P<0.05$, respectively) in SPG.

Di-hommo-γ-linoleic acid (DGLA, 20:3n-6) the immediate precursor for AA, was elevated significantly ($P<0.0001$) at term in all phosphoglycerides (Tables 3.4-3.7), particularly in IPG (85%) and SPG (134%). Consequently, the term placentae had a higher ratio of total AA precursors/AA [∑(AA precurs)/AA] compared with the earlier in gestation ($P<0.0001$) (Tables 3.4-3.7).

3.3.2.1.5. Sphingomyelin- fatty acids

The percentage of C24 ($P<0.005$), C20 ($P<0.05$), C22 and ∑SFA ($P<0.0001$) was higher at term compared with earlier in gestation (Table 3.8). Whilst, C24:1 ($P<0.01$), oleate and ∑Mono ($P<0.0001$) were reduced at term.

3.3.2.2. Free fatty acids and neutral lipids

3.3.2.2.1 Free fatty acids

The free fatty acids (FFA) had higher percentage of palmitoleate, oleate and Σmono ($P<0.005$) at term compared with earlier in gestation (Table 3.9). Likewise, ∑SFA, palmitate and C20 ($P<0.005$), stearate, C22 and C24 ($P<0.05$) were enhanced at term.

DPA n-6 ($P<0.05$), DTA and ∑n-6 ($P<0.0001$) were lower while DGLA ($P<0.0001$), DPA/DTA n-6 ($P<0.05$), LA and ALA ($P<0.005$) were higher at term compared with earlier in gestation. The proportions of DHA ($P<0.005$) and AA ($P<0.0001$) were lower, hence, AA/DHA ($P<0.01$) and AA/LA ($P<0.0001$) were reduced at term in placental FFA (Table 3.9).

3.3.2.2.2. Triacylglycerols- fatty acids

In placental triacylglycerols (TG), oleate and ∑mono (42%), palmitate (37%) and ∑SFA (20%) were markedly increased at term compared with earlier in gestation ($P<0.0001$) (Table 3.10). At term, the increase in palmitoleate and C24:1 was also significant ($P<0.0001$).

In TG, the proportions of AA, DTA, DPA n-6, ∑n-6 and the ratio AA/LA were lower at term compared with earlier in gestation ($P<0.0001$) (Table 3.10). However, the marked reduction in DHA (57%) and ∑n-3 (52%) ($P<0.0001$), enhanced the ratios AA/DHA, ∑met n6/∑met n-3 (50%) and ∑n-6/∑n-3 (69%) significantly at term ($P<0.0001$). In placental TG, the percentage 20:2n-6 ($P<0.05$) and ALA was higher while EPA and DPA n-3 was lower ($P<0.0001$) at term compared with earlier in gestation (Table 3.10).

3.3.2.2.3. Cholesterol esters- fatty acids

In cholesterol esters (CE), the decrease in stearate and ∑SFA (69% and 29% respectively) was marked at term compared with earlier in gestation, $P<0.0001$ (Table 3.11). Palmitate ($P<0.05$), oleate, C20:1 and C24:1 ($P<0.0001$), C22:1 and ∑mono ($P<0.005$) were reduced, whereas palmitoleate ($P<0.0001$) was elevated at term in CE.

In placental CE, the parent EFA, LA (73%, $P<0.0001$) was markedly increased (Table 3.11) and as a result ∑n-6, ∑n-6/∑n-3 and ∑(AA precursors)/AA were enhanced (42%, 86% and 82% respectively) at term compared with earlier in gestation ($P<0.0001$). By contrast, AA ($P<0.05$), AA/LA, DGLA and DTA ($P<0.0001$) were reduced at term.

EPA ($P<0.05$), DPA n-3, DHA and Σn-3 ($P<0.0001$) were lower, whereas ALA ($P<0.05$), DPA n-6/DHA ($P<0.01$), ∑met n-6/∑met n-3, AA/DHA and DPA/DTA n-6 ($P<0.0001$) were higher at term compare with earlier in gestation (Table 3.11).

% total fatty acids

	EARLY	TERM
16:0	34.1±3.65	39.0±2.91[d]
18:0	8.99±2.18	7.90±1.00[c]
20:0	0.09±0.04	0.06±0.03[c]
22:0	0.09±0.04	Trace
24:0	0.09±0.04	Trace
ΣSFA	43.2±2.73	47.0±3.09[d]
16:1	1.51±0.37	1.29±0.21[d]
18:1	13.1±2.43	9.85±1.45[d]
20:1	0.21±0.06	0.10±0.03[d]
22:1	0.08±0.02	Trace
24:1	0.07±0.04	Trace
Σmono	14.8±2.75	11.2±1.60[d]
18:2 n-6	11.8±1.59	13.4±3.01[c]
18:3 n-6	0.13±0.05	0.10±0.03[b]
20:2 n-6	0.56±0.16	0.49±0.14[a]
20:3 n-6	2.41±0.57	4.00±0.71[d]
20:4 n-6	18.9±3.12	17.2±2.44[c]
22:4 n-6	0.67±0.20	0.31±0.08[d]
22:5 n-6	0.40±0.16	0.22±0.07[d]
Σ n-6	34.7±3.89	35.7±3.42
Σ(AA precursors)/AA	0.79±0.16	1.08±0.30[d]
18:3 n-3	0.07±0.03	0.07±0.03
20:5 n-3	0.16±0.06	0.18±0.06
22:5 n-3	0.26±0.10	0.22±0.09[a]
22:6 n-3	1.81±0.54	1.64±0.58
Σ n-3	2.25±0.61	1.99±0.74
AA/LA	1.63±0.34	1.36±0.39[d]
AA/DHA	11.1±2.67	11.1±3.43
DPA n-6/DHA	0.21±0.07	0.13±0.08[d]
DPA/DTA n-6	0.56±0.18	0.67±0.14[c]
Σmet n-6/ Σmet n-3	10.9±2.45	12.0±3.68
Σ n-6/ Σ n-3	16.3±3.86	18.5±5.81[a]

Table 3.4. Percent fatty acid composition of choline phosphoglycerides of early (8-14 wk) and term (38-41 wk) placentae, ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σ(AA precursors)/AA; total arachidonic metabolites-to-arachidonic acid ratio, Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA n-6/DHA; docosapentaenoic (n-6)-to-docosahexaenoic acid, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3; total metabolites n-6 PUFA-to-total metabolites n-3 PUFA; Σn-6/ Σn-3; total n-6 PUFA-to-total n-3 PUFA.

Values are means ± SD, n=63 (Early); n=40 (Term). The term differed from the early gestation (Student's unpaired t-test), [a]$P<0.05$; [b]$P<0.01$; [c]$P<0.005$; [d]$P<0.0001$.

% total fatty acids

	EARLY	TERM
16:0	7.13±1.21	7.72±0.99[a]
18:0	11.4±1.88	12.5±1.27[c]
20:0	0.10±0.03	0.08±0.02[b]
22:0	0.11±0.03	0.08±0.03[c]
24:0	0.07±0.03	0.12±0.07[a]
ΣSFA	18.6±2.32	20.3±1.62[d]
16:1	0.45±0.15	0.58±0.15[d]
18:1	8.18±1.43	9.30±1.39[d]
20:1	0.21±0.05	0.19±0.06
22:1	0.07±0.02	0.06±0.02
24:1	0.10±0.04	Trace
Σmono	8.83±1.53	10.0±1.43[d]
18:2 n-6	4.73±1.08	6.32±1.70[d]
18:3 n-6	0.09±0.03	0.25±0.14[b]
20:2 n-6	0.37±0.13	0.38±0.11
20:3 n-6	1.92±0.50	3.47±0.56[d]
20:4 n-6	28.5±2.12	24.7±1.83[d]
22:4 n-6	3.90±0.80	2.41±0.45[d]
22:5 n-6	2.37±0.74	1.60±0.56[d]
Σ n-6	41.7±2.67	38.9±2.54[d]
Σ(AA precursors)/AA	0.25±0.05	0.42±0.09[d]
18:3 n-3	0.09±0.04	0.08±0.03
20:5 n-3	0.29±0.13	0.27±0.10
22:5 n-3	1.43±0.40	1.68±0.39[c]
22:6 n-3	8.24±1.80	8.87±1.99
Σ n-3	9.97±1.89	10.8±2.23[a]
AA/LA	6.29±1.31	4.20±1.27[d]
AA/DHA	3.60±0.75	3.00±1.07[c]
DPA n-6/DHA	0.29±0.09	0.19±0.10[d]
DPA/DTA n-6	0.62±0.20	0.65±0.13
Σmet n-6/ Σmet n-3	3.83±0.66	3.22±1.08[d]
Σ n-6/ Σ n-3	4.31±0.76	3.85±1.32[a]

Table 3.5. Percent fatty acid composition of ethanolamine phosphoglycerides of early (8-14 wk) and term (38-41 wk) placentae, ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σ(AA precursors)/AA; total arachidonic precursors-to-arachidonic acid ratio, Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA n-6/DHA; docosapentaenoic (n-6)-to-docosahexaenoic acid ratio, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3; total metabolites n-6 PUFA-to-total metabolites n-3 PUFA ratio; Σn-6/ Σn-3; total n-6 PUFA-to-total n-3 PUFA ratio.
Values are means ± SD, n=63 (Early); n=40 (Term). The term differed from the early gestation (Student's unpaired t-test), [a]$P<0.05$; [b]$P<0.01$; [c]$P<0.005$; [d]$P<0.0001$.

	% total fatty acids	
	EARLY	**TERM**
16:0	10.0±2.03	13.6±2.25[c]
18:0	30.7±2.57	27.0±2.31[c]
20:0	0.14±0.04	0.13±0.04
22:0	0.43±0.13	0.66±0.15[b]
24:0	0.50±0.17	1.26±0.45[c]
ΣSFA	41.2±1.89	41.8±3.15
16:1	0.34±0.11	0.33±0.10
18:1	5.50±0.93	4.76±0.88[c]
20:1	0.15±0.03	0.09±0.03[c]
22:1	0.16±0.04	0.19±0.06[a]
24:1	0.64±0.22	0.92±0.30[c]
Σmono	6.47±1.02	6.03±1.08
18:2 n-6	2.27±0.34	2.23±0.53
18:3 n-6	0.13±0.03	0.09±0.03[a]
20:2 n-6	0.63±0.20	0.60±0.20
20:3 n-6	3.71±0.83	6.87±1.03[c]
20:4 n-6	35.9±2.58	31.9±2.48[c]
22:4 n-6	1.57±0.24	1.11±0.21[c]
22:5 n-6	0.60±0.18	0.61±0.22
Σ n-6	44.4±2.32	43.1±3.22[a]
Σ(AA precursors)/AA	0.18±0.03	0.30±0.04[c]
18:3 n-3	0.13±0.04	0.07±0.02[b]
20:5 n-3	0.48±0.17	0.56±0.30
22:5 n-3	0.64±0.19	0.82±0.24[c]
22:6 n-3	2.61±0.70	2.22±0.69[a]
Σ n-3	3.61±0.88	3.43±0.92
AA/LA	16.3±3.24	15.1±3.59
AA/DHA	14.8±4.40	15.8±5.04
DPA n-6/DHA	0.25±0.11	0.31±0.16
DPA/DTA n-6	0.40±0.13	0.56±0.19[c]
Σmet n-6/ Σmet n-3	12.4±2.89	12.8±3.47
Σ n-6/ Σ n-3	13.0±3.06	13.4±3.66

Table 3.6. Percent fatty acid composition of inositol phosphoglycerides of early (8-14 wk) and term (38-41 wk) placentae, ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σ(AA precursors)/AA; total arachidonic metabolites-to-arachidonic acid ratio, Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA n-6/DHA; docosapentaenoic (n-6)-to-docosahexaenoic acid, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3; total metabolites n-6 PUFA-to-total metabolites n-3 PUFA; Σn-6/ Σn-3; total n-6 PUFA-to-total n-3 PUFA.

Values are means ± SD, n=46 (Early); n=40 (Term). The term differed from the early gestation (Student's unpaired t-test), [a]$P<0.05$; [b]$P<0.005$; [c]$P<0.0001$.

	% total fatty acids	
	EARLY	**TERM**
16:0	13.7±3.06	10.5±1.93[d]
18:0	31.2±2.83	34.0±4.48[c]
20:0	0.37±0.10	0.16±0.03[d]
22:0	0.23±0.07	0.17±0.04[c]
24:0	0.47±0.15	0.38±0.12[a]
ΣSFA	45.5±1.96	45.0±5.31
16:1	0.74±0.21	0.42±0.10[d]
18:1	17.7±2.16	13.2±1.73[d]
20:1	0.30±0.09	0.20±0.05[d]
22:1	0.22±0.07	0.26±0.09
24:1	0.62±0.20	0.41±0.14[d]
Σmono	19.3±2.27	14.3±1.77[d]
18:2 n-6	5.96±1.33	7.04±1.83[c]
18:3 n-6	0.13±0.02	0.14±0.04
20:2 n-6	0.25±0.09	0.17±0.06[c]
20:3 n-6	3.96±0.91	9.15±1.44[d]
20:4 n-6	11.2±1.90	10.8±1.52
22:4 n-6	1.95±0.55	1.67±0.38[b]
22:5 n-6	1.33±0.44	1.16±0.33[a]
Σ n-6	24.5±2.92	30.0±3.60[d]
Σ(AA precursors)/AA	0.93±0.23	1.53±0.27[d]
18:3 n-3	Trace	Trace
20:5 n-3	0.68±0.23	0.55±0.20[a]
22:5 n-3	0.50±0.18	0.69±0.23[d]
22:6 n-3	3.02±0.69	3.62±1.10[c]
Σ n-3	3.88±0.93	4.69±1.40[c]
AA/LA	1.94±0.45	1.63±0.44[c]
AA/DHA	3.84±0.84	3.23±1.34[a]
DPA n-6/DHA	0.47±0.17	0.33±0.15[d]
DPA/DTA n-6	0.69±0.23	0.69±0.13
Σmet n-6/ Σmet n-3	5.04±1.19	5.29±1.98
Σ n-6/ Σ n-3	6.64±1.55	6.93±2.81

Table 3.7. Percent fatty acid composition of serine phosphoglycerides of early (8-14 wk) and term (38-41 wk) placentae, ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σ(AA precursors)/AA; total arachidonic metabolites-to-arachidonic acid ratio, Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA n-6/DHA; docosapentaenoic (n-6)-to-docosahexaenoic acid, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3; total metabolites n-6 PUFA-to-total metabolites n-3 PUFA; Σn-6/ Σn-3; total n-6 PUFA-to-total n-3 PUFA.

Values are means ± SD, n=46 (Early); n=40 (Term). The term differed from the early gestation (Student's unpaired t-test), [a]$P<0.05$; [b]$P<0.01$; [c]$P<0.005$; [d]$P<0.0001$.

	% total fatty acids	
	EARLY	**TERM**
16:0	36.5±5.35	36.8±4.11
18:0	9.93±2.13	8.84±2.70
20:0	1.71±0.29	2.00±0.61[a]
22:0	8.01±1.95	10.7±2.56[d]
24:0	11.0±3.59	13.8±3.04[c]
ΣSFA	67.4±3.64	72.6±5.14[d]
16:1	0.33±0.10	0.34±0.13
18:1	4.30±1.58	2.90±1.12[d]
20:1	Trace	0.25±0.08
22:1	0.43±0.12	0.48±0.17
24:1	9.39±2.26	7.90±2.13[b]
ΣMono	14.2±1.81	11.3±2.68[d]
18:2 n-6	2.82±1.07	2.77±1.01

Table 3.8: Percent fatty acid composition of sphingomyelin of early (8-14 wk) and term (38-41 wk) placentae, ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids.
Values are means ± SD, n=46 (Early); n=40 (Term). The term differed from the early gestation (Student's unpaired t-test), [a]$P<0.05$; [b]$P<0.01$; [c]$P<0.005$; [d]$P<0.0001$.

	% total fatty acids	
	EARLY	**TERM**
16:0	21.1±2.77	22.8±2.36[c]
18:0	11.8±2.48	12.7±1.60[a]
20:0	0.19±0.06	0.25±0.10[c]
22:0	0.30±0.09	0.37±0.15[a]
24:0	0.19±0.06	0.31±0.14[a]
ΣSFA	33.8±4.31	36.7±3.51[c]
16:1	0.97±0.20	1.14±0.27[c]
18:1	12.0±1.77	13.2±1.77[c]
20:1	0.34±0.11	0.33±0.09
22:1	0.19±0.07	0.18±0.05
24:1	0.47±0.17	0.54±0.23
Σmono	13.7±1.95	15.1±1.91[c]
18:2 n-6	10.0±1.96	11.5±2.49[c]
18:3 n-6	0.15±0.04	0.17±0.05
20:2 n-6	0.29±0.11	0.38±0.16[a]
20:3 n-6	3.17±0.88	4.64±1.08[d]
20:4 n-6	25.3±2.94	18.8±2.79[d]
22:4 n-6	1.49±0.40	1.09±0.37[d]
22:5 n-6	0.90±0.30	0.77±0.27[a]
Σ n-6	41.7±6.04	37.2±3.84[d]
Σ(AA precursors)/AA	0.54±0.12	0.90±0.21[d]
18:3 n-3	0.17±0.05	0.23±0.10[c]
20:5 n-3	0.51±0.20	0.58±0.22
22:5 n-3	0.83±0.27	0.84±0.28
22:6 n-3	5.04±1.02	4.33±1.27[c]
Σ n-3	6.39±1.29	5.84±1.62
AA/LA	2.71±0.96	1.72±0.49[d]
AA/DHA	5.28±1.17	4.50±1.45[b]
DPA n-6/DHA	0.19±0.08	0.19±0.09
DPA/DTA n-6	0.63±0.22	0.75±0.24[a]
Σmet n-6/ Σmet n-3	5.18±1.11	4.64±1.41[a]
Σ n-6/ Σ n-3	6.73±1.35	6.53±2.21

Table 3.9. Percent fatty acid composition of free fatty acids of early (8-14 wk) and term (38-41 wk) placentae, ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σ(AA precursors)/AA; total arachidonic metabolites-to-arachidonic acid ratio, Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA n-6/DHA; docosapentaenoic (n-6)-to-docosahexaenoic acid, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3; total metabolites n-6 PUFA-to-total metabolites n-3 PUFA; Σn-6/ Σn-3; total n-6 PUFA-to-total n-3 PUFA.
Values are means ± SD, n=54 (Early); n=40 (Term). The term differed from the early gestation (Student's unpaired t-test), [a]$P<0.05$; [b]$P<0.01$; [c]$P<0.005$; [d]$P<0.0001$.

% total fatty acids		
	EARLY	**TERM**
16:0	19.5±3.94	26.5±3.50[b]
18:0	10.3±1.99	9.50±2.71
20:0	0.36±0.13	0.31±0.17
22:0	0.27±0.11	0.25±0.11
24:0	0.16±0.05	Trace
ΣSFA	31.0±5.31	37.1±5.27[b]
16:1	1.41±0.38	2.05±0.59[b]
18:1	14.5±3.27	20.1±5.04[b]
20:1	0.47±0.16	0.43±0.17
22:1	0.26±0.11	0.23±0.08
24:1	0.21±0.06	0.47±0.13[b]
Σmono	16.5±3.67	22.7±5.58[b]
18:2 n-6	12.0±2.36	13.3±4.32
18:3 n-6	0.21±0.06	0.19±0.05
20:2 n-6	0.18±0.05	0.27±0.09[a]
20:3 n-6	4.24±1.46	4.11±1.29
20:4 n-6	15.1±3.10	10.3±2.95[b]
22:4 n-6	2.77±0.82	1.38±0.38[b]
22:5 n-6	1.17±0.55	0.67±0.27[b]
Σ n-6	35.5±3.86	30.0±5.88[b]
Σ(AA precursors)/AA	1.14±0.30	1.83±0.64[b]
18:3 n-3	0.26±0.10	0.38±0.16[b]
20:5 n-3	0.57±0.23	0.37±0.13[b]
22:5 n-3	1.43±0.32	0.84±0.26[b]
22:6 n-3	7.33±2.26	3.14±1.14[b]
Σ n-3	9.46±2.66	4.52±1.49[b]
AA/LA	1.32±0.43	0.85±0.34[b]
AA/DHA	2.25±0.85	3.81±1.93[b]
DPA n-6/DHA	0.21±0.10	0.24±0.09
DPA/DTA n-6	0.44±0.22	0.49±0.18
Σmet n-6/ Σmet n-3	2.73±0.87	4.25±1.55[b]
Σ n-6/ Σ n-3	4.02±1.15	6.98±2.64[b]

Table 3.10. Percent fatty acid composition of triacylglycerols of early (8-14 wk) and term (38-41 wk) placentae, ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σ(AA precursors)/AA; total arachidonic metabolites-to-arachidonic acid ratio, Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA n-6/DHA; docosapentaenoic (n-6)-to-docosahexaenoic acid, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3; total metabolites n-6 PUFA-to-total metabolites n-3 PUFA; Σn-6/ Σn-3; total n-6 PUFA-to-total n-3 PUFA.
Values are means ± SD, n=54 (Early); n=40 (Term). The term differed from the early gestation (Student's unpaired t-test), [a]$P<0.05$; [b]$P<0.0001$.

	% total fatty acids	
	EARLY	**TERM**
16:0	14.3±2.70	13.0±2.21[a]
18:0	5.43±1.86	1.72±0.44[d]
20:0	0.38±0.17	Trace
22:0	0.36±0.15	0.28±0.09
24:0	0.36±0.15	Trace
ΣSFA	21.2±4.12	15.2±2.35[d]
16:1	2.85±0.85	3.71±1.11[d]
18:1	23.8±4.39	19.9±4.01[d]
20:1	0.43±0.13	0.22±0.09[d]
22:1	0.42±0.18	0.29±0.11[c]
24:1	0.58±0.17	0.28±0.06[d]
Σmono	27.4±4.72	23.9±4.85[c]
18:2 n-6	23.6±4.73	40.9±6.99[d]
18:3 n-6	0.38±0.12	0.54±0.14[d]
20:2 n-6	0.39±0.14	0.37±0.13
20:3 n-6	3.05±1.07	2.25±0.53[d]
20:4 n-6	8.59±2.96	7.46±2.05[a]
22:4 n-6	1.58±0.64	0.63±0.23[d]
22:5 n-6	0.60±0.25	0.51±0.21
Σ n-6	37.7±5.64	52.2±6.42[d]
Σ(AA precursors)/AA	3.52±1.25	6.37±2.23[d]
18:3 n-3	0.41±0.18	0.53±0.17[a]
20:5 n-3	0.46±0.16	0.35±0.11[a]
22:5 n-3	0.76±0.27	0.38±0.13[d]
22:6 n-3	2.26±1.01	1.26±0.56[d]
Σ n-3	3.59±1.50	2.09±0.77[d]
AA/LA	0.39±0.19	0.19±0.07[d]
AA/DHA	4.13±1.59	6.12±1.84[d]
DPA n-6/DHA	0.26±0.11	0.36±0.15[b]
DPA/DTA n-6	0.41±0.20	0.84±0.26[d]
Σmet n-6/ Σmet n-3	4.94±2.11	6.79±2.22[d]
Σ n-6/ Σn-3	12.3±5.81	22.4±6.92[d]

Table 3.11. Percent fatty acid composition of cholesterol esters of early (8-14 wk) and term (38-41 wk) placentae, ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σ(AA precursors)/AA; total arachidonic metabolites-to-arachidonic acid ratio, Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA n-6/DHA; docosapentaenoic (n-6)-to-docosahexaenoic acid, DPA/DTA n-6; docosapentaenoicn-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3; total metabolites n-6 PUFA-to-total metabolites n-3 PUFA; Σn-6/ Σn-3; total n-6 PUFA-to-total n-3 PUFA.
Values are means ± SD, n=54 (Early); n=40 (Term). The term differed from the early gestation (Student's unpaired t-test), [a]$P<0.05$; [b]$P<0.01$; [c]$P<0.005$; [d]$P<0.0001$.

3.4. Discussion

In this study, total weight (wt), total lipid content and fatty acid composition of phospholipids (PL) and neutral lipids (NL) of early and term placental tissues were investigated. The placentae were obtained from healthy women who underwent elective abortion between gestational ages (GA), 8wk and 14 wk or delivered a healthy baby at term. The women were from the borough of Newham, which is economically deprived inner-city area of London. To our knowledge such comparative study has not been reported before.

3.4.1. Placental weight in human healthy pregnancy

The human placenta grows exponentially during gestation, from an average of 6g at 3wk (or 20 days that placental basic organization is present) to 15g at 9wk, to 83g at 14wk, to 138g at 18wk and usually to 500-650g at term (Page, 1993; Harding & Bocking, 2001; O'Connor & Covacs, 2003; Pocock et al, 2004). In this study, the mean total placental weight (wt) at term was 541.3± 125.1 (g) while the actual reported wt range of the term placenta among other studies has been considerably variable, 440-802g (Page, 1993; Harding & Bocking, 2001; Shekhawat et al, 2003; Taricco et al, 2003; Gilbert-Barness & Debich-Spicer, 2004; Pocock et al, 2004; Wang et al, 2005; Alonso et al, 2006; Varastehpour et al, 2006).

Moreover, Makhseed et al (2004) and Rahman et al (2006) reported ranges at term (400-500 and 260-496g, respectively) analogous to the placental wt usually seen in preeclampsia, low birth weight or preterm and accelerated villous maturation (Gilbert-Barness & Debich-Spicer, 2004; Wang et al, 2005; Kocak et al, 2006).

3.4.2. Total lipid in the early and term placenta

Bayon et al (1993) reported that the total lipid content in the term placenta was 18.0 mg/g (1.8kg/100kg tissue) vs. 14.00 mg/g (this study). In contrast, the total lipid in 8-14 wk of gestation was 8.5mg/g tissue (blotted). Nonetheless, the high fat content at the end of pregnancy might be due to elevated placental NL as a reflection of the higher fetal demand for energy during the growth spurt in the last trimester.

3.4.3. Placental fatty acids in early and term pregnancy

Data on the early placenta are limited. Nikolasev et al (1973) investigated the fatty acid composition of choline (CPG), ethanolamine (EPG) and inositol (IPG) phosphoglycerides, and sphingomyelin (SPM) from 5 pregnancies (5- and 12- wk of gestation) in Szeged. Oppose to our UK study, they defined the early membranes as predominantly saturated. C16 was 66% and 59% in CPG at 5wk and 12wk and 45% and 18% in EPG at 5wk and 12 wk, respectively. C18 was 42% and 49% in placental IPG at 5wk and 12 wk and 29% in EPG at 12wk. Total saturated fatty acids (SFA) were 65%, 47%, 74% and 79% in CPG, IPG, EPG and SPM of the 12wk placentae (Nikolasev et al, 1973). SPM is expected to be high in SFA (67% total SFA, this study). They also reported arachidonic acid (AA) level at ≤1.0% and 20:4n-3 range between 6% and 21% in the PL subclasses, which we suspect were due to a methodological error.

The data on term placentae is in agreement with other term data (Percy et al, 1991; Bayon et al 1993; Lakin et al, 1998; Matorras et al, 2001; Klingler et al, 2003; Jain et al, 2004; Varastehpour et al, 2006), demonstrating that AA is quantitatively an important placental membrane constituent (Table 3.12A&B). Even in serine phosphoglycerides (SPG), AA was greater than the total n-3 polyunsaturated fatty acids (PUFA), which are usually reported to be concentrated in docosahexaenoic acid (DHA) rather than AA. Moreover, the study of Powell et al (1999) on the fatty acid composition of the microvillous (MVM) and basal (BM) membranes, suggested that the AA content is relevant to the syncytiotrophoblast membranes that comprise the epithelial barrier to transport across the human placenta. AA was significantly higher in BM, 24% vs. 19% in MVM while DHA was lower in the transporting endothelium in the placenta, 5% in BM vs. 6.5% in MVM (Powell et al, 1999).

The studies reported above, with the exception of Nikolasev et al (1973) agreed on a high preference of AA for the placental membranes. However, these were either studies of a small sample size or focused on the total PL only. A disadvantage of analysis of total PL is that the proportion of FA in total PL can change with a variation in lecithin-to-sphingomyelin ratio (L/SPM). This ratio is a marker for respiratory distress syndrome (Wijnberger et al, 2003; Poggi et al, 2003). Total PL analysis also misses variation in individual membrane lipids, each of which has a separate membrane function. In contrast, this study provides reference levels of essential fatty acids (EFA) in the placental PL components, derived from an ethnically mixed population in East London. The data of the individual PL might be used as biological markers for comparative investigations and nutritional interventions.

Another advantage of this study is that human placenta was analysed for the individual NL components and free fatty acids (FFA). The compositional data, with exception of triacylglycerols (TG), are in agreement with the Klingler's study, suggesting that AA is the most abundant PUFA in FFA and linoleic acid (LA) in cholesterol esters, CE (Figure 3.1). The quantified dominant nonesterified AA and DHA in the human placenta were reported to be 42.3 and 9 μg/g (Wang et al, 2005). The reported percent AA and DHA in total NL were 7.4±0.40 and 1.10±0.15, respectively (Varastehpour et al, 2006).

The studies of Bayon et al (1993), Lakin et al (1998), Matorras et al (2001), Klingler et al (2003), and Varastehpour et al (2006) on the term placenta suggested that differences in the maternal diet reflect different placenta AA and DHA levels amongst countries (Table 3.12A&B). On the other hand, the DHA level was greatly varied within the two Spanish groups (Table 3.12B), implying that diet might have a more direct effect on DHA than AA, as it is an indispensable n-3 PUFA (Cunnane et al, 1999). In a large across-country cohort study early in pregnancy, the reference Dutch group had lower DHA in plasma PL compared with the Ghanaian due to the higher fish intakes in the latter group and higher DHA than the Turkish due to the lower fish consumption in the Turkish group (van Eijsden et al, 2009).

However, our findings (Table 3.1) suggest that this rapidly growing vascular network, the human placentae, conserve AA, independent differences in maternal origin and culture. The high velocity of placental growth, will also impose a demand for AA to satisfy the proliferation of its vasculature. This implies that nutritional differences dominate over genetic contrasts. Similarly, there were no significant differences in plasma PL fatty acids between subpopulations (Creole vs. Hindustani) allowing for inclusion in one Surinamese group, despite the heterogeneity in genetic makeup, history, culture or dietary preferences within ethnic groups (van Eijsden et al, 2009).

The unique capacity of the placenta to concentrate AA, has further been demonstrated in rats fed on EFA-deficient diets during pregnancy (Menon et al, 1981). Likewise, Amusquivar & Herrera (2003) showed that when rats fed on diets free of AA (palm, sunflower, olive oil and fish oil) during pregnancy, AA was the most abundant PUFA (15-20% between groups) in the placental membranes and its proportions were not different in any of the diet groups, with exception the fish oil. The human and the rat placenta lack desaturase activity to explain these high proportions of AA (Chambaz et al, 1985; Duta-Roy, 2000b; Elmes et al, 2004).

3.4.4. Arachidonic acid in the placenta

AA was significantly reduced at term, but still the proportions are substantially higher than in maternal and neonatal (cord) plasma and red blood cells (RBC), umbilical arteries and brain (Figure 3.2) (Crawford et al, 1990, 1997; Lakin et al, 1998; Bohles et al, 1999; Rum & Hornstra, 2002; Min et al, 2004, 2005b). The abundance of AA in the placental membranes may suggest an important role in maintaining membrane structure, function and integrity. The high proportions of AA in IPG is consistent with its role as a mediator in cell signalling, activation of protein kinase C (PKC) and control of imprinting of the genes for placental and fetal growth (Das, 1999; Hindenes et al, 2000; Min & Crawford, 2004). It has also been postulated that the AA metabolites may influence uterine vascularisation and placental blood flow with a favourable balance of prostacyclin to thromboxane (Benirschke & Kauffmann, 1995; Sorem & Siler-Khodr, 1995). Since intrauterine nutrition is critical and the developing fetus depends on placental PUFA transport, a role for EFA and their long chain derivatives, in organogenesis and proper growth has been implicated (Powell et al, 1999; Mattoras et al, 2001).

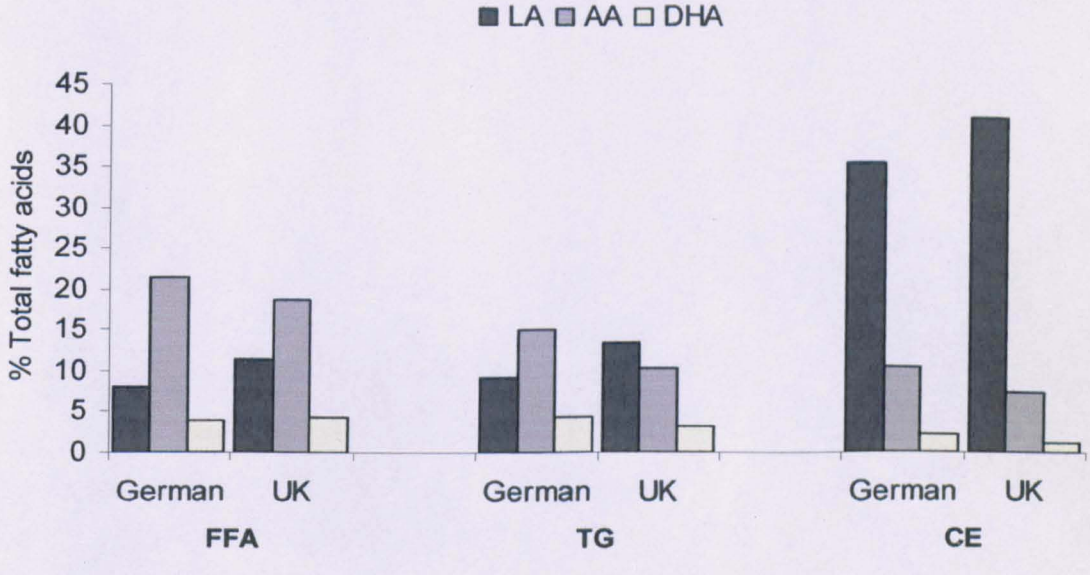


Figure 3.1. Mean percent linoleic (LA), arachidonic (AA) and docosahexaenoic (DHA) acids in free fatty acids (FFA), triacylglycerols (TG) and cholesterol esters (CE) of the German (Klingler et al, 2003) and UK placenta (this study). (Because the German data was expressed as coefficient of variation in%, statistical comparisons could not be undertaken).

% Total fatty acids		Swedish	EU (Batch A*)	UK (this study)
CPG	OA	12.5±0.9	9.5±0.0	9.85±1.5
	Σmono	-	13.0±0.2	11.2±1.6
	DGLA	4.8±0.4	4.6±0.0	4.0±0.7
	AA	20.1±1.5	18.0±0.3	17.2±2.4
	Σn-6	36.6±1.5	38.0±0.5	35.7±3.4
	DHA	2.6±0.5	1.6±0.0	1.6±0.6
	Σn-3	3.1±0.7	1.8±0.0	1.99±0.7
EPG	OA	14.4±0.6	7.4±0.1	9.30±1.4
	Σmono	-	9.7±0.3	10.0±1.4
	DGLA	4.4±0.4	3.4±0.1	3.47±0.6
	AA	28.6±2.3	28.0±0.3	24.7±1.8
	Σn-6	43.8±3.0	40.2±0.7	38.9±2.5
	DHA	10.2±1.5	8.5±0.0	8.9±2.0
	Σn-3	12.0±1.5	10.3±0.1	10.8±2.2
IPG	OA	NA	4.9±0.1	4.76±0.9
	Σmono	NA	7.4±0.2	6.03±1.1
	DGLA	NA	7.1±0.1	6.87±1.0
	AA	NA	29.2±0.4	31.9±2.5
	Σn-6	NA	39.8±0.6	43.1±3.2
	DHA	NA	1.4±0.0	2.22±0.7
	Σn-3	NA	1.9±0.1	3.43±0.9
SPG	OA	NA	15.2±0.1	13.2±1.7
	Σmono	NA	16.9±0.2	14.3±1.8
	DGLA	NA	7.5±0.3	9.15±1.4
	AA	NA	10.4±0.4	10.8±1.5
	Σn-6	NA	26.3±1.2	30.0±3.6
	DHA	NA	2.6±0.1	3.6±1.1
	Σn-3	NA	3.1±0.2	4.69±1.4

Table 3.12A. Percent unsaturated fatty acids in choline (CPG),ethanolamine (EPG), serine (SPG) and inositol (IPG) phosphoglycerides of Swedish, EU and UK term placentae, OA; oleic acid, Σmono; total monounsaturated fatty acids (MONO), DGLA; di-hommo-γ-linolenic acid, AA; arachidonic acid, Σn-6; total n-6 polyunsaturated fatty acids (PUFA), DHA; docosahexaenoic acid, Σn-3; total n-3 PUFA. Values are means ± SD, n=7 (Swedish; Percy et al, 1991); n=4 (mothers from different European countries in France; Batch A*: UK, France, Belgium, Spain; Bayon et al, 1993); n=40 (this study). NA= not analysed.

	% Total fatty acids				
	[1]British (n=10)	[2]German (n=8)	[3]Spanish (Study 1 n=78)	[4] Spanish (Study 2, n=6)	[5]French* (n=15)
AA	22.5±0.8	25.0±2.7	22.5±2.9	20.1±0.7	19.3±0.7
DHA	5.1±0.6	4.7±5.5	5.6±0.9	3.8±0.3	3.3±0.4

Table 3.12B. Percent arachidonic (AA) and docosahexaenoic (DHA) acids in total phospholipids of British, German, Spanish (2 studies) and French term placentae. Values are means ± SD. [1] Lakin et al (1998), [2] Klingler et al (2003), [3] Matorras et al (2001), [4]Varastehpour et al (2006), [5]Bayon et al (1993). *Placentae were collected in France from mothers that differ in origin (UK, France, Belgium, Spain, Thailand, Egypt, Sri Lanka, Austria, USA, Scandinavia, Pakistan, Chile and the former Soviet Union).

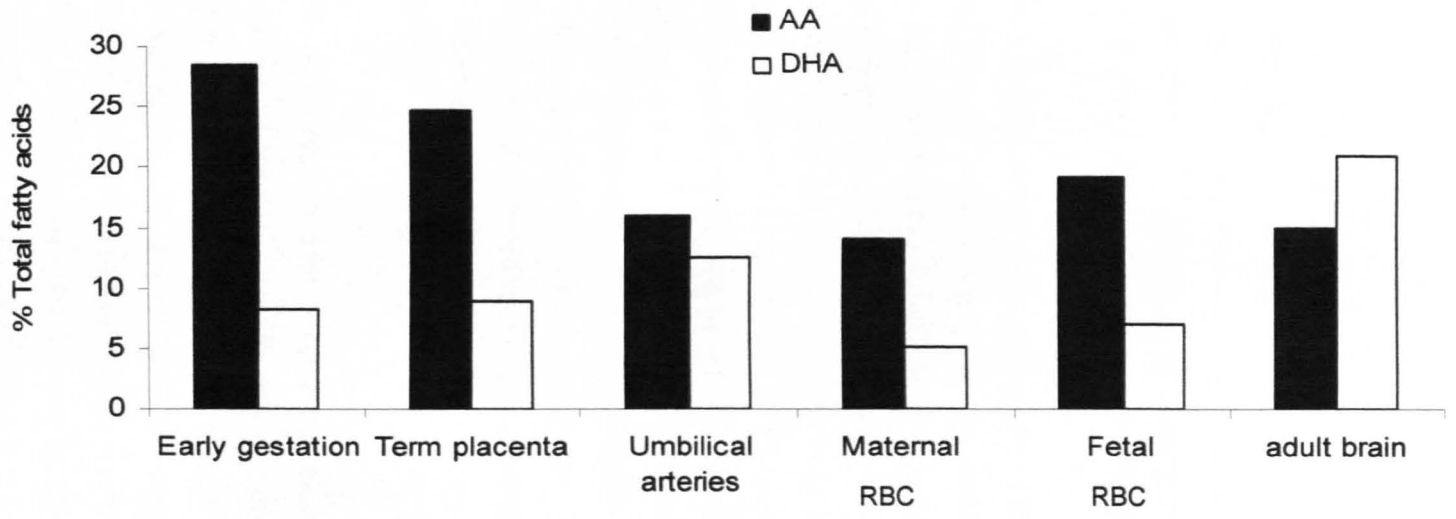


Figure 3.2. Mean percent arachidonic (AA) and docosahexaenoic (DHA) acids in ethanolamine (EPG) phosphoglycerides of early (gestation, n=63) and term (n=40) placentae, umbilical arteries (Crawford et al, 1990), maternal and fetal red blood cells, RBC (Min et al, 2005b) and adult brain (Crawford et al, 2003). (Because data on the umbilical arteries was based on a very small sample size, statistical comparisons could not be undertaken).

The lipid analysis of the early placentae is especially interesting. During this transient period of development, which is associated with uteroplacental vascularisation and organogesesis (Burrows et al, 1996; Pijnenborg, 1998; kingdom et al, 2000a; Dantzer et al, 2000; Kliman, 2000) the data showed that independent of differences in maternal origin and ethnicity the placenta captures remarkably high amounts of AA from its inception. This finding is in line with several experimental studies supporting an important role for AA and AA metabolites in the mediation of metabolic and endocrine function of ovarian and placental cell membranes and in the establishment and maintenance of pregnancy (Linton & Whitehead, 1980; Hellberg et al, 1996; Pawlosky & Salem, 1996; Lim et al, 1997, 1999; Mikuni et al, 1998a&b; Niswender et al, 2000; Paria et al, 2000; Gaytan et al, 2003, 2006; Li et al, 2006; Burns et al, 2008; Kurusu et al, 2009) or human and animal studies showing that the n-6 PUFA are involved testes development (Retterstol et al, 2001; Merrells et al, 2009) and that exogenous AA, in human capacitated spermatozoaria, can induce the reaction of sperm acrosome (Mack et al, 1992).

AA was incorporated in high proportions (18.9-35.9%) in most phospholipids between 8 weeks (wk) and 14wk of gestation. The embryoplacental circulation is effective by 10 wk and the first extra-embryonic vessels are paired veins and arteries communicating directly with the embryonic cardiovascular system (Kingdom et al, 2000b). The first heartbeat can be detected by highly sensitive ultrasound techniques as early as four and a half weeks of gestation (Kingdom et al, 2000b). Moreover, PKC is involved in AA-induced human placental lactogen (hPL) release from trophoblast cells (Boone et al, 1993). hPL is a peptide hormone that contributes to the proliferative changes in the mammary tissue and stimulates an increase in the maternal plasma levels of glucose (the so-called anti-insulin or diabetogenetic effect), amino acids and FFA to ensure optimal placental transport of essential metabolites to the fetus (Pocock et al, 2004). hPL is released at around 10 wk of gestation (Pocock et al, 2004) and it is not dependent on further AA metabolism (Boone et al, 1993).

There is also evidence that AA but not eicosapentaenoic acid (EPA) or DHA induces differentiation of uterine stromal and decidual cells (Tessier-Prigent et al, 1999). Its turnover in nuclear membranes in stromal cells during proliferation is particularly high (Delton-Vandenbroucke et al, 2004). The early membranes –enriched in AA may further support the role of AA cascade in early neurogenesis. The AA metabolite, prostaglandin E2 (PGE_2) is a lipid messenger and its generation increased in early mice embryo organogenesis for a brief period preceding neural tube closure (Piddington et al, 1996; Higa et al, 2007). It has also been postulated that PGE_2 may participate in the control of fetal breathing movements and be involved in the change in control of breathing at birth and in the postnatal control of breathing (Kitterman, 1987). The high AA in the placental

membranes (this study), cord plasma in the late preterm (GA=33wk) (Sabel et al, 2009) and in cord blood mononuclear cell (CBMC) membranes at term (Moodley et al, 2009) may further imply that AA is important for placentofetal vasculature development, and fetal and neonatal growth.

Thus, the placenta is an in utero developed fetal organ (Pardi & Cetin, 2006) and a defect in the invasion process of trophoblasts may result in uteroplacental insufficiency leading to vascular injury and increased capillary permeability associate with preeclampsia, miscarriage, preterm labour and IUGR (Norwitz, 2006). Similarly, loss of the modulatory role of endothelium and enhanced vasoconstriction eicosanoids predominate in the diabetic vasculopathy, and vascular dysfunction in the placenta may progress the development of gestational diabetes mellitus, GDM (Lappas et al, 2004; Akamine et al, 2006). GDM, a good example of *in utero* disturbances, is associated with abnormal lipid metabolism (Lindegaard et al, 2006) and, hence, the following study investigates placental PUFA in diabetic and early labour pregnancy.

3.4.5. Early Vs term placental arachidonic acid

A secondary aim of this study was to test if the AA content of the placental membranes increased towards term consistent with increased vascularisation and reached a critical level that could initiate parturition through prostaglandins (PG) derived from AA. The data presented here showed that the proportions of AA decreased and the only eicosanoid precursor to increase in any phosphoglycerides was DGLA (Table 3.4-3.7). The DGLA derivative PGE_1 is functionally opposite of the muscle contracting and vascular constrictor properties of thromboxane A_2 (TXA_2) and prostaglandin F2- alpha ($PGF_{2\alpha}$).The AA derived $PGF_{2\alpha}$ has been used routinely as an abortifacient (Boone et al, 1993). The reduction of AA and increase of DGLA suggests a physiological design more relevant to muscle relaxation and the prevention of vascular occlusive events.

Kuhn et al (1990) found that little or no muscle contracting eicosanoids were produced from AA in the *ex vivo* perfusion of the human placental lobe. However, successive wave pressures quickly elicited prostaglandin production. Our results support Kuhn's conclusion that the high content of AA maybe also be a 'fail safe' mechanism rather than an initiator of parturition. Additionally, culture systems demonstrated that AA is more readily released to its nonesterified state than it can be converted to prostanoids by cyclooxygenase, COX (Ogburn et al, 1988). Moreover, oppose to its adverse effect as an inflammatory and vasoconstrictor agent, it is evident that AA itself causes relaxation of blood vessels in the presence of the endothelium and also that AA, itself in the presence of diabetes, does not

induce vasoconstriction of the perfused rat kidney (Kamata et al, 2006a).

In contrast, when ewes were fed on a diet supplemented with LA for six wk during pregnancy, they went on early labor due to the increased AA metabolites, PGE_2 and PGF_{2a} within the placenta (Elmes et al, 2004). However, the placenta AA composition did not show prominent changes to suggest a cause of early labor and, similarly to the human data (herein), there was a two-fold increase in DGLA in the fetal allantochorion (Elmes et al, 2004). Considering that enhanced PG production in maternal circulation increases near term (Boone et al, 1993), increased mobilization of AA in the preparatory phase 1 of parturition, remains to be established.

3.4.6. Docosahexaenoic acid in the placenta

Once FFA enter the placenta are re-esterified in the different placental lipid classes PL, TG and CE and hydrolysed for later release in the fetal circulation (Buhl et al, 1995; Lindegaard et al, 2006). The data at 8-14 wk of gestation showed a preferential incorporation of DHA (7.33%) in TG. This is in line with other reports (Campbell et al, 1997; Waterman et al, 1998; Dutta-Roy, 2000b) suggesting that once FA are taken up by trophoblast cells, DHA is preferentially incorporated into placental TG. Additional data obtained from studies on the comparative incorporation of AA and DHA into retina cells and rat brain and placenta, agreed that DHA is preferentially used over AA for the synthesis of NL (Furchgott, 1984; Crabtree et al, 1998; Amusquivar & Herrera, 2003).

Moreover, similarly to the PUFA composition of placental PL and TG in early human pregnancy (herein), the yolk sac membrane of the chicken embryo showed that AA is incorporated into PL and DHA in the TG (Speake et al, 2003). The similarity between these two studies is striking. It was proposed that the presence of DHA in TG most likely reflects the distribution of this n-3 PUFA among the lipid classes of the nascent VLDL and may be related to the need to distribute this FA to specific embryonic tissues (Speake et al, 2003).

At term, DHA was reduced in NL, but markedly in TG (57%). It is conceivable that the placenta may contribute to the hypertriglycaemia of pregnancy by producing TG in vivo and releasing them to the maternal circulation (Ogburn et al, 1988), suggesting a primary role of placental TG in the transport of DHA into the fetal circulation (Campbell et al,1997; Crabtree et al, 1998; Dutta-Roy, 2000b; Haggarty, 2004). Hence, if the placenta is involved in initiating the mobilisation of FA from the maternal adipose tissue in response to fetal needs (Haggarty, 2004), then placental TG may act as a reservoir of DHA during the

third trimester that fetal fat deposition is enhanced (Bonet et al, 1992; Waterman et al, 1998; Herrera, 2002; Haggarty, 2002; Magnusson et al, 2004; Gauster et al, 2007).

The reduction in esterified AA (PL and NL) and DHA (NL) at term may suggest that placental lipases are important for transplacental FA transport (Smith et al, 1992; Bonnet et al, 1992; Buhl et al, 1995; Waterman et al, 1998; Campbell et al, 1998a; Dutta-Roy, 2000b; Handling & Bocking, 2001; Herrera, 2002; Amusquivar & Herrera, 2003; Larque et al, 2003; Magnusson et al, 2004; Lindegaard et al, 2006; Gauster et al, 2007). Through this mechanism maternal plasma TG are hydrolysed and taken up by the placenta, where re-esterification and intracellular hydrolysis facilitate diffusion of the released PUFA to the fetus, and their subsequent transport to fetal liver (Herrera, 2002).

3.5. Conclusion

The remarkably high proportions of AA in early placenta raises the speculation that AA may participate in early developmental processes such as implantation, vascular growth and hence organogenesis. The sequestering of AA and enrichment of term membranes with DGLA in normal pregnancies, suggests a role for the n-6 PUFA favoring blood flow. With the current popular enthusiasm for n-3 PUFA, the significance of the n-6 PUFA both in relation to developmental mechanisms and the nutritional requirements for the mother and fetus needs re-evaluation. On the other hand, the NL composition suggests that the preferential incorporation of DHA in placental TG could support an important role in providing DHA to the fetus.

CHAPTER 4. PLACENTAL FATTY ACIDS IN PREGNANCIES COMPLICATED WITH GESTATIONAL DIABETES MELLITUS AND PREMATURITY: A COMPARATIVE STUDY

4.1. Introduction

Prematurity is the most common cause of perinatal death, neonatal morbidity (O'Connor & Kovacs, 2003; Gilbert-Barness & Debich-Spicer, 2004) and childhood disability (Kingdom et al, 2000a). The haemorrhage of the chick cerebellum, during nutritional encephalomalacia, resembles the clinical disorder of periventricular haemorrhage in babies and the haemolysis in marmosets is similar to that developed by the preterm infants (Harbige et al, 1990; Phylactos et al, 1994). Preterm birth occurs in 5-10% of all pregnancies (Challis et al, 2002; Robertson et al, 2006).

In the later part of pregnancy, both first and second phase insulin secretion increases (Buchanan et al, 1990) in order to compensate for pregnancy-induced insulin resistance and to maintain normal carbohydrate tolerance. Those women, 3-5% (Hollingsworth, 1985; Engelgau et al, 1995), who fail to respond to the changes in insulin resistance, develop transient diabetes, GDM that resolves after delivery. However, rates of prevalence vary from 1-14%, due to differences in the criteria used to diagnose GDM and the population studied (Kyriakidis et al, 2005; Perkins et al, 2007; Bentley-Lewis et al, 2008). In addition, with the increase in maternal body mass index in pregnancy, the risk of preterm delivery and other maternal complications increases, suggesting a complex obesity-prematurity relationship (Madan et al, 2010).

In human (Type 1 and 2) and experimental diabetes, the activity of Δ-6 and -5 desaturases, vital for the synthesis of arachidonic (AA, 20:4n-6) and docosahexaenoic (DHA, 22:6n-3) acids are impaired (El Boustani et al, 1989; Arisaka et al, 1991; Brenner et al, 2000) and the levels of the fatty acids (FA) are reduced in membranes (Tilvis & Miettinen, 1985; Mikhailidis et al, 1986; Igal et al, 1991). DHA is the essential cell membrane constituent of the brain and visual system and AA of the vascular endothelium, brain (Anderson & Maude, 1972; Crawford & Sinclair, 1972; Uauy & Hoffman, 2000; Crawford et al, 2003) and the placenta (Chapter 3), and the requirements of both AA and DHA are increased during pregnancy (Clandinin et al, 1980; Martinez, 1992).

The fetus and neonate are able to synthesise AA from linoleic acid (LA) and DHA from α-linolenic acid (ALA) (Chambaz et al, 1985; Poisson et al, 1993; Descomps & Rodriguez,

1995; Salem et al, 1996; Carnielli et al, 1996), however, the rate of synthesis is not fast enough to support optimal growth and development (Farquharson et al, 1992; Makrides et al, 1994). Indeed, the high neonatal demand for AA was best demonstrated in an IBCHN study, revealing that the plasma AA and DHA were reduced by about 47% and 32%, respectively, between birth and postnatal age of 2 wk in preterm baby fed on formula milk containing LA (Leaf et al, 1992a; Ghebremeskel et al, 1995). Moreover, the biomagnifications process (Crawford et al, 1976) which enhances the 20 and 22 carbon chain length polyunsaturated fatty acids (PUFA) for the fetus also reduces the precursors of AA and DHA which in the case of the n-3 PUFA results in negligible amounts in the fetal circulation and liver making any conversion capability academic.

The IBCHN data also showed that the preterm and low birthweight (LBW) infants were born with reduced and greatly variable proportions of AA and DHA (Leaf et al, 1992b; Crawford et al, 1989, 2003) and of low antioxidant defence mechanisms (Phylactos et al, 1994). Additionally, the IBCHN provided with evidence that the proportions of these long chain polyenoic FA were compromised in neonates of Type 1 (Ghebremeskel et al, 2004), Type 2 diabetic (Min et al, 2005a) and gestational diabetic women (Min et al, 2005b; Thomas et al, 2005). Women with gestational diabetes mellitus (GDM) had lower AA and DHA in red cells (Min et al, 2004) but not in plasma (Thomas et al, 2004) as oppose to the controls. Since maternal plasma AA and DHA, which is the main source for the fetus, was unaltered in the GDM women, it is unclear whether the observed compromise in the level of the two PUFA in the neonates (Min et al, 2005b; Thomas et al, 2005) was due to reduced supply, impaired transfer or both.

As the placenta is solely responsible for the selective transfer of AA and DHA to the foetus, the placenta of gestational diabetics and mothers who delivered preterm may retain the nutrients instead of transferring to the foetus, hence, the aim of this study was to investigate whether GDM or preterm delivery, known to be associated with vascular disorders, are both associated with a distortion in the placental membrane lipids, especially with regard to AA and DHA.

4.2. Methods

4.2.1. Subjects and Selection criteria

Total eighty, twenty-nine preterm, eleven gestational diabetic and forty control (Chapter 3) women with uncomplicated singleton pregnancy were enrolled on admission for delivery at Newham General Hospital, London, UK (Ethical approval see section 2.2.1.).

All subjects had no obstetric or clinical complications. The placental, cord and fetal membranes were examined in the delivery room by the physician in charge. The preterm delivery presented accelerated placental maturation, abnormal bleeding, chorioamnionitis or prolonged rupture of the membranes, but the mothers were not diabetics or had any other condition that was not related to their pregnancy. Their history was checked from the clinic's antenatal record. Additional data from the antenatal record included maternal ethnicity, age and body mass index (BMI) at first visit, fetal and placental measurements and mode of delivery; however as in Perry (1995), complete data from the antenatal and labour ward record were not always available. The missing data could also be explained by the absence of systematic validation of the midwife during certain periods (Ego et al, 2006).

The dating criteria used to calculate gestational age (GA) were based on the patient's last menstrual period (LMP) and was confirmed or corrected by routine ultrasonography (Blake et al, 2001; Hafner et al, 2003; Di Cianni et al, 2005; Kale et al, 2005; Swamy et al, 2008), usually late in gestation (18-20wk) to establish maturity and to exclude fetal anomalies (Lao & Ho, 2001). Exceptions were few preterm cases, particularly mothers with emergency caesarean section, where GA was based on LMP only. Both GDM and control delivered at term (37-41 wk of gestation). GA of the preterm group ranged between 24 and 36 wk and those with a GA below 30 wk were classified as very preterm (n=7). The preterm babies were healthy in that they were not suffering nosocomic infection nor infection acquired *in utero* or inflammation reactions (Moodley et al, 2009) or congenital malformations (Sabel et al, 2009) and the GDM in that they had no signs of fetal malformations or abnormal karyotypes or signs of distress (Ortega-Senovilla et al, 2009).

Twenty one (n=21) of the women in pre-term labor were administered dexamethasone (12 mg) prior to delivery and a second dose (n=14) was received approximately 12h after the first dose (Moodley et al, 2009) to those who remained undelivered and were still at risk of preterm labor. Two preterm pregnant (n=2) were not administered steroids and six (n=6) were

undeclared due to incomplete notes or spontaneous preterm labour, prelabour rapture of the membranes or elective preterm delivery.

Where reported last ultrasound examination (within 4-7d before delivery), provided an assessment of several foetal biometric parameters (listed in section 4.2.3.). Eventhough, ultrasound measurements provide only estimates of these measures as oppose to growth charts or measurements at birth (Fenton, 2003), these data were only available from the antenatal record. On the other hand, birthweight (BWT) and placental weight (wt) were assessed at delivery. Infants with BWT above 2,499 (g) were classified as normal, those with BWT between 1,500 and 2,499 (g) as low birthweight (LBW), those below 1,500 (g) as very low birthweight (VLBW) (Jewell et al, 2001; O' Connor & Kovacs, 2003) and those <1000g, based on international standards, as extremely-LBW (Jewell et al, 2001).

With regards to GA, the most commonly used threshold for identifying small for gestational age (SGA) is the 10th percentile (Lao & Ho, 2001; Fenton, 2003; Di Cianni et al, 2005; Ego et al, 2006; Mori et al, 2006; Espinoza et al, 2007a&b; Mazaki-Tovi et al, 2009; Ayaz et al, 2009; Moodley et al, 2009). Herein, SGA were defined as those with a BWT at the 10th percentile or below in order to include the whole spectrum of SGA infants (Goldenberg et al, 1998; Moodley et al, 2009); because SGA fetuses may be constitutionally small (40%) and not pathologically growth restricted (also 40%) (http://emedicine.medscape.com/article/261226-overview). The remaining 20% of the SGA fetuses are intrinsically small secondary to a chromosomal or environmental etiology (http://emedicine.medscape.com/article/261226-overview).

Infants with BWT lying above the 10th and below the 90th percentile were appropriate for gestational age (AGA) and those above the 90th percentile were large for gestational age (LGA) calculated from a birth chart customized for sex, BWT and GA (Lao & Ho, 2001; Di Cianni et al, 2005; Mori et al, 2006; Espinoza et al, 2007a&b; Mazaki-Tovi et al, 2009; Ayaz et al, 2009; Moodley et al, 2009). Macrosomia was diagnosed for neonatal body wt≥4Kg or as an LGA infant (Buchanan et al, 1994; Di Cianni et al, 2005; Boney et al, 2005; Kale et al, 2005; Jansson et al, 2006; Khan et al, 2007; Ayaz et al, 2009).

4.2.2. Diagnosis of diabetes and treatment

Usually, pregnant women underwent a glucose challenge test (GCT) between 24th and 30th wk of gestation (Ramachandran et al, 1998, Lepercq et al, 1999; WHO, 2000; Di Cianni et al, 2005; Pietryga et al, 2006; Mazaki-Tovi, 2009).

In this study, however, all of the women were assessed for diabetes as part of antenatal care at 16 wk of gestation due to a high prevalence of the disease in East London. Those women considered to be at high risk – BMI greater than 30, polycystic ovary syndrome (PCOS) and a history GDM, insulin resistance (IR), pregnancy-induced hypertension, stillbirth and macrosomia - were screened for GDM with an oral glucose tolerance test (OGTT) at gestation wk 28. Similarly, Cosson et al (2006), women with a history of GDM or who had more than two risk factors of GDM were screened at 15 wk and if negative, it was repeated after 24 wk of gestation.

Blood glucose level was determined after an overnight fast. The women with a fasting blood glucose value of greater than 7 mmol/l were given Polycal (Nutricia Zoetermeer, the Netherlands), a high-energy carbohydrate supplement (Table 4.1) equivalent to 75g glucose load by diluting a 113 ml of Polycal to 200 ml with water; the supplement residuals were rinsed with 100 ml of water. GDM was diagnosed if the glucose concentration of blood taken at 120 min was greater than 7.8 mmol/l (World Health Organisation criteria, WHO) (WHO, 2000; Schmidt et al, 2001).

Herein, women with PCOS were considered at high risk (Ong & Dunger, 2004; Perkins et al, 2007), because PCOS is associated with an increased incidence of obesity. The metabolic, hormonal and reproductive alterations associated with PCOS can be influenced by dietary modification and wt loss (Kind et al, 2006). Subsequent to diagnosis, four of the GDM subjects were treated with diet, four with insulin, one with both insulin and diet and two did not receive any treatment. Self-monitoring of blood-glucose level or insulin administration and dietary counselling are the current treatments for GDM, when necessary (Lao & Ho, 2001; Harizopoulou et al, 2008; Ayaz et al, 2009; Mazaki-Tovi et al, 2009). Insulin therapy is given if dietary readjustment (>2wk) fails to maintain the fasting glucose levels less than 6.0mmol/L or postprandial glucose less than 7.1mmol/L (American Diabetes Association, 2000, 2003, 2009; Lao & Ho, 2001; Kyriakidis et al, 2005; Ayaz et al, 2009) or to normalize levels (Kale et al, 2005).

4.2.3. Foetal biometric parameters

a) **Birth and placental weight:** measured to the nearest gram (g).

b) **Placental ratio:** calculated as the placental weight (wt) divided by the birthweight (BWT). Placental ratio considered high, if > 0.2095 (Lao & Ho, 2001).

c) **Fetal:placental wt:** reverse placental ratio, calculated by dividing BWT with placental wt. Normal ratio =6, at delivery (Pardi & Cetin, 2006).

d) **Biparietal diameter (cm):** The transverse width of the head measured from the leading edge to the leading edge of the bones (the diameter between the two sides of the head) (www.ob-ultrasound.net).

e) **Head circumference (cm):** measured with a non-stretchable tape to the nearest 0.1 cm as the maximum occipito-frontal circumference (Dewbury et al, 1993).

f) **Abdominal circumference (cm):** measured at the widest point in the abdomen, through the liver at the level of the left portal vein or stomach. Abdominal circumference (AC) reflects fetal size and weight rather than fetal GA (www.ob-ultrasound.net).

g) **Femur length (cm):** a measurement of the longest bone in the body, reflecting the longitudinal growth of the fetus (www.ob-ultrasound.net). Femur length (FL), also known as femur diaphysis length (Salpou et al, 2008), is reliable and best measured after 14 wk (Dewbury et al, 1993).

4.3. Sample collection

Term GDM, control (37-41 wk) and preterm placentae (24-36 wk of gestation) sample collection at delivery and treatment was in agreement with other studies' protocol (Percy et al, 1991; Lafond et al, 1993; Bayon et al, 1993; Anand et al, 1996; Powell et al, 1999; Wheeler et al, 1999; Lao & Ho, 2001; Klingler et al, 2003; Jain et al, 2004; Lappas et al, 2004, 2005; Wang et al, 2005; Talas et al, 2008; Natarajan et al, 2010); all agreed that placentae should be collected between 10 and 30 minutes after delivery (section 2.2.1.2.).

4.3.1. Sample size and statistics

These are described at sections 2.4.1. & 2.4.2.

4.4. Results

4.4.1. The morphology of the human placentae

An assessment of total nineteen cases of the control group, showed that the mature normal placentae varied in shape [discoid (n=10) or oval (n=9)], diameter (between 13.0 x16.0 and 21.0x22.0cm) and thickness (2.0-3.0 cm), umbilical cord length (32-64 cm) and cord diameter (0.8-2.0 cm). Nonetheless, a placenta of larger (4 cm) thickness and one of smaller thickness (1.5cm) than the range (above) and a case with long cord (~70cm) were reported. Placental insertions of the cord were either marginal or marginal and eccentric, but not markedly eccentric. In two cases placental insertion of the cord was central and in another two cases was defined as eccentric and partially velamentous.

Four (n=4) GDM mothers, treated with either diet or diet and insulin, delivered large placentae (>650g up to 820g). Additionally, one of these was characterised by adherence of clots and had a cord with marginal and velamentus origin of insertion. Additionally, a case with true knot on the cord and one case with long cord (>70cm) with a knot and adherence of clots were reported. Of the pre-term group, eight delivered small placentae (250-400g), including a placenta with long stringy cord and a true knot and two vessels only (emergency caesarean). Accelerated placental maturation, abnormal bleeding, infection, chorioamnionitis, prolonged rupture of the membranes, failure of pregnancy to progress, breech presentation and continuous uterine contractions associated with cord abnormalities resulted in preterm or very preterm birth. Four mothers had elective and twelfth had emergency caesarean.

4.4.2. Clinical data

Baseline characteristics of the population studies are shown in Table 4.2 [Abdominal circumference (AC), biparietal diameter (BPD) and femur length (FL) measurements or mean birhtweight (BWT) at term are agreeable to published data (Dewbury et al, 1993; www.ob-ultrasound.net) or to the reported average BWT (3.2 Kg) of the full-term infant (www.moreproductinfo.com/manuals/2663.pdf)]. The majority of GDM who delivered at term were Asians whilst small numbers were from other ethnic groups (Table 4.2). The women

with a preterm birth were mainly Asians and Caucasians and a small number were Africans (n=3).

The GDM mothers were older and heavier (pre-pregnancy BMI) than the control, $P<0.05$ (Table 4.2). The term GDM did not match the control with respect to gestational age, GA ($P<0.0001$) and BPD ($P<0.05$). Nonetheless, placental wt (g), BWT, placental/fetal wt, placental ratio (fetal/placental wt ratio), head circumference (HC), AC and head-to-abdominal circumference ratio (HC/AC) (Table 4.2) were comparable between the two groups ($P>0.05$).

Whilst, the preterm matched with the control group with respect to age in years (y), pre-pregnancy BMI (Kg/m^2), placental ratio, and HC/AC $P>0.05$ (Table 4.2). The two groups (preterm vs. control) differed though in femur length (FL, $P<0.005$) and placental/fetal wt ratio ($P<0.05$). Moreover, the preterm differed from both control and GDM, with respect to BWT ($P<0.0001$ in both groups), placental wt ($P<0.005$ and $P<0.05$), HC and AC ($P<0.0001$ and $P<0.005$) and BPD ($P<0.0001$ and $P<0.05$ respectively).

4.4.3. Relationship of birth and placental weight with the biometric measurements of the fetus

Correlation coefficients for the relationship between maternal, fetal and placental characteristics are presented in Table 4.3. Maternal age, BMI, ethnicity, gender and mode of delivery show no significant correlations with any of the foetal measurements of biometry, placental wt or ratio ($P>0.05$).

In multiple regression analysis, the contribution of GA was significant for the prediction of BWT ($r^2=0.52$), placental wt ($r^2=0.21$) and placental ratio ($r^2=0.37$), $P<0.005$ (Table 4.4). In the stepwise multivariate regression analysis in which placental wt was the continuous variable to be explained (Table 4.5), when BWT entered the model, BWT was significantly associated with placental wt ($r^2=0.91$, $P<0.0001$). Similarly, when placental ratio was the dependent variable in the regression model of analysis, the contribution of the preterm birth ($r^2=0.47$, $P<0.05$) and HC ($r^2=-0.94$, $P<0.005$) was significant for the prediction of placental ratio (Table 4.5). However, these relationships (Table 4.5) between BWT and placental wt and between prematurity, HC and placental ratio were not significant when adjusting placental and foetal characteristics for AGA, $P>0.05$.

Average contents		Per 100ml	Per 100Kcal
Energy :	Kcal	247	100
	KJ	1050	420
Protein:	g	-	-
Carbohydrates:	g	61.9	25.1
Polysacharides	g	50.7	20.5
Sugars:		10.6	4.3
Glucose	g	2.2	0.9
Maltose	%	8.4	3.40
% of total Energy	%	100	100
Fat:	g	-	-
Dietary Fibre:	g	-	-
Minerals:	mg (mmol)		
Sodium		≤5 (≤0.22)	≤2.02 (≤0.09)
Potassium		≤5 (≤0.13)	≤2.02 (≤0.05)
Chloride		≤5 (≤0.14)	≤2.02 (≤0.06)
Calcium		≤5 (≤0.13)	≤2.02 (≤0.02)
Phosphorus		≤5 (≤0.16)	≤2.02 (≤0.06)
Magnesium		≤5 (≤0.21)	≤2.02 (≤0.08)
Water:	g	61	25
Osmolarity	mOsmol/l	900	900
Osmolality	mOsmol/Kg H_2O	1470	1470
Potential renal solute load	mOsmol/l	1	1

Table 4.1. Nutrient composition of Polycal liquid; a high energy carbohydrate supplement used in diagnosis of diabetes (section 4.2.2.) based on glucose syrup, presented in 200ml crown cap, glass bottle (Nutricia Zoetermeer, The Netherlands).

	GDM (n=11)	Pre-term (n=29)	Term (n=40)
Maternal characteristics			
Age (y)	32.6±3.27	30.0±5.31 (n=27)	29.1±5.58 (n=26)
Range	(27-37)	(18-40)	(20-41)
Pre-pregnancy BMI (Kg/m^2)	30.1±3.54	26.5±7.24 (n=20)	24.4±4.59 (n=19)
<25	1	12	12
>25	10	8	7
Ethnicity			
Caucasian	-	11	8
African	4	3	10
Asian	7	15	22
Mode of delivery (c/v)	8/3	16/12	9/31
Undeclared	-	1	-
Ultrasound measurements	37-39 wk	24-36 wk	37-41 wk
Head circumference (HC, cm)	33.6±1.71 (n=10)	29.0±4.68 (n=23)	33.5±1.16 (n=21)
Abdominal circumference (AC, cm)	30.7±2.44 (n=9)	24.8±5.47 (n=16)	31.8±1.89 (n=12)
HC/AC	1.07±0.08 (n=8)	1.13±0.12 (n=16)	1.05±0.05 (n=12)
Biparietal diameter (BPD, mm)	85.8±5.72 (n=9)	71.6±16.1 (n=17)	90.3±2.52 (n=11)
Femur length (FL, mm)	67.0 (n=4)	53.0±14.1 (n=14)	70.4±5.64 (n=9)

Table 4.2. Relevant clinical data, GDM; gestational diabetes mellitus, y; years, BMI; body mass index, c/v; caesarean/vaginal delivery, HC/AC; head-to-abdominal circumference ratio.

	GDM (n=11)		Pre-term (n=29)		Term (n=40)	
Neonatal measurements						
Gestational age (GA) at delivery	38.0 ±0.78		32.3±3.72		39.8±1.19	
Term (≥37 wk)	11		-		40	
Pre-term (30 wk -36 wk and 6d)	-		22		-	
Very pre-term (<30 completed wk)	-		7		-	
BWT (g):	3251.8±674.6		2123.5±696.2 (n=28)		3212.2±530.8 (n=40)	
Normal (>2499)	9		7		38	
LBW (1500-2499)	2		16		2	
VLBW (<1500)	-		5		-	
Size for gestational age:						
LGA (>90th centile)	3		5		7	
AGA (90th < GA >10th centiles)	8		21		31	
SGA (<10th centile)	-		2		2	
Placental wt (g)	640.0±176.7	(n=7)	404.1±99.6	(n=15)	541.3±125.1	(n=24)
Placental ratio and when	0.19±0.02	(n=7)	0.25±0.14	(n=14)	0.17±0.04	(n =22)
placental ratio adjusted for AGA	0.18±0.03	(n=4)	0.28±0.15	(n=10)	0.17±0.04	(n=21)
or fetal/placental wt and when	5.42±0.63	(n=7)	5.09±2.36	(n=14)	6.06±1.24	(n=22)
fetal/placental wt adjusted for AGA	5.61	(n=4)	4.38±1.91	(n=10)	6.01±1.30	(n=21)
Sex (M/F)	5M/6F		13M/16F		21M/19F	

Table 4.2. (continuous) Relevant clinical data, GDM; gestational diabetes mellitus, BWT; birhtweight, LBW; low birthweight, VLBW; very low birthweight, LGA; large for gestational age, AGA; appropriate for gestational age, SGA; small for gestational age, wt; weight, M/F; male/female.

Study groups			r	n	P
GDM	AC vs.	BWT	+0.88	10	<0.005
Preterm	HC vs.	BWT	+0.73	22	<0.0001
	AC vs.	BWT	+0.85	15	<0.0001
	HC/AC vs.	BWT	-0.60	15	<0.0001
	FL vs.	BWT	+0.66	13	<0.05
	Placental ratio vs.	BWT	-0.88	14	<0.0001
	HC vs.	AC	+0.78	16	<0.0001
	HC vs.	FL	+0.95	14	<0.0001
	HC vs.	BPD	+0.84	16	<0.0001
	FL vs.	AC	+0.91	14	<0.0001
	FL vs.	BPD	+0.93	14	<0.0001
	BPD vs.	AC	+0.81	16	<0.0001
Control (Term)	HC vs.	BWT	+0.66	21	<0.005
	AC vs.	BWT	+0.78	12	<0.005
	HC vs.	AC	+0.69	12	<0.05

Table 4.3. Correlations between foetal measurements of biometry and birth, placental weight (wt) and placental ratio in study groups and control, GDM; gestational diabetes mellitus, AC; abdominal circumference, BWT; birthweight, HC; head circumference, HC/AC; head-to-abdominal circumference ratio, FL; femur length, BPD; biparietal diameter, r; strength of associations (Pearson's coefficient or Spearman's rank coefficient when sample size was less than 20, n<20).

		r^2	B	95% CI for B			T	*P*
				Lower		Upper		
BWT	vs.:							
	GA	0.52	+0.72	111.9	-	174.8	9.06	<0.0001
Placental wt	vs.:							
	GA	0.21	+0.45	5.91	-	23.9	3.34	<0.005
Placental ratio	vs.:							
	GA	0.37	-0.61	-0.02	-	-0.01	-5.08	<0.0001

Table 4.4. Factors associated with gestational age (GA) in normotensive women who delivered between 24-41 wk of gestation (n=69), BWT; birthweight, r^2; regression squared, B; standardised coefficients, CI; confidence intervals, T; slope coefficient/standard error (multiple regression analysis).

	B	T	95% CI			P
Placental wt (g):			Lower		Upper	
Preterm birth (1/0)+	0.24	1.57	-27.9	-	189.1	>0.05
BWT (g)	0.91	4.40	0.10	-	0.28	<0.0001
HC	-0.26	-1.41	-30.5	-	6.05	>0.05
Placental ratio:						
Preterm birth (1/0)+	0.47	2.20	0.002		0.10	<0.05
BWT (g)	0.0 2	0.06	0.000		0.000	>0.05
HC	-0.94	-3.65	-0.02		-0.006	<0.005

Table 4.5. Factors associated with placental weight (wt) and placental ratio in normotensive women delivered at term vs. preterm (n=69), BWT; birthweight, HC; head circumference, B; standardised coefficients, CI; confidence intervals, T; slope coefficient/standard error (Multiple regression analysis). +Preterm=1; Term (GDM and control) =0. However, when adjusted for AGA, $P>0.05$.

4.4.4. Placental lipid content

Placental total lipid (mg/ g tissue, blotted) was higher in the GDM (n=11, 15.8±1.02) compared with the control (n=40, 13.8±2.51) and the preterm (n=29, 10.8±1.76) groups ($P<0.05$ and $P<0.0001$, respectively). The preterm had lower total lipid compared with the control group ($P<0.0001$).

4.4.5. Placental fatty acid composition

Percentage linoleic (LA) arachidonic (AA) and docosahexaenoic (DHA) acids in relation to ethnicity are presented in Table 4.6. The pre-term and the very-preterm groups or the appropriate for gestational age (AGA) and the low for gestational age (LGA) newborn of the control mothers had comparable placental LA, AA and DHA content ($P>0.05$). Fatty acid composition (% total fatty acids) of the individual placental lipid components is presented in Table 4.7-4.14.

4.4.5.1.Phosholipids

4.4.5.1.1. Choline phosphoglycerides- fatty acids

Of the LA metabolites, in choline phosphoglycerides (CPG), the proportions of 20:2n-6, AA, docosatetraenoic (DTA) and docosapentaenoic (DPA n-6) acids n-6 were higher in the gestational diabetes mellitus (GDM) compared with the control (P<0.01, P<0.05, P<0.005, P<0.005) and the preterm groups (P<0.0001, P<0.005, P<0.01, P<0.05 respectively). Consequently, total n-6 (Σn-6) polyunsaturated fatty acids (PUFA) were enhanced in the former group (P<0.005) (Table 4.7). Likewise, 20:1, docosapentaenoic acid n-3 (DPA n-3) and DHA were elevated in the GDM compared with the control (P<0.0001, P<0.01, P<0.05) and the preterm (P<0.0001, P<0.005, P<0.0001 respectively) groups (Table 4.7). Similar to the GDM, the control had higher proportions of DHA compared with the preterm group (P<0.05).

In placental CPG, the percentage total n-3 PUFA (∑n-3) was higher in the GDM compared with the preterm (P<0.005). However, the diabetics had lower 18:3n-6 compared with the preterm (P<0.01), palmitoleate compared with the control (P<0.005) and palmitate (P<0.0001) and total saturated fatty acids (∑SFA, P<0.005) compared with both the control and the preterm groups (Table 4.7). The preterm had higher proportions of the parent α-linolenic acid (ALA) and the ratios arachidonic-to-docosahexaenoic acid (AA/DHA), total metabolites (n-6)-to-total metabolites (n-3) PUFA (∑met n-6/∑met n-3) and total (n-6)-to- total (n-3) PUFA (∑n-6/n-3) compared with the control (P<0.01, P<0.005, P<0.01, P<0.05) and the GDM (P<0.01, P<0.005, P<0.0001, P<0.005 respectively). Similarly, the preterm had higher oleate, total monounsaturated fatty acids (∑mono, P<0.05) and docosapentaenoic (n-6)-to-docoahexaenoic acid (DPAn-6/DHA, P<0.01) compared with the control group. Stearate was enhanced in the preterm (P<0.05) as well as in the GDM (P<0.005) compared with the control group (Table 4.7).

4.4.5.1.2. Ethanolamine phosphoglycerides – fatty acids

In ethanolamine phosphoglycerides (EPG), the percentage palmitoleate, oleate, ∑mono (P<0.0001), palmitate and di-hommo-γ-linolenic acid (DGLA, P<0.005) were lower in the GDM compared with the control and the preterm groups (Table 4.8). By contrast, the GDM had higher proportions of DTA, arachidonic-to-linoleic acid ratio (AA/LA), DHA and ∑n-3 compared with both control (P<0.0001, P<0.0001, P<0.01, P<0.01) and preterm (P<0.005, P<0.05, P<0.005, P<0.005 respectively). Both the GDM (P<0.0001, P<0.05 correspondingly)

and the preterm ($P<0.01$) had lower LA and higher AA compared with the controls (Table 4.8). However, the preterm had lower 20:2n-6 compared with the control ($P<0.0001$) and the GDM ($P<0.005$).

4.4.5.1.3. Inositol phosphoglycerides- fatty acids

Compared with the control, both GDM and the preterm had higher proportions of stearate ($P<0.01$) and ΣSFA ($P<0.0001$) (Table 4.9). However, the preterm had lower proportions of C24:1 ($P<0.0001$), DPA n-3 ($P<0.01$) and DPA n-6/DHA ($P<0.05$) compared with the control and lower 20:2n-6 compared with both the GDM and the control ($P<0.0001$). On the other hand, the GDM had lower ∑n-3 compared with the control ($P<0.005$).

4.4.5.1.4. Serine phosphoglycerides- fatty acids

In serine phosphoglycerides (SPG), the proportions of DPA n-3 and ∑n-3 were elevated in the control compared with the GDM ($P<0.01$, $P<0.05$ respectively) and the preterm ($P<0.0001$) groups (Table 4.10). However, in the former group the percentage C20, C22, 20:2n-6 ($P<0.0001$), ∑SFA ($P<0.01$), oleate and ∑mono ($P<0.05$, $P<0.005$ respectively) was lower compared with both GDM and preterm. Likewise, the proportions of 20:2n-6 ($P<0.05$) and DTA ($P<0.005$) were lower in the preterm compared with the GDM group (Table 4.10).

In placental SPG, the preterm compared with the control had higher proportions of palmitate ($P<0.005$), C20:1 ($P<0.05$) and palmitoleate ($P<0.01$) and lower AA ($P<0.01$), Σn-6 ($P<0.05$) and DHA ($P<0.0001$). Consequently, the fatty acid ratios, AA/DHA, DPAn-6/DHA ($P<0.05$), ∑met n-6/∑met n-3 and ∑n-6/∑n-3 ($P<0.0001$) were elevated in the preterm group (Table 4.10). On the other hand, the GDM had enhanced proportions of stearate and C24 compared with the control group ($P<0.05$).

4.4.5.1.5. Sphingomyelin- fatty acids

Both GDM ($P<0.0001$) and preterm ($P<0.01$) had elevated proportions of stearate compared with the control group (Table 4.11). In addition, the preterm had higher ∑SFA compared with the control group ($P<0.05$) and palmitate compared with both GDM ($P<0.0001$) and control

(P<0.05). On the other hand, the GDM had lower palmitate compared with the control (P<0.005) and higher C24 and C24:1 compared with the preterm group (P<0.05).

4.4.5.2. Free fatty acids and neutral lipids

4.4.5.2.1. Free fatty acids

The free fatty acid (FFA) data showed that the GDM had lower proportions of stearate and C20:1 and higher AA, ∑n-6 and DHA compared with the control (*P*<0.0001, *P*<0.005, *P*<0.0001, *P*<0.0001, *P*<0.0001) and the preterm (*P*<0.05, *P*<0.005, *P*<0.05, *P*<0.05, *P*<0.005 respectively) groups (Table 4.12). Similar to the GDM, the preterm had lower percentage stearate (*P*<0.005) and higher AA and ∑n-6 (*P*<0.0001) compared with the control. Consequently, the fatty acid ratio AA/DHA was elevated in the preterm compared with the GDM (*P*<0.01) and the control (*P*<0.05) groups. In placental FFA, oleate and ALA were also higher in the preterm compared with either control (*P*<0.05) or GDM (*P*<0.005) group as well as the ratios ∑met n-6/∑met n-3 (*P*<0.0001) and ∑n-6/∑n-3 (*P*<0.01) compared with the control (Table 4.12). By contrast, 18:3n-6 was lower in the preterm compared with the control (*P*<0.01).

Both the GDM and preterm placental FFA had higher the proportions of 20:2n-6 and DGLA and lower palmitate, ∑SFA and eicosapentaenoic acid (EPA) compared with the controls (*P*<0.0001). The percentage of DPA n-6 was elevated in the GDM compared with the control (*P*<0.01). The preterm had higher ∑mono (*P*<0.01) and lower ∑n-3 (*P*<0.05) compared with the GDM group (Table 4.12).

4.4.5.2.2. Triacylglycerols- fatty acids

The GDM had higher proportions of C22 compared with the controls (P<0.005), LA (P<0.01) and palmitoleate (P<0.05) compared with the preterm and DPA/DTA n-6 compared with both controls (P<0.01) and preterm (P<0.05) (Table 4.13). By contrast, the GDM had lower percentage C20:1 and DPA n-3 compared with the controls (P<0.05), AA (P<0.005) and DTA (P<0.05) compared with the preterm, and DGLA compared with both the control (P<0.01) and the preterm (P<0.005) groups (Table 4.13). The parent n-3, ALA (P<0.0001, P<0.05) and the ratio AA/LA (P<0.05, P<0.0001 respectively) were elevated in the preterm compared with

either control or GDM (Table 4.13). Moreover, 20:2n-6 was higher in the preterm compared with the control group ($P<0.05$).

4.4.5.2.3. Cholesterol esters- fatty acids

The preterm had higher $\sum$n-6/$\sum$n-3 ($P<0.05$) compared with the controls, Σmono ($P<0.01$) compared with the GDM, and stearate ($P<0.005$, $P<0.05$) and oleate ($P<0.05$, $P<0.005$ respectively) compared with both control and GDM groups (Table 4.14). However, the preterm had lower percentage of LA compared with the controls and $\sum$n-6 compared with either the GDM or the controls ($P<0.05$). In contrast, the GDM had elevated proportions of AA compared with the preterm and the control groups ($P<0.05$).

4.4.6. Relationship between fatty acids and placental weight and ratio and the biometric measurements of the fetus

Correlation coefficients for the relationship between fatty acids (FA) of the individual placental phospholipids [choline (CPG), ehanolamine (EPG) and inositiol (IPG) phosphoglycerides], placental weight (wt) and placental ratio and foetal measurements of growth are presented in Table 4.15. Maternal age, body mass index (BMI), gender and mode of delivery showed no significant correlations with any placental FA and FA ratios, $P>0.05$.

For placental EPG and IPG, when these variables (Table 4.15) were included in a stepwise linear regression model with each FA and FA ratios as dependent variables, none of the independent variables BWT, gestational age (GA), abdominal (AC) and head (HC) circumeference, placental wt and placental ratio were associated with neither placental FA nor FA ratios. In contrast in CPG, when BWT, GA, AC, HC, placental wt and placental ratio were included in a stepwise linear regression model with each FA and FA ratios as dependent variables, only GA ($r^2=0.07$, $P<0.05$) remained independently was associated with placental DHA (Fig 4.1).

%Total fatty acids

	Caucasians		Asians		
	Control[a] (n=8)	Preterm[b] (n=11)	Control[c] (n=22)	GDM[d] (n=7)	Preterm[e] (n=15)
CPG					
LA	12.0±2.40	12.2±1.29	14.2±3.00	14.4±0.48	13.2±1.88
AA	16.2±3.09	15.5±1.81	17.5±2.22[d]	19.7±0.98[c,e]	16.9±1.79[d]
DHA	1.30±0.32	1.29±0.72	1.69±0.59[d,e]	2.35±0.40[c,e]	1.32±0.36[c,d]
EPG					
LA	5.83±1.49	5.07±0.89	6.41±1.49[d,e]	4.16±0.46[c,e]	5.46±1.24[c,d]
AA	25.5±1.64	25.3±1.00	24.6±2.00[d,e]	26.1±1.22[c,e]	26.3±1.35[c,d]
DHA	8.45±0.78	7.68±1.50	8.90±2.24[d]	11.5±0.78[c,e]	8.90±1.55[d]
TG					
LA	11.2±2.90	10.2±1.62[e]	14.4±4.66[d]	16.7±1.84[c,e]	12.6±2.16[b,d]
AA	12.4±4.45	12.4±3.86	9.58±2.29[e,d]	6.78±2.14[c,e]	11.7±3.57[c,d]
DHA	2.65±0.99	2.34±0.53	3.07±1.19	2.24±0.79	3.39±1.43

Table 4.6. Percent placental linoleic (LA), arachidonic (AA) and docosahexaenoic (DHA) acids in relation to ethnicity, GDM; gestational diabetes mellitus, CPG; choline phosphoglycerides, EPG; ethanolamine phosphoglycerides, TG; triacylglycerols. Values are means ± SD. Means that share a superscript letter differ; $P<0.05$ (non-parametric tests).

% total fatty acids

	Control[a]	GDM[b]	Pre-term[c]
16:0	39.0±2.91[b]	32.6±3.70[a,c]	38.6±3.99[b]
18:0	7.90±1.00[b,c]	9.27±0.59[a]	8.68±1.50[a]
20:0	0.06±0.03	Trace	Trace
22:0	Trace	Trace	0.06±0.02
24:0	Trace	Trace	0.27±0.08
ΣSFA	47.0±3.09[b]	41.9±3.56[a,c]	47.5±4.01[b]
16:1	1.29±0.21[b]	1.01±0.17[a]	1.19±0.23
18:1	9.85±1.45[b,c]	10.7±0.80[a]	11.1±2.13[a]
20:1	0.10±0.03[b]	0.20±0.07[a,c]	0.12±0.04[b]
22:1	Trace	Trace	Trace
24:1	Trace	Trace	Trace
Σmono	11.2±1.60[c]	11.9±0.92	12.3±2.21[a]
18:2n-6	13.4±3.01	14.1±1.52	12.9±1.76
18:3n-6	0.10±0.03	0.08±0.02[c]	0.12±0.04[b]
20:2n-6	0.49±0.14[b]	0.63±0.16[a,c]	0.44±0.12[b]
20:3n-6	4.00±0.71	4.48±0.68	4.10±0.99
20:4n-6	17.2±2.44[b]	19.4±1.75[a,c]	16.5±1.92[b]
22:4n-6	0.31±0.08[b]	0.56±0.19[a,c]	0.32±0.09[b]
22:5n-6	0.22±0.07[b]	0.38±0.13[a,c]	0.24±0.09[b]
Σn-6	35.7±3.42[b]	39.6±2.16[a,c]	34.5±3.43[b]
18:3n-3	0.07±0.03[c]	0.06±0.02[c]	0.11±0.05[a,b]
20:5n-3	0.18±0.06	0.23±0.08	0.18±0.07
22:5n-3	0.22±0.09[b]	0.31±0.10[a,c]	0.20±0.06[b]
22:6n-3	1.64±0.58[b,c]	2.09±0.50[a,c]	1.25±0.55[a,b]
Σn-3	1.99±0.74	2.56±0.59[c]	1.60±0.67[b]
AA/LA	1.36±0.39	1.41±0.32	1.30±0.20
AA/DHA	11.1±3.43[c]	9.77±2.63[c]	14.7±4.90[a,b]
DPA n-6/DHA	0.13±0.08[c]	0.19±0.08	0.23±0.12[a]
DPA/DTA n-6	0.67±0.14	0.64±0.16	0.80±0.38
Σmet n-6/Σmet n-3	12.0±3.68[c]	10.6±2.57[c]	15.0±4.13[a,b]
Σn-6/ Σn-3	18.5±5.81[c]	16.2±3.37[c]	23.4±6.46[a,b]

Table 4.7. Percent fatty acid composition of placental choline phosphoglycerides, GDM; gestational diabetes mellitus. ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA n-6/DHA; docosapentaenoic (n-6)-to-docosahexaenoic acid, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3 ratio; total metabolites n-6 PUFA-to-total metabolites n-3 PUFA ratio; Σn-6/ Σn-3; total n-6 PUFA-to-total n-3 PUFA ratio.
Values are means ± SD, n=11 (GDM); n=29 (Preterm); n=40 (Control). Means that share a superscript letter differ; $P<0.05$ (ANOVA).

% total fatty acids

	Control[a]	GDM[b]	Pre-term[c]
16:0	7.72±0.99[b]	6.30±1.39[a,c]	7.84±1.35[b]
18:0	12.5±1.27	13.3±1.83	13.4±2.19
20:0	0.08±0.02	Trace	0.05±0.01
22:0	0.08±0.03	Trace	0.06±0.01
24:0	0.12±0.07	Trace	0.28±0.11
ΣSFA	20.3±1.62	19.7±2.95	21.4±3.03
16:1	0.58±0.15[b]	0.14±0.03[a,c]	0.55±0.12[b]
18:1	9.30±1.39[b]	6.81±1.34[a,c]	8.85±1.15[b]
20:1	0.19±0.06	0.18±0.04	0.16±0.05
22:1	0.06±0.02	Trace	0.06±0.03
24:1	Trace	Trace	0.02±0.01
Σmono	10.0±1.43[b]	7.03±1.40[a,c]	9.53±1.14[b]
18:2n-6	6.32±1.70[b,c]	4.35±0.82[a]	5.25±1.09[a]
18:3n-6	0.25±0.14	Trace	0.06±0.02
20:2n-6	0.38±0.11[c]	0.35±0.14[c]	0.23±0.06[a,b]
20:3n-6	3.47±0.56[b]	2.94±0.31[a,c]	3.71±0.86[b]
20:4n-6	24.7±1.83[b,c]	26.2±1.09[a]	25.9±1.25[a]
22:4n-6	2.41±0.45[b,c]	3.27±0.50[a,c]	2.68±0.46[a,b]
22:5n-6	1.60±0.56	1.85±0.65	1.94±0.67
Σn-6	38.9±2.54	38.9±1.16	39.7±1.87
18:3n-3	0.08±0.03	Trace	0.08±0.03
20:5n-3	0.27±0.10	Trace	0.22±0.06
22:5n-3	1.68±0.39	2.12±0.75	1.64±0.38
22:6n-3	8.87±1.99[b]	10.7±1.59[a,c]	8.50±1.62[b]
Σn-3	10.8±2.23[b]	12.9±2.02[a,c]	10.4±1.93[b]
AA/LA	4.20±1.27[b,c]	6.22±1.22[a,c]	5.11±0.96[a,b]
AA/DHA	3.00±1.07	2.50±0.46	3.17±0.69
DPA n-6/DHA	0.19±0.10	0.19±0.09	0.25±0.12
DPA/DTA n-6	0.65±0.13	0.57±0.19	0.73±0.25
Σmet n-6/Σmet n-3	3.22±1.08	2.72±0.52	3.47±0.78
Σn-6/ Σn-3	3.85±1.32	3.09±0.62	3.98±0.90

Table 4.8. Percent fatty acid composition of placental ethanolamine phosphoglycerides, GDM; gestational diabetes mellitus. ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA n-6/DHA; docosapentaenoic (n-6)-to-docosahexaenoic acid, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3 ratio; total metabolites n-6 PUFA-to-total metabolites n-3 PUFA ratio; Σn-6/ Σn-3; total n-6 PUFA-to-total n-3 PUFA ratio.
Values are means ± SD, n=11 (GDM); n=29 (Preterm); n=40 (Control). Means that share a superscript letter differ; $P<0.05$ (ANOVA).

% total fatty acids

	Control[a]	GDM[b]	Pre-term[c]
16:0	13.6±2.25	14.9±1.50	14.9±3.09
18:0	27.0±2.31[b,c]	29.5±2.53[a]	29.9±2.59[a]
20:0	0.13±0.04[c]	0.19±0.08	0.26±0.08[a]
22:0	0.66±0.15	Trace	0.66±0.17
24:0	1.26±0.45[c]	1.51±0.64[c]	0.67±0.27[a,b]
ΣSFA	41.8±3.15[b,c]	45.9±2.70[a]	45.8±3.99[a]
16:1	0.33±0.10	0.33±0.05	0.35±0.11
18:1	4.76±0.88	4.36±0.66	4.99±1.15
20:1	0.09±0.03	Trace	0.06±0.02
22:1	0.19±0.06	Trace	0.14±0.04
24:1	0.92±0.30[c]	0.84±0.30	0.62±0.22[a]
Σmono	6.03±1.08	5.49±0.62	5.94±1.15
18:2n-6	2.23±0.53	2.09±0.40	2.08±0.49
18:3n-6	0.09±0.03	Trace	0.05±0.01
20:2n-6	0.60±0.20[c]	0.64±0.26[c]	0.22±0.07[a,b]
20:3n-6	6.87±1.03	6.31±0.67	6.85±1.39
20:4n-6	31.9±2.48	31.2±1.95	30.3±3.93
22:4n-6	1.11±0.21	1.09±0.21	1.04±0.22
22:5n-6	0.61±0.22	0.53±0.25	0.54±0.25
Σn-6	43.1±3.22	41.6±2.94	40.9±4.61
18:3n-3	0.07±0.02	Trace	0.09±0.03
20:5n-3	0.56±0.30	Trace	0.06±0.01
22:5n-3	0.82±0.24[c]	0.69±0.22	0.64±0.19[a]
22:6n-3	2.22±0.69	2.08±0.27	2.25±0.93
Σn-3	3.43±0.92[a]	2.77±0.40[b]	2.92±0.93
AA/LA	15.1±3.59	15.5±3.29	15.1±2.65
AA/DHA	15.8±5.04	15.2±1.72	14.7±5.57
DPA n-6/DHA	0.31±0.16[c]	0.29±0.15	0.22±0.07[a]
DPA/DTA n-6	0.56±0.19	0.54±0.27	0.55±0.31
Σmet n-6/Σmet n-3	12.8±3.47	14.5±2.04	14.8±4.89
Σn-6/ Σn-3	13.4±3.66	15.3±2.08	15.4±5.07

Table 4.9. Percent fatty acid composition of placental inositol phosphoglycerides, GDM; gestational diabetes mellitus. ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA n-6/DHA; docosapentaenoic (n-6)-to-docosahexaenoic acid, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3 ratio; total metabolites n-6 PUFA-to-total metabolites n-3 PUFA ratio; Σn-6/ Σn-3; total n-6 PUFA-to-total n-3 PUFA ratio.
Values are means ± SD, n=11 (GDM); n=29 (Preterm); n=40 (Control). Means that share a superscript letter differ; $P<0.05$ (ANOVA).

% total fatty acids

	Control[a]	GDM[b]	Pre-term[c]
16:0	10.5±1.93[c]	11.4±1.42	12.4±2.78[a]
18:0	34.0±4.48[b]	36.8±1.36[a]	35.4±1.80
20:0	0.16±0.03[b,c]	0.44±0.07[a]	0.55±0.18[a]
22:0	0.17±0.04[b,c]	0.81±0.23[a]	0.75±0.11[a]
24:0	0.38±0.12[b]	0.50±0.12[a]	0.42±0.16
ΣSFA	45.0±5.31[b,c]	49.8±1.62[a]	49.0±3.93[a]
16:1	0.42±0.10[c]	0.46±0.09	0.50±0.10[a]
18:1	13.2±1.73[b,c]	14.2±0.85[a]	14.8±1.96[a]
20:1	0.20±0.05[c]	0.21±0.05	0.23±0.06[a]
22:1	0.26±0.09	0.21±0.06	0.22±0.06
24:1	0.41±0.14[b]	0.27±0.09[a]	0.37±0.13
Σmono	14.3±1.77[b,c]	15.3±0.97[a]	16.0±2.07[a]
18:2n-6	7.04±1.83	6.03±1.56	6.50±1.40
18:3n-6	0.14±0.04	Trace	0.11±0.01
20:2n-6	0.17±0.06[b,c]	0.51±0.14[a,c]	0.34±0.11[a,b]
20:3n-6	9.15±1.44	8.08±0.77	8.58±2.16
20:4n-6	10.8±1.52[c]	10.7±1.15	9.67±1.62[a]
22:4n-6	1.67±0.38	1.93±0.49[c]	1.44±0.42[b]
22:5n-6	1.16±0.33	1.09±0.32	1.03±0.48
Σn-6	30.0±3.60[c]	28.3±2.76	27.5±4.46[a]
18:3n-3	Trace	Trace	0.08±0.02
20:5n-3	0.55±0.20	Trace	0.12±0.05
22:5n-3	0.69±0.23[b,c]	0.53±0.10[a]	0.48±0.18[a]
22:6n-3	3.62±1.10[c]	3.22±0.71	2.54±0.89[a]
Σn-3	4.69±1.40[b,c]	3.78±0.72[a]	3.09±1.00[a]
AA/LA	1.63±0.44	1.87±0.50	1.52±0.23
AA/DHA	3.23±1.34[c]	3.46±0.72	4.23±1.56[a]
DPA n-6/DHA	0.33±0.15[c]	0.32±0.10	0.44±0.19[a]
DPA/DTA n-6	0.69±0.13	0.57±0.09	0.73±0.31
Σmet n-6/Σmet n-3	5.29±1.98[c]	5.99±0.98	7.38±2.02[a]
Σn-6/ Σn-3	6.93±2.81[c]	7.72±1.35	9.63±2.89[a]

Table 4.10. Percent fatty acid composition of placental serine phosphoglycerides, GDM; gestational diabetes mellitus. ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA n-6/DHA; docosapentaenoic (n-6)-to-docosahexaenoic acid, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3 ratio; total metabolites n-6 PUFA-to-total metabolites n-3 PUFA ratio; Σn-6/ Σn-3; total n-6 PUFA-to-total n-3 PUFA ratio.
Values are means ± SD, n=11 (GDM); n=29 (Preterm); n=40 (Control). Means that share a superscript letter differ; $P<0.05$ (ANOVA).

% total fatty acids

	Control[a]	GDM[b]	Pre-term[c]
16:0	36.8±4.11[b,c]	32.7±2.39[a,c]	39.8±5.15[a,b]
18:0	8.84±2.70[b,c]	12.9±3.47[a]	11.1±2.94[a]
20:0	2.00±0.61	2.37±0.53	2.02±0.48
22:0	10.7±2.56	12.0±2.72	10.6±2.41
24:0	13.8±3.04	15.3±3.15[c]	12.3±3.47[b]
ΣSFA	72.6±5.14[c]	75.4±4.62	75.8±3.79[a]
16:1	0.34±0.13	0.45±0.16	0.37±0.15
18:1	2.90±1.12	3.48±1.31	3.29±1.08
20:1	0.25±0.08	Trace	0.12±0.04
22:1	0.48±0.17	Trace	0.26±0.12
24:1	7.90±2.13	9.42±1.90[c]	7.35±2.21[b]
ΣMono	11.3±2.68	13.1±2.74	11.2±2.41
18:2n-6	2.77±1.01	3.21±1.48	2.62±0.88

Table 4.11. Percent fatty acid composition of placental sphingomyelin, GDM; gestational diabetes mellitus. ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids.
Values are means ± SD, n=11 (GDM); n=29 (Preterm); n=40 (Control). Means that share a superscript letter differ; $P<0.05$ (ANOVA).

% total fatty acids

	Control[a]	GDM[b]	Pre-term[c]
16:0	22.8±2.36[b,c]	19.1±1.55[a]	19.9±2.84[a]
18:0	12.7±1.60[b,c]	9.72±1.43[a,c]	11.2±1.68[a,b]
20:0	0.25±0.10	Trace	0.23±0.09
22:0	0.37±0.15	Trace	0.15±0.04
24:0	0.31±0.14	0.23±0.06	Trace
ΣSFA	36.7±3.51[b,c]	29.2±2.73[a]	31.6±3.88[a]
16:1	1.14±0.27	0.95±0.19	1.02±0.24
18:1	13.2±1.77[c]	12.6±1.22[c]	14.7±2.37[a,b]
20:1	0.33±0.09[b]	0.23±0.08[a,c]	0.34±0.08[b]
22:1	0.18±0.05	Trace	Trace
24:1	0.54±0.23	Trace	Trace
Σmono	15.1±1.91	13.8±1.37[c]	16.1±2.59[b]
18:2n-6	11.5±2.49	12.9±0.79	11.7±1.84
18:3n-6	0.17±0.05[c]	0.14±0.01	0.12±0.06[a]
20:2n-6	0.38±0.16[b,c]	0.72±0.16[a]	0.64±0.18[a]
20:3n-6	4.64±1.08[b,c]	6.45±1.02[a]	6.09±1.58[a]
20:4n-6	18.8±2.79[b,c]	24.7±2.27[a,c]	22.3±2.96[a,b]
22:4n-6	1.09±0.37	1.15±0.27	1.19±0.38
22:5n-6	0.77±0.27[b]	1.07±0.35[a]	0.94±0.29
Σn-6	37.2±3.84[b,c]	47.1±3.70[a,c]	42.8±4.99[a,b]
18:3n-3	0.23±0.10[c]	0.16±0.05[c]	0.33±0.16[a,b]
20:5n-3	0.58±0.22[b,c]	0.27±0.10[a]	0.37±0.15[a]
22:5n-3	0.84±0.28	0.64±0.17	0.79±0.34
22:6n-3	4.33±1.27[b]	6.00±1.01[a,c]	4.32±1.24[b]
Σn-3	5.84±1.62	7.02±1.03[c]	5.58±1.57[b]
AA/LA	1.72±0.49	1.91±0.17	1.93±0.26
AA/DHA	4.50±1.45[c]	4.23±0.84[c]	5.52±1.54[a,b]
DPA n-6/DHA	0.19±0.09	0.19±0.10	0.23±0.08
DPA/DTA n-6	0.75±0.24	0.93±0.17	0.80±0.19
Σmet n-6/Σmet n-3	4.64±1.41[c]	5.06±1.04	6.28±2.04[a]
Σn-6/ Σn-3	6.53±2.21[c]	6.88±1.37	8.32±2.78[a]

Table 4.12. Percent fatty acid composition of placental free fatty acids, GDM; gestational diabetes mellitus. ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA n-6/DHA; docosapentaenoic (n-6)-to-docosahexaenoic acid, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3 ratio; total metabolites n-6 PUFA-to-total metabolites n-3 PUFA ratio; Σn-6/ Σn-3; total n-6 PUFA-to-total n-3 PUFA ratio.
Values are means ± SD, n=11 (GDM); n=29 (Preterm); n=40 (Control). Means that share a superscript letter differ; $P<0.05$ (ANOVA).

% total fatty acids

	Control[a]	GDM[b]	Pre-term[c]
16:0	26.5±3.50	28.1±1.39	26.2±3.67
18:0	9.50±2.71	9.06±3.36	10.7±3.54
20:0	0.31±0.17	0.27±0.08	0.32±0.14
22:0	0.25±0.11[b]	0.44±0.14[a]	0.35±0.14
24:0	Trace	Trace	Trace
ΣSFA	37.1±5.27	38.1±4.06	37.8±6.75
16:1	2.05±0.59	2.29±0.77[c]	1.73±0.66[b]
18:1	20.1±5.04	22.2±6.52	17.8±7.56
20:1	0.43±0.17[b]	0.30±0.09[a]	0.33±0.13
22:1	0.23±0.08	Trace	Trace
24:1	0.47±0.13	Trace	Trace
Σmono	22.7±5.58	24.9±7.11	19.9±8.11
18:2n-6	13.3±4.32	15.8±3.08[c]	11.7±2.45[b]
18:3n-6	0.19±0.05	0.19±0.05	0.17±0.06
20:2n-6	0.27±0.09[c]	0.52±0.13	0.60±0.27[a]
20:3n-6	4.11±1.29[b]	2.73±1.15[a,c]	4.45±1.38[b]
20:4n-6	10.3±2.95	7.77±3.08[c]	12.0±3.60[b]
22:4n-6	1.38±0.38	1.08±0.68[c]	1.48±0.43[b]
22:5n-6	0.67±0.27	0.56±0.22	0.78±0.37
Σn-6	30.0±5.88	28.7±2.73	30.8±4.52
18:3n-3	0.38±0.16[c]	0.40±0.12[c]	0.63±0.18[a,b]
20:5n-3	0.37±0.13	0.32±0.15	0.37±0.12
22:5n-3	0.84±0.26[b]	0.56±0.21[a]	0.81±0.38
22:6n-3	3.14±1.14	2.44±0.77	3.15±1.34
Σn-3	4.52±1.49	3.40±1.15	4.27±1.90
AA/LA	0.85±0.34[c]	0.54±0.33[c]	1.09±0.46[a,b]
AA/DHA	3.81±1.93	3.35±1.37	4.47±2.70
DPA n-6/DHA	0.24±0.09	0.31±0.15	0.26±0.09
DPA/DTA n-6	0.49±0.18[b]	0.75±0.32[a,c]	0.50±0.22[b]
Σmet n-6/Σmet n-3	4.25±1.55	4.64±1.82	5.46±2.50
Σn-6/ Σn-3	6.98±2.64	8.64±2.88	8.33±3.88

Table 4.13. Percent fatty acid composition of placental triacylglycerols, GDM; gestational diabetes mellitus. ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA n-6/DHA; docosapentaenoic (n-6)-to-docosahexaenoic acid, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3 ratio; total metabolites n-6 PUFA-to-total metabolites n-3 PUFA ratio; Σn-6/ Σn-3; total n-6 PUFA-to-total n-3 PUFA ratio.
Values are means ± SD, n=11 (GDM); n=29 (Preterm); n=40 (Control). Means that share a superscript letter differ; $P<0.05$ (ANOVA).

% total fatty acids

	Control[a]	GDM[b]	Pre-term[c]
16:0	13.0±2.21	13.2±1.99	13.4±2.09
18:0	1.72±0.44[c]	1.71±0.37[c]	2.20±0.62[a,b]
20:0	Trace	Trace	Trace
22:0	0.28±0.09	0.31±0.06	Trace
24:0	Trace	Trace	Trace
ΣSFA	15.2±2.35	15.8±2.19	16.1±2.49
16:1	3.71±1.11	3.62±1.05	3.85±1.20
18:1	19.9±4.01[c]	18.1±3.14[c]	22.4±3.12[a,b]
20:1	0.22±0.09	Trace	0.19±0.06
22:1	0.29±0.11	Trace	0.40±0.18
24:1	0.28±0.06	0.40±0.10	Trace
Σmono	23.9±4.85	21.8±3.94[c]	26.5±3.39[b]
18:2n-6	40.9±6.99[c]	40.4±4.24	37.0±5.84[a]
18:3n-6	0.54±0.14	0.63±0.21	0.50±0.16
20:2n-6	0.37±0.13	0.35±0.08	0.36±0.21
20:3n-6	2.25±0.53	2.34±0.65	2.48±0.90
20:4n-6	7.46±2.05[b]	8.99±1.13[a,c]	7.16±1.55[b]
22:4n-6	0.63±0.23	0.53±0.18	0.74±0.34
22:5n-6	0.51±0.21	0.52±0.17	0.59±0.19
Σn-6	52.2±6.42[c]	53.5±5.17[c]	48.1±5.80[a,b]
18:3n-3	0.53±0.17	0.40±0.11	0.52±0.20
20:5n-3	0.35±0.11	Trace	0.45±0.20
22:5n-3	0.38±0.13	0.25±0.06	0.33±0.14
22:6n-3	1.26±0.56	1.43±0.52	1.43±0.73
Σn-3	2.09±0.77	2.02±0.70	2.03±1.04
AA/LA	0.19±0.07	0.22±0.03	0.20±0.05
AA/DHA	6.12±1.84	7.01±2.38	6.56±2.85
DPAn-6/DHA	0.36±0.15	0.42±0.22	0.50±0.28
DPA/DTA n-6	0.84±0.26	1.05±0.21	0.90±0.37
Σmet n-6/Σmet n-3	6.79±2.22	8.60±3.22	7.39±2.96
Σn-6/ Σn-3	22.4±6.92[c]	28.8±8.28	30.8±11.4[a]

Table 4.14. Percent fatty acid composition of placental cholesterol esters, GDM; gestational diabetes mellitus. ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA n-6/DHA; docosapentaenoic (n-6)-to-docosahexaenoic acid, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3 ratio; total metabolites n-6 PUFA-to-total metabolites n-3 PUFA ratio; Σn-6/ Σn-3; total n-6 PUFA-to-total n-3 PUFA ratio.
Values are means ± SD, n=11 (GDM); n=29 (Preterm); n=40 (Control). Means that share a superscript letter differ; $P<0.05$ (ANOVA).

Outcome	PL- fatty acids	vs.	Fetal & placental characteristics	r	n	*P*
GDM	EPG					
	LA	vs.	BWT	0.65	11	<0.05
	AA/LA	vs.	BWT	-0.62	11	<0.05
Preterm	EPG					
	DGLA	vs.	AC	0.50	16	<0.05
	AA/DGLA	vs.	AC	-0.51	16	<0.05
	AA/LA	vs.	Placental ratio	0.51	16	<0.05
	CPG					
	DHA	vs.	FL	0.64	14	<0.05
	∑n-3	vs.	FL	0.65	14	<0.05
	AA/DHA	vs.	FL	-0.63	14	<0.05
	∑n-6/∑n-3	vs.	FL	-0.58	14	<0.05
	AA/DGLA	vs.	AC	-0.52	23	<0.05
	AA/DGLA	vs.	HC/AC	-0.57	16	<0.05
	IPG					
	AA/DGLA	vs.	AC	-0.51	23	<0.05
	AA/DHA	vs.	FL	0.56	16	<0.05
	DPA n-6/DHA	vs.	FL	0.58	16	<0.05
	∑n-6/∑n-3	vs.	FL	0.52	16	<0.05
Term	EPG					
	AA	vs.	Placental ratio	0.44	22	<0.05
	AA/LA	vs.	Placental ratio	0.52	22	<0.01
	DPA/DTA n-6	vs.	AC	-0.62	11	<0.05
	CPG					
	DHA	vs.	AC	0.71	11	<0.05
	DGLA	vs.	HC	0.56	21	<0.01
	DPA/DTA n-6	vs.	Placental ratio	0.58	13	<0.05
	DPA n-6/DHA	vs.	Placental ratio	0.62	13	<0.05
	IPG					
	DGLA	vs.	HC	0.47	21	<0.05

Table 4.15. Correlation coefficients between placental fatty acids and one of the foetal measurements of growth, GDM; gestational diabetes mellitus, PL; phospholipids, CPG; choline phosphoglycerides, EPG, ethanolamine phosphoglycerides, IPG; inositol phosphoglycerides, BWT; birthweight, AC; head circumferences, FL; femur length; HC/AC; head-to-abdominal circumference ratio, LA; linoleic acid; AA; arachidonic acid, DGLA; di-homo-γ-linolenic acid, AA/LA; arachidonic-to-linoleic acid ratio, AA/DGLA; arachdonic-to-DGLA ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA n-6/DHA; docosapentaenoic (n-6)-to-docosahexaenoic acid ratio, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, ∑met n-6/∑met n-3; total metabolites n-6 polyunsaturated fatty acids (PUFA)-to-total metabolites n-3 PUFA; ∑n-6/ ∑n-3; total n-6 PUFA-to-total n-3 PUFA. r; strength of associations (Pearson's coefficient or Spearman's rank coefficient if sample size < 20).

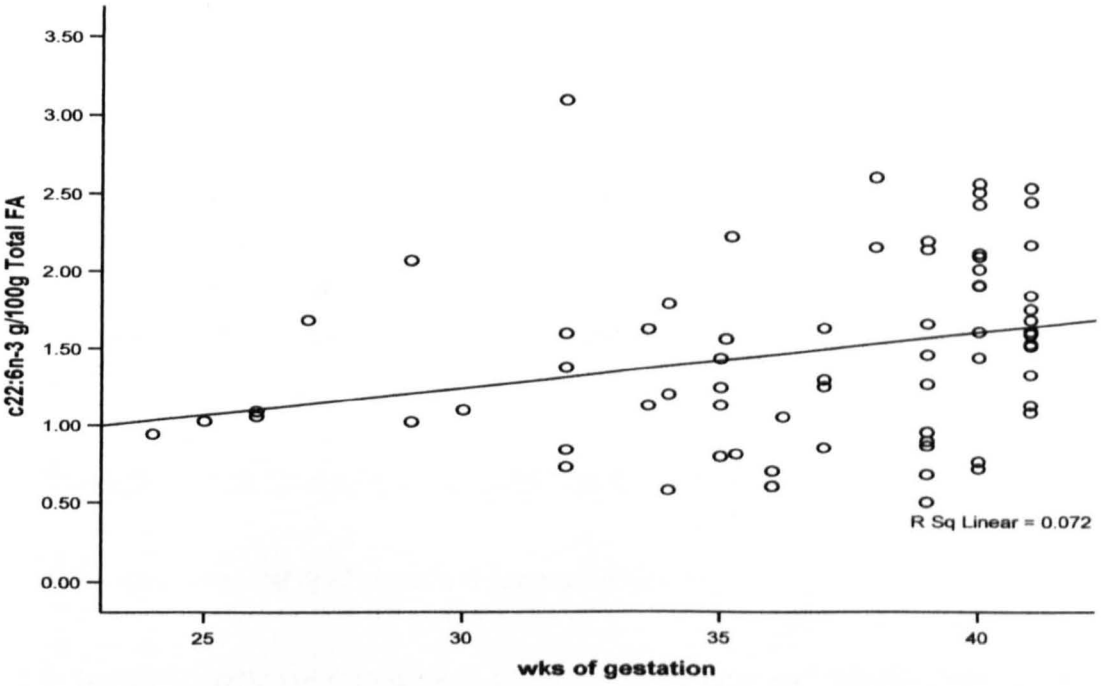


Figure.4.1. Percent docosahexaenoic acid (DHA, 22:6n-3) vs. gestational age in weeks (wk). Linear regression: y=0.183+0.035x, r=0.27, r^2=0.07, n=69, r=regression, r^2; regression squared.

4.5. Discussion

This study investigated total weight (wt), total lipid content and fatty acid composition of phospholipids (PL) and neutral lipids (NL) of placental tissues derived from preterm birth (24-36 wk of gestation) or term (37-41 wk of gestation) pregnancies complicated with gestational diabetes mellitus (GDM). Similar to the study described in Chapter 3, these women were from the borough of Newham, which is economically deprived inner-city area of London with high incidence of GDM and prematurity. Additionally, the relationship between maternal characteristics, foetal measurements of biometry and placental fatty acids (FA) were assessed.

4.5.1. Placental weight, total lipid content and fatty acids in preterm delivery

Placental weight and total lipid content in preterm delivery

The functional maturation of the placenta as an endocrine organ occurs progressively through gestation. The significant increase in total placental surface area and decreased thickness allow sufficient nutrient transport in order to meet the needs for proper fetal growth (Cetin et al, 2005). Hence, the lower total weight (wt) and total lipid content of the preterm placenta may relate to the earlier stage of placental growth and of brain growth and development of the preterm baby.

Placental fatty acids in preterm delivery

To our knowledge data on placental fatty acids (FA) in preterm birth are limited. Reece et al (1997) reported that arachidonic (AA) and docosahexaenoic (DHA) acids content in the placenta were unaltered in preterm delivery. Whilst, an earlier report in the preterm placenta focused on AA only, suggested that chorioamnionitis was the cause of the elevated AA in the preterm placenta (Delmis et al, 1989). They reported 25.9% total phospholipid AA in preterm placenta with chorioamnionitis and 20% in preterm without the presence of chorioamnionitis. Whilst, the high AA in triacylglycerols, TG (Delmis et al, 1989) could be due to a methodological error (Table 4.16). Herein, preterm pregnancy resulted in a reduced DHA in the placenta PL. AA was also reduced in serine (SPG) phosphoglycerides, but it increased in ethanolamine phosphoglycerides (EPG) and in free fatty acids (FFA) of the human placenta, delivered between 24 and 36 wk of gestation, suggesting an abnormal polyunsaturated fatty acid (PUFA) vascular composition.

NL	Preterm (this study)	Preterm [1]	Preterm with [1] Chorioamnionitis
FFA	22.3±2.96	18.0 ±2.9	25.6±7.0
TG	12.0±3.60	8.9±4.3	24.3±14.0
CE	7.16±1.55	9.0±13.0	8.6±4.5

Table 4.16. Percent arachidonic acid (AA) in placental neutral lipids (NL) and free fatty acids (FFA) from preterm or preterm pregnancies with chorioamnionitis, TG; triacylglycerols, CE; cholesterol esters. Values are means ± SD, n=29 (this study); n=9 (preterm[1]); n=6 (preterm with chorioamnionitis[1]).
[1] Delmis (1989).

A limitation of this study was that food intake was not assessed. DHA, in particular, is indispensable (Pontes et al, 2006; Klingler et al, 2006) and low maternal intakes could explain as to why even the term infant could be born with lower DHA than their mothers (Pontes et al, 2006). Moreover, short height reflecting long-term poorer nutrition together with other socio-demographic determinants may result in unfavorable pregnancy outcome, low birthweight (LBW) and preterm birth in the non-Dutch vs. the Dutch population (Troe et al, 2007).

Conversely, herein, the preterm mothers were mainly Asian and Caucasians and they shared a comparable AA and DHA profile in their placenta. Similarly, ethnicity had no effect on placental PUFA of healthy pregnant (8-14 wk of gestation or term) women (Chapter 3) nor significant ethnic differences between African mothers (i.e. age & BMI) were reflected in foetal biometric measurements (i.e. head circumference or femur length) at second trimester (Salpou et al, 2008). In a contemporary well nourished cohort in UK- from a 10% random sample of the Avon longitudinal study of pregnancy and childhood, maternal diet was adequate and was not related to size at birth (Ong et al, 2000). Moreover, a study of British and South Korean women showed similar associations between AA and DHA in maternal red cell membrane PL despite the different genetic and nutritional background (Ghebremeskel et al, 1997).

Arachidonic and docosahexaenoic acids in the preterm placenta

It is evident that the preterm infants are born with reduced AA and DHA and increased markers of essential fatty acid (EFA) deficiency (Crawford et al, 1989, 2003; Leaf et al, 1992b; Foreman-van Drongelen et al, 1995 Bitsanis et al, 2000). In contrast, the marked increase of maternal red blood cells (RBC) and trophoblast tissue (amnion) with AA and docosapentaenoic acid n-6 (DPA n-6) suggested that DHA is spared for fetal transport in the preterm delivery (Reece et al, 1997).

However, that the preterm mothers had low DHA in plasma, but high in RBC compared with their corresponding controls at 34 wk of gestation (Reece et al, 1997) might be in contrast to the reports of increased mobilisation or depletion of DHA from maternal sources at term. Alternatively, it might reflect a decreased bioavailability for DHA to the placenta and the fetus (Burdge et al, 2006). Though plasma and red blood cells (RBC) PL have been regularly measured as surrogate markers of tissue PL fatty acid composition, they are not ideal (Hunt et al, 1996). During intrauterine growth, the human placenta selectively transfers PUFA to the fetus, preformed from the maternal stores (Crawford et al, 1976, 1992; Crawford, 1992, 2000). Hence, the human placenta is a very important vascular system to study.

In this study, preterm delivery reduced DHA, 24% in choline phosphoglycerides (CPG) and 30% in SPG and markedly increased AA/DHA (32% in CPG & 31% in SPG), DPA n-6/DHA (77% in CPG & 33% in SPG) and total (n-6)-to-total (n-3) PUFA (27% in CPG & 39% in SPG) ratios, and this placental profile could be indicative of a reduced n-3 PUFA availability or supply, mainly in DHA to the premature infant. In support of this, studies in midterm abortions, resembling preterm delivery, showed that the transplacental gradient in proportions of AA and DHA are greater at term than mid-term (Crawford, 2000). Similarly, the elevated AA and DPA n-6/DHA in fetal growth retardation (FGR) placenta (particularly in the asymmetric FGR) suggested a low DHA availability to the fetus (Mattoras et al, 2001).

Previous study (Chapter 3) showed that in early human pregnancy (8-14wk of gestation), DHA is highly incorporated into placental TG (Speake et al, 2003). Similarly, the yolk sac membrane of the chicken embryo showed that DHA is incorporated into TG (Speake et al, 2003). The initial incorporation of DHA into TG is a crucial stage in the transport of this PUFA to its functional locations in the membrane PL of the neurons and the retinal photoreceptors (Speake et al, 2003). However, that the high DHA in TG in early gestation was reduced at term placenta (Chapter 3) and that term and preterm (herein) had a comparable DHA level in the TG, is in line with the suggestion that assimilation of DHA into brain PL and neuronal

maturation are initiated in mid-gestation in humans (Hunt et al, 1996). The preterm newborn offspring had also low DHA and AA in PL cord blood mononuclear cell (CBMC) membranes together with reduced absolute lymphocyte count and CD4+ and CD8+ T-cell subsets (Moodley et al, 2009), suggesting a potent role for DHA and AA as immunoregulators and that in a deficiency state may compromise the immune system of very preterm infants. Quattrucci et al (2009) suggested that the reduced membrane AA in cystic Fibrosis may resulted in insufficient production of lipid mediators involved in the resolution of lung inflammation.

Lipoxins (LX) and aspirin-triggered LX with the unique trihydroxytetraene structure (Chiang et al, 2006) are endogenously produced from AA (Bannenberg et al, 2004; Chiang et al, 2003, 2006; Serhan et al, 2008a). These eicosanoids represent a distinct class of lipid mediators, function as "stop signals" in inflammation to regulate excessive leukocyte trafficking and to promote resolution (Bannenberg et al, 2004; Chiang et al, 2006; Serhan & Chiang, 2008; Serhan et al, 2008a&b). The native LXA_4 and LXB_4, and the stable ATL analog ATLa2 and the ZK-994 of the series of 3-oxa-ATL analogs were potent orally inhibitors of inflammation *in vivo* in the zymosan A-induced peritonitis mice model (Bannenberg et al, 2004). In addition to LX, the resolvins 18R E-series (RvE1 & RvE2) derived from eicosapentaenoic acid (EPA), and the resolvins 17R and 17-S D-series (RvD1 & RvD2) and protectins (neuroprotectin D1/protectim D1; NPD1/PD1) derived from DHA as well as their aspirin-triggered epimeric forms are novel chemical agonists of resolution in the area of immunomodulation and tissue protection (Haworth & Levy, 2007; Schwab et al, 2007; Serhan & Chiang, 2008; Serhan et al, 2008a&b; Merched et al, 2008).

Moreover, though a small lipid component, SPG is a marker for apoptotic cells in different systems (Kingdom et al, 2000a; Vance & Vance, 2004). SPG is located exclusively to the inner leaflet of most cells and loss of PL asymmetry and exposure of SPG to the outer leaflet appears to be a universal feature of apoptotic cells (Fadok et al, 1998). Similarly, in the placenta membranes, triggering the apoptotic cascade will lead to the phosphatidylserine expression on the surface of cytotrophoblasts (Kingdom et al, 2000a). The expression of this PL is associated with membrane-to-membrane fusion leading to the formation of synchytiotrophoblast (Kingdom et al, 2000a), the primary barrier for transplancental transport in humans (Powell et al, 1999). Whether the low DHA and AA in SPG could be linked to the apoptotic placental cascade leading to preterm delivery, it requires further investigation.

Nonetheless, the increased AA in FFA or altered AA in membrane PL may induce apoptosis (Klingler et al, 2006), because impaired FA oxidation may lead to accumulation of fatty acids in the placentae. Similarly, AA was increased in placental FFA of mothers with acute fatty liver of pregnancy (AFLP) (Natarajan et al, 2010). In contrast, when mothers were supplemented with fish oil (DHA) in combination with folic acid, the placental trophoblastic tissue showed enhanced proliferation (Klingler et al, 2006).

Arachidonic and docosahexaenoic acids, fetal measurements of biometry and prematurity

The low levels of AA and DHA in preterm neonates are greatly variable (Crawford et al, 1989, 2003; Leaf et al, 1992b) At birth large part of the variation for AA is explained by birthweight (BWT) and for DHA by gestational age (GA), with both related to head circumference, HC (Leaf et al, 1992b; Crawford et al, 2003). In agreement with the low DHA in the infants of shorter GA and the known role of DHA to prolong gestation, placental DHA was associated with GA (Figure 4.1), suggesting that the low DHA level might be attributed to the preterm delivery rather than to reduced fetal growth rates, since the preterm infants were mainly appropriate for gestational age (AGA) and had no signs of intrauterine growth retardation (this study).

The umbilical (Crawford et al, 2003) and placental PUFA data (this study) greatly support that these variations are likely to be prenatal in origin. Indeed, the positive association of DHA in early pregnancy with BWT and HC (van Eijsden et al, 2008; Dirix et al, 2009), but not during pregnancy or at delivery (Dirix et al, 2009) may suggest a fetal-growth programming potential of maternal DHA. In line with the above, at three months of age, the low AA and EFA index in umbilical artery was associated with an unfavourable neurological condition in the healthy infants as measured by quality of general movements, a highly accurate predictor of the development of cerebral palsy (Bouwstra et al, 2006).

Moreover, follow up studies showed that the lower neonatal AA and DHA status at preterm delivery remained lower at the expected date of delivery, irrespective whether the newborn were breastfed or formula fed (Leaf et al, 1992a; Ghebremeskel et al, 1995). This could also be explain due to the fact that GA at delivery (very preterm) and/or insulin diabetes mellitus strongly compromised the onset of lactogenesis II in humans (Hartmann & Cregan, 2001; Henderson et al, 2008). In a case of late prematurity in Sweden, where the premature infants given feeds of breast milk derived from the milk bank within 1-2h of birth and gradually replaced with their own mothers' milk within three days, at 1 wk postnatal age, the infant

plasma had higher linoleic (LA) and α-linolenic (ALA) acids and lower di-hommo-γ-linolenic acid (DGLA, 20:3n-6), AA and DHA compared with the cord plasma (Sabel et al, 2009).

4.5.2. Placental weight, total lipid content and fatty acids in gestational diabetic pregnancies

Placental weight in gestational diabetes mellitus

Diabetic and impaired gestational glucose tolerance (IGGT) women deliver large placentas (Phillips, 1998; Makhseed et al, 2004; Alonso et al, 2006; Jansson et al, 2006); 573g placental wt in IGGT (Makhseed et al, 2004), 603 g in non- macrosomic and 765 g in macrosomic insulin-dependent diabetes mellitus (IDDM) (Lepercq et al, 2001), and between 592.0-656.0 g in GDM (Taricco et al, 2003; Ashfaq et al, 2005; Alonso et al, 2006; Varastehpour et al, 2006). In this study, the mean total placental wt in GDM was within the reported range (640±176.7, g).

Total lipid content in the placenta in gestational diabetes mellitus

In agreement with Daimant & Kissilevitz (1983), the first study to show that maternal diabetes in late pregnancy was accompanied by metabolic changes in placental tissue, and with Varastehpour et al (2006), the lipid content of the placenta was elevated in GDM (this study).

It was reported that the amount of TG was enhanced in the human Type-1 diabetes and GDM placentae (Daimant & Kissilevitz, 1983; Coleman, 1989; Lindegaard et al, 2006), leading to a greater lipid mass due to maternal diabetes. Similarly, the placenta of the STZ-diabetic rat showed enhanced TG and cholesterol esters (CE) levels, expressed as μg/mg protein (Capobianco et al, 2008). On the other hand, total PL (mg/tissue) were decreased in the placenta of the GDM and insulin-treated women, 21.4 ±0.4 vs. 24.5 ± 0.9 in controls (Varastehpour et al, 2006).

Placental fatty acids in pregnancy complicated with gestational diabetes mellitus

GDM resulted in elevated AA and DHA and reduced monounsaturated fatty acids, pamlitoleate and oleate in the major placental PL subclasses, choline (CPG) and ethanolamine (EPG) (this study). On the other hand, it was reported that AA and DHA were

unaltered in the placenta of obese neonates born to GDM insulin-treated women compared with control (Varastehpour et al, 2006) while reduced DHA and normal AA levels were reported in the placenta of type 1 diabetic women (Lakin et al, 1998).

The contrast between our findings and that of Lakin et al (1998) and Varastehpour et al (2006) could be a reflection of the two different disease entities. In the latter studies, fatty acids (FA) of placental total lipids (PL and TG) or total PL and total NL rather than of the individual lipid fractions were determined. Hence, it is conceivable that the real effect of diabetes on the FA composition of the individual PL might have been masked by the disproportion contribution of the neutral lipids, primarily triacylglycerols (TG). For instance, Varastehpour et al (2006) reported that in total NL of the placenta, DHA was increased, 1.60±0.15 vs. 1.10±0.15, controls. However, we believe that this increase in DHA was contributed to FFA content of the placenta rather than to NL components, TG and CE. Herein, DHA was elevated in FFA and was unaltered in TG and CE of the GDM placentae. Moreover, the advantage of FA analysis of the individual NL and FFA fraction rather than total NL is further demonstrated by the greater proportions of AA in FFA and CE (this study) in the GDM. Whilst, Varastehpour et al (2006) reported a comparable AA in total NL of the GDM and non-GDM placentae.

The differences in placental AA and DHA between the GDM and non-GDM women (this study) were unlikely to have been a reflection of their dietary background since AA was reduced by 25% in placental TG of GDM relative to that of the non-GDM women. The GDM group comprised of four Africans and seven Asians, and the non-diabetics of eight Caucasians, ten Africans and twenty-two Asians. It is conceivable that the observed difference between the two groups could be due to ethnic imbalance of the populations studied. However, the difference in the pattern of the major PUFA in CPG, EPG and TG between the GDM and non-GDM Asians was similar to that of the total GDM and non-GDM groups. Similarly, in a British and Korean cohort, where the habitual diet in South Korean is high in n-3 PUFA and low in total fat, all GDM or controls (British vs. Korean) pregnant women had a comparable red blood cell, RBC-CPG AA and DHA content at delivery, which suggest that the reduced membrane AA an DHA in GDM might be attributed to the disease *per se*, regardless of ethnicity, obesity or diet (Min et al, 2006).

Similarly, it was reported that the reduced n-6 and n-3 PUFA profile in FFA of the preeclamptic placenta was relate to the metabolism and not dietary intake of the mothers (von Versen-Hoeynck & Powers, 2007). Moreover, Thomas et al (2006) showed that the higher DHA intakes in GDM as oppose to non-GDM was most likely due to the effect of

dietary advice given to GDM, rather a reflection of the different habitual diets. Though fish intakes were increased, still DHA was below the recommended DHA level in pregnancy (Thomas et al, 2006).

Arachidonic and docosahexaenoic acids in placenta of gestational diabetic women

In previous communications, the IBCHN data reported that red cells of women with gestational diabetes mellitus (GDM) at diagnosis (Min et al, 2004), and plasma (Thomas et al, 2005) and red cells (Min et al, 2005b) of their neonates at birth had an abnormal FA composition. More currently, low AA and DHA was also reported in the umbilical artery plasma of the GDM infants compared with the non-GDM infants (Ortega-Senovilla et al, 2009). Two questions arose from these studies: (a) Whether this abnormality is a generalised phenomenon or restricted to red cells of the GDM mother and plasma and red cells of their newborn babies? (b) Why do the seemingly non-diabetic healthy babies of women with GDM have abnormal blood AA and DHA?

The placenta is a very important tissue to investigate in relation to maternal-fetal AA and DHA status and balance. First, the human placenta is a vascular organ rich in phospholipids (Nikolasev et al, 1973; Bayon et al, 1993; Anand et al, 1996; Klingler et al, 2003; Varastehpour et al, 2006), which in turn are rich in PUFA, particularly AA (Chapter 3). Second, the placenta lacks desaturase activity (Chambaz et al, 1985) and is thought to be primarily dependent on maternal circulation for the supply of these vital PUFA. Consequently, diabetes-induced abnormality in supply and/or uptake would be expected to alter placental AA and DHA composition.

In contrast to plasma (Thomas et al, 2004; Ortega-Senovilla et al, 2009) and red cells (Min et al, 2004), the placenta of the GDM women had higher levels of both AA and DHA in the major lipid components, CPG (outer cell membrane) and EPG (inner cell membrane) compared with the non-diabetics. AA was elevated by 13% and 6%, and DHA by 27% and 21% in CPG and EPG of the diabetics, respectively. The high AA and DHA in CPG and EPG of the GDM group could not be explained by enhanced synthesis since the placenta is thought to be devoid of desaturase activity (Chambaz et al, 1985). As maternal circulation is the primary source of placental AA and DHA, our data suggests that the uptake of these two long chain polyunsaturated fatty acids (LCPUFA) is enhanced in gestational diabetes.

There is also evidence of alteration of the transporters and transport of LCPUFA in experimental animals (Luiken et al, 2002; Chabowski et al, 2004) and enhanced expression of the liver fatty acid binding protein in placental homogenates of the GDM women (Magnusson et al, 2004). Bonen et al (2004) also hypothesised that the intramuscular accumulation in human obesity and type-2 diabetes is attributable to an increased rate of LCPUFA transport as the result of an increase in the FA transporters, fatty acid translocase protein (FAT/CD36) and/or plasma-membrane associated fatty acid-binding protein (FABPpm). Actually, the importance of both FAT/CD36 and FABPpm in regulating LCPUFA uptake and metabolism in the heart of obese, Type 1 & 2 diabetes has been clearly demonstrated (Chabowski et al, 2008).

It is also conceivable that the fetal circulation was the source of the high PUFA proportions in the placenta. Indeed, when supply of AA is high then the fetus discharged AA in the placenta and may act as a source of maternal AA (Hendrickse et al, 1985). However, the mothers with GDM had comparable AA in their plasma TG and CPG (Thomas et al, 2004; Min et al, 2006) to suggest that fetal AA and DHA resulted in increased PUFA content in the placenta. On the contrary, diabetes alters the acylation and/or deacylation process of membrane PL (Min et al, 2006) and the GDM mothers had low AA and DHA in their RBC- PL (Min et al, 2004).

In parallel with the report that fetal lipid accumulation is caused by an increased maternal-to-fetal lipid concentration gradient, diabetic women may enhance the supply of FFA and/or TG to the syncytiotrophoblast (Lindegaard et al, 2006). The lipoprotein lipase (LPL) activity of the placenta is increased in diabetes (Magnusson et al, 2004) and could contribute to its high content in the long chain polyenoic FA (this study). In fact, FFA cross the placenta to a lesser extend (Di Cianni et al, 2005), hence the dissociation of FFA from maternal TG by placental LPL is important for delivering lipid nutrients to the fetus (Lindegaard et al, 2006). On the other hand, whether the reduction of AA in TG (25%, $P>0.05$) could indicate selectively transfer of TG AA to the fetus requires further investigation. Bonet et al (1992) showed a ten-fold preference in uptake of FA from TG compared with uptake of albumin-bound fatty acids by isolated placental trophoblasts. Also, it could be that AA was utilised for eicosanoid production (Kuhn et al, 1990), hydrolysed and re-esterified into lipid components or both. AA was elevated in placental PL and CE (This study).

The question is, whether the abnormal FA composition of the placenta of the GDM women, which was manifested by higher levels of AA and DHA, would help explain the reduced levels of the two LCPUFA in the newborn offspring of women with GDM (Min et al, 2005b; Thomas et al, 2005; Ortega-Senovilla et al, 2009). The fetus and neonate are able to synthesise AA

and DHA (Poisson et al, 1993; Descomps & Rodriguez, 1995; Salem et al, 1996; Carnielli et al, 1996). However, because of the slow rate of synthesis and the consequential inability to support optimal development (Farquharson et al, 1992; Makrides et al, 1994), it is acknowledged that maternal circulation is the major source of AA and DHA for the fetus. The enhanced levels of the two LCPUFA in the placenta and the concomitant reduction in the fetus suggest that the two LCPUFA are taken up by the former and retained after esterification mainly into PL instead of being transferred to the fetus. The amount of AA and DHA retained in GDM is likely to be significant since in the placenta, PL account for about 80% of total lipids, EPG and CPG for 60% of the PL and LCPUFA for 40% of the PL fatty acids (Nikolasev et al, 1973; Klingler et al, 2003; Bayon et al, 1993).

Conversely, Ortega –Senovilla et al (2009) was the first study to show that umbilical vein plasma AA and DHA (placental supply) was unaltered in the GDM, suggesting that placental transfer was unimpaired. However, Ortega-Senovilla et al. did not analyse placental FA composition. Whilst, animal studies showed that increasing maternal glycaemia is associated with a decrease in the unidirectional transfer of FA suggesting that the uteroplacental blood flow is compromised in diabetic pregnancies (Honda et al, 1990).

The limitations of the GDM study were the ethnic heterogeneity of the subjects and the lack of FA intake data. Nevertheless, in spite of these shortcomings, the investigation reveals, in contrast to fetal blood, the placenta of women with pregnancy-induced transient diabetes mellitus has elevated levels of the vital fatty acids AA and DHA.

4.6. Conclusion

Preterm delivery or pregnancy-induced transient diabetes altered placental AA and DHA composition. The diverse PUFA status in the two clinical conditions, low DHA in preterm and enhanced AA and DHA in GDM, may suggest a different placental response to the intrauterine lipid metabolic changes that resulted to preterm delivery or during the development of GDM and that other factors might be involved such as GA. It is conceivable that the low DHA and altered AA could reflect a compromised PUFA status of the placental and fetal vasculature expressed in placental malfunction and the vascular related complications of prematurity postnatally. Conversely, in women with GMD the placenta has elevated levels of AA and DHA, suggesting that the uptake of these two fatty acids is enhanced. However, the mechanism involved and the effect of this phenomenon on the long-term fetal growth and development remain to be elucidated.

CHAPTER 5: Liver and heart fatty acid composition of the offspring born to diabetic and non-diabetc dams fed on a high-lard diet during pregnancy

5.1. Introduction

Epidemiological studies have strongy suggested that maternal, fetal and neonatal nutrition may programme the individual, setting the risk for coronary heart disease (CHD), hypertension, stroke and non-insulin dependent diabetes mellitus (NIDDM) in adulthood (Barker et al, 1990, 1993; Barker, 1994). The high intake of saturated fatty acids (SFA) or hydrogenated fats rich in *trans* fatty acids and SFA in Western diets has been linked to endemic coronary vascular disease and several studies up to date have demonstrated the athero-and thrombo-genicity of SFA and *trans* fatty acids (Spady et al, 1993; Mustad et al, 1997; Simopoulos et al, 1999; Kris-Etherton et al, 2002; Das, 2008; Pisani et al, 2008).

Arachidonic (AA, 20:4n-6) and docosahexaenoic (DHA, 22:6n3) acids are vital structural lipid components in biomembranes. AA is the major essential fatty acid component of the inner cell membrane lipid in human vascular endothelium (Crawford et al, 1997) and DHA is an important component of the photoreceptor (Anderson & Maude, 1972; Crawford & Sinclair, 1972; Uauy & Hoffman, 2000), synaptosomes (Suzuki et al, 1997), and has a crucial function in signal transduction and amplification. PUFA are also involved in inflammation and regulation of cellular function. There is evidence to suggest that pre- and postnatal deficits of AA and DHA may partly contribute to the vascular and neurodevelopmental complications of prematurity (Crawford et al, 1998). The developing fetus and neonate are dependent on the maternal supply of AA and DHA (Crawford et al, 1976; Ruyle et al, 1990) and the human placenta selectively transfers AA and DHA from the maternal to the fetal circulation (Crawford et al, 1976; Crawford, 2000).

AA and DHA are synthesized from their respective parent essential fatty acids (EFA), linoleic (LA, 18:2n-6) and a-linolenic (ALA, 18:3n-3), by a process of desaturation and chain elongation. In human Type 1 & 2 diabetes and experimental diabetes the activity of Δ-5 and Δ-6 desaturases is impaired (Brenner et al, 2000; Vessby, 2000). Suppressed conversion of LA to AA in various tissue of experimental diabetes (Huang et al, 1984; Igal et al, 1991; Shin et al, 1995) and reduced level of AA and DHA in human diabetes (Arisaka et al, 1991), pregnant diabetic women and their neonates have been reported (Min et al, 2004; Ghebremeskel et al, 2004; Min et al, 2005a&b). Moreover, maternal diabetes may have

adverse effects on cardiovascular function of the next generation, since the offspring born to diabetic mothers demonstrated overt insulin resistance in adulthood (Holemans et al, 1991).

The previous study (Chapter4) showed that GDM (a good example of *in utero* disturbances) or preterm pregnancy *per se*, altered the AA and DHA content in the human placenta. In addition, the uteroplacental blood flow was compromised in the rat diabetic pregnancy (Honda et al, 1990). Because changes in maternal metabolism may modify the metabolism of EFA to their long chain polyunsaturated fatty acids (LCPUFA) and promote deleterious consequences to the offspring, such as predisposition to develop cardiovascular and metabolic diseases (Das, 2008; Pisani et al, 2008), the uniqueness of this study is that it was set up to investigate the effect of a maternal high-fat diet or the combined effect of a high-fat diet and maternal diabetes during pregnancy on liver and heart EFA of their offspring; the neonates reflecting intrauterine growth and development and the suckling pups reflecting lactation period. The investigation was particularly concerned with AA and DHA because of their crucial role in the structure and function of vascular and neural membranes.

5.2. Methods

Animal growth and husbandry were kindly performed by our collaborator Dr. E. Koukkou from St. Thomas' Hospital (Ethical approval see section 2.2.2.). The streptozotocin (STZ)-induced diabetic rat was used which demonstrates many of the characteristics and complications of the human diabetes (Lopez-Soldado & Herrera, 2003; Mir et al, 2008; Zhnag et al, 2008; Sinzato et al, 2009). This rat model has been used successfully for several years in investigations of diabetic pregnancy by our collaborators at St.Thomas' hospital (Honda et al, 1990; Thomas & Lowy, 1992; Koukkou et al, 1998).

5.2.1. Animal husbandry and dietary protocol

Briefly: Female Sprague-Dawley rats of breeding age (12 - 14 wk old; Charles River Laboratories, UK) were habituated in a 12h light: dark cycle with food and water freely available *ad libitum*, intake and body wt monitored daily. This is a universally accepted procedure (Innis & de la Presa Owens, 2001; Amusquivar & Herrera, 2003; Taylor et al, 2003; Khan et al, 2003, 2005; Friesen & Innis, 2006; Armitage et al, 2007; Pisani et al, 2008).

Female rats were fed on either a standard breeding diet of normal laboratory chow (4.28% fat [corn oil]; Rat and Mouse Diet [RM], Special Diet Services, Witham, Essex, UK) or a diet high in fat (animal lard 30% was used for the formulation of this diet; final analysis: 32.9% fat) for 10d prior to mating, through pregnancy and lactation. To offset the dilution effect of the lard, the levels of protein and essential micronutrients in the high fat diet were adjusted. The composition of fatty acids and other essential nutrients of the two experimental diets are shown in Table 5.1&5.2.

5.2.1.1. Induction of diabetes

On days 1-2 of pregnancy, diabetes was induced in half animals by caudial injection of streptozotocin (30 mg/kg; Upjohn Co., Kalamazoo, MI, USA). This low dose of STZ-induced diabetes was within the reported ranges potent to induce hyperglycaemia (Koh et al, 2007; Okamoto et al, 2008; Cunha et al, 2009). Diabetes was confirmed by the demonstration of glycosuria (using glucostix®; Boehringer Mannheim, Lewes, East Sussex, UK) 48h after injection. Likewise, diabetes was confirmed by measurements of glucosuria and blood glucose levels (Veerkamp et al, 1996). The severity of diabetes was monitored for glucose and ketones every 2d during pregnancy using glucostix® and ketostix® (Boehringer Mannheim, Germany) respectively, and in order to partially control the diabetes, insulin was given in the form of an implant (half tablet; release 2U/24h per implant for >40 d; Limplant, Mollegard, Denmark) which was inserted subcutaneously when ketonuria was first detected.

5.2.2. Sample collection and fatty acid analysis

Total of fifty-nine (n=59) pregnant rats entered the study and divided into four groups: Low-fat control (LFC, n=15); Low-fat diabetic (LFD, n=10); High-fat control (HFC, n=24) and High-fat diabetic (HFD, n=10). Of those control and diabetic animals that fed the 32.9% fat diet and experienced difficulty in labour, were humanely sucrified. Of the offspring, total of forty-one (n=41) newborn and total of thirty-five (n=35) suckling pups entered the study and categorized in the same four groups as follows: a) newborn of the LFC mothers (n=10); newborn of the LFD mothers (n=9); newborn of the HFC mothers (n=16) and newborn of the HFD mothers (n=6) and b) suckling pups of the LFC mothers (n=16); suckling pups of the LFD mothers (n=8); suckling pups of the HFC mothers (n=7) and suckling pups of the HFD

mothers (n=4). In The later group, due to the severity of the combination of maternal high-fat diet and diabetes and the increased mortality rates, only a small number of their offspring could be followed up to day 15.

The rats were weighed every 2 days (d) until 20-21d of pregnancy and their food and water intakes were monitored regularly. At delivery, a proportion of each litter was killed in order that five to six pups were left with the mother in all four groups. In each group, the same number of neonates was left to wean up to postpartum day 15 in order the neonates to have comparable milk intake and to avoid cannibalization of the pups. The suckling pups were killed at 15d and the mothers at 16d, postnatally. Animals were killed by CO_2 inhalation and cervical dislocation (Koukou et al, 1998; Capobianco et al, 2008).

Fresh samples (liver of the dams and their offspring and heart of all offspring) (section 2.2.2) were washed several times in cold saline to remove all traces of blood, quickly blotted and homogenized with high-speed tissue homogeniser (Bourre et al, 1990; Orellana et al, 1997; Merzouk et al, 2000; Friesen & Innis, 2006; Perez et al, 2006; Hsu & Huang, 2007) and processed for fatty acid analysis (section 2.3).

5.2.3. Sample size and statistics

These are described at sections 2.4.1. & 2.4.2.

5.3. Results

Data are presented as means±SD (Table 5.3-5.18) and as medians with interquartile range in Appendix A (Tables 5.3.A-5.18A). Herein, due to the vast data only the n-6 and n-3 poyunsaturated fatty acids (PUFA) composition of the vascular tissues (% Total fatty acids) and their fatty acid ratios will be described; refer to Table 5.-5.6 for dams' liver and Table 5.7-5.18 for offpsring's liver and heart lipid components % monounsaturated (MONO) and saturated (SFA) fatty acid distribution. In addition, due to the severity of the high-fat diet and maternal diabetes and the increased mortality rates, only a small number (n=4) of their offspring could be followed up to day (d) 15 in the high-fat diabetic (HFD) group. For this reason, significant differences (*P*-values) in liver and heart fatty acids between HFD suckling pups (15d) and their corresponding high-fat control (HFC) or low-fat diabetic (LFD) matched for age groups would not be stated.

5.3.1. Maternal liver phospholipid and neutral lipid-fatty acids

5.3.1.1. Liver choline and ethanolamime phosphoglycerides of the dams at 16 days

5.3.1.1.1. Low-fat fed dams (LFC vs. LFD)

In liver ethanolamine phosphoglycerides (EPG), arachidonic-to-linoleic acid ratio, AA/LA (Table 5.4) was lower in the LFD compared with the LFC dams ($P<0.05$).

5.3.1.1.2. High-fat fed dams (HFC vs. HFD)

In liver choline phosphoglycerides, CPG (Table 5.3), the proportions of docosatetraenoic (DTA) and eicosapentaenoic (EPA) acids ($P<0.05$) were lower in the HFD vs. HFC. The effect of diabetes and a high-lard diet was more pronounced in liver EPG (Table 5.4) n-6 PUFA. Linoleic acid (LA) was higher ($P<0.05$), whereas AA, di-hommo-γ-linolenic (DGLA), docosapentaenoic n-6 (DPA n-6) ($P<0.05$), DTA ($P<0.01$) acids and AA/LA ($P<0.005$) were lower in the HFD compared with the HFC mothers.

5.3.1.1.3. Low-fat vs. high-fat fed dams at 16 days

5.3.1.1.3.1. Control dams (LFC vs. HFC)

In liver CPG (Table 5.3), the HFC had lower percentage of 18:3n-6 compared with the LFC dams ($P<0.01$). In both lipid components (CPG and EPG), docosahexaenoic acid, DHA ($P<0.05$, CPG; $P<0.005$, EPG) and AA ($P<0.0001$, CPG; $P<0.005$, EPG) were elevated in the HFC mothers (Table 5.3&5.4). Likewise, in CPG and particularly in EPG, DPAn-6, DTA and docosapentaenoic-to-docosatetraenoic acid (n-6) ratio (DPA/DTA n-6) were markedly higher in the HFC compared with the LFC group ($P<0.0001$). In contrast, the proportions of the essential fatty acids (EFA), LA ($P<0.0001$; CPG and EPG) and α-linolenic acid, ALA ($P<0.005$, CPG; $P<0.0001$ EPG), DGLA, EPA and docosapentaenoic (n-3) acid, DPA n-3 ($P<0.0001$) were significantly lower in the HFC compared with the LFC mothers (Table 5.3&5.4). Thus, arachidonic-to-docosahexaenoic acid ratio, AA/DHA ($P<0.005$ in CPG), total metabolites n-6 (PUFA)-to-total metabolites n-3 (PUFA) ratio, Σmet n-6/Σmet n-3 ($P<0.005$; CPG and EPG) and AA/LA ($P<0.0001$; CPG and EPG) were enhanced in the HFC dams.

5.3.1.1.3.2. Diabetic dams (LFD vs. HFD)

In liver CPG, AA ($P<0.005$) was substantially higher in the HFD compared with the LFD (Table 5.3). In both liver CPG and EPG, the proportions of LA ($P<0.0001$), ALA($P<0.005$, CPG; $P<0.0001$, EPG), DGLA ($P<0.005$, CPG; $P<0.0001$, EPG), EPA ($P<0.0001$) and DPA n-3 ($P<0.05$, CPG; $P<0.0001$, EPG) were significantly lower, whereas DHA ($P<0.05$, CPG; $P<0.005$, EPG), DTA ($P<0.05$, CPG; $P<0.0001$, EPG) and DPA n-6 ($P<0.0001$) were higher in the HFD compared with the LFD (Table 5.3&5.4). Consequently, AA/LA and DPA/DTA n-6 were enhanced in the HFD group in both CPG ($P<0.0001$, $P<0.005$ respectively) and EPG ($P<0.0001$).

5.3.1.2. Liver free fatty acids of the dams at 16 days

5.3.1.2.1. Low-fat fed dams (LFC vs. LFD)

In liver free fatty acids (FFA), of the n-6 PUFA (Table 5.5.), the enhanced proportions of LA, AA and total n-6 PUFA (Σn-6) in the LFD group were striking ($P<0.005$). Moreover, DGLA ($P<0.005$) and DTA ($P<0.01$) were higher in the LFD compared with the LFC dams. Of the n-3 PUFA, the proportions of ALA, DPA n-3, total n-3 (PUFA), Σn-3 ($P<0.05$) and especially DHA ($P<0.01$) were elevated in the liver FFA of the LFD mothers.

5.3.1.2.2. Low-fat vs. high-fat fed dams at 16 days

5.3.1.2.2.1. Control dams (LFC vs. HFC)

In liver FFA, the enhanced proportions of LA, AA ($P<0.0001$) and Σn-6 ($P<0.0001$) were striking in the HFC dams (Table 5.5). DTA ($P<0.0001$) and DPA n-6 ($P<0.05$) were higher while EPA ($P<0.0001$), Σn-3 and DPA/DTA n-6 ($P<0.05$) were lower in the HFC compared with the LFC dams. AA/DHA, Σmet n-6/Σmet n-3 and total n-6 (PUFA) –to- total n-3 (PUFA), Σn-6/Σn-3 were markedly enhanced in the liver FFA of the HFC mothers ($P<0.0001$).

5.3.1.2.2.2. Diabetic dams (LFD vs. HFD)

The HFD had higher DTA ($P<0.005$) and lower DGLA ($P<0.0001$), ALA ($P<0.01$), EPA ($P<0.005$) and Σn-3 ($P<0.05$) compared with the LFD group in maternal liver FFA (Table 5.5).

Hence, Σmet n-6/Σmet n-3 and Σn-6/Σn-3 were enhanced in the HFD mothers ($P<0.05$). Although, there were no differences in the proportions of AA and DHA in liver FFA between the two groups, the ratio AA/DHA was higher in the HFD vs. LFD dams ($P<0.05$) (Table 5.5).

5.3.1.3. Liver triacylglycerols of the dams at 16 days

5.3.1.3.1. Low-fat dams (LFC vs. LFD)

In liver triacylglycerols (TG), of the n-6 PUFA, the elevated proportions of LA ($P<0.005$) and Σn-6 ($P<0.01$) were striking of the LFD dams (Table 5.6). DGLA ($P<0.005$) and AA ($P<0.05$) were also higher in the LFD vs. LFC dams. Likewise, of the n-3 PUFA, percent EPA, DHA ($P<0.005$), DPA n-3 and Σn-3 ($P<0.0001$) were elevated significantly in liver TG of the LFD mothers. As a result, compared with the LFC, AA/DHA ($P<0.01$), DPA/DTA n-6 ($P<0.05$), Σmet n-6/Σmet n-3 ($P<0.0001$) and Σn-6/Σn-3 ratios ($P<0.005$) were reduced in the LFD group (Table 5.6).

5.3.1.3.2. High-fat dams (HFC vs. HFD)

In liver TG, the enhanced proportions of AA (Table 5.6) contributed to the elevated AA/LA ratio in the HFD mothers ($P<0.05$). Moreover, DGLA and EPA, DPA n-3, DHA and Σn-3 ($P<0.0001$) were higher in the HFD vs. HFC group. Hence, AA/DHA ($P<0.05$), Σmet n-6/Σmet n-3 and Σn-6/Σn-3 ratios ($P<0.0001$) were reduced in the HFD dams (Table 5.6).

5.3.1.3.3. Low-fat vs. high-fat fed dams at 16 days

5.3.1.3.3. 1. Control dams (LFC vs. HFC)

In liver TG, percent LA ($P<0.0001$) and LA metabolites, 18:3n-6 ($P<0.005$), DGLA, AA, DTA, DPA n-6 and Σn-6 ($P<0.0001$) were elevated in the HFC dams (Table 5.6). Of the n-3 PUFA, the proportions of EPA ($P<0.0001$) were lower while DPA n-3 ($P<0.005$) and Σn-3 ($P<0.05$) were higher in the HFC compared with the LFC mothers. As a result, AA/LA, AA/DHA and Σmet n-6/Σmet n-3 were elevated in the former group ($P<0.0001$).

5.3.1.3.3.2. Diabetic dams (LFD vs. HFD)

Of the n-3 PUFA, the percentage of EPA ($P<0.005$) in liver TG was significantly lower in the HFD compared with the LFD mothers (Table 5.6). Whilst, of the n-6 PUFA, 18:3n-6 ($P<0.01$), AA, DTA and DPA n-6 ($P<0.005$) were higher in the HFD vs. LFD group. Consequently, DPA/DTA n-6 ($P<0.005$), AA/LA, AA/DHA and Σmet n-6/Σmet n-3 ($P<0.0001$) were enhanced in the liver TG of the HFD dams (Table 5.6).

5.3.2. Liver and heart fatty acids of the newborn (1d) and suckling (15d) pups

5.3.2.1. Liver choline phosphoglycerides

5.3.2.1.1. Offspring of the low-fat fed dams (LFC vs. LFD)

In liver CPG, all LFD offspring had lower AA ($P<0.01$ at 1d; $P<0.005$ at 15d) compared with the offspring of the LFC group, matched for age (Table 5.7&5.8). Moreover, at 15d suckling pups, the proportions of DTA ($P<0.05$), Σn-6 ($P<0.005$), AA/LA ($P<0.005$), AA/DHA, Σmet n-6/Σmet n-3 ($P<0.01$) and Σn-6/Σn-3 ($P<0.05$) were lower in LFD vs. LFC group (Table 5.8).

5.3.2.1.2. Low-fat vs. high-fat group

5.3.2.1.2.1. Control offspring (LFC vs. HFC)

In liver CPG, all offspring of the HFC group had higher proportions of DTA ($P<0.05$ at 1d; $P<0.005$ at 15d), DPA n-6 ($P<0.0001$) and DPA/DTAn-6 ($P<0.0001$ at 1d; $P<0.005$ at 15d) compared with their corresponding LFC group at 1d and 15d (Table 5.7&5.8). Of the n-3 PUFA, DPA n-3 ($P<0.005$ at 1d; $P<0.0001$ at 15d), DHA and Σn-3 ($P<0.0001$) were lower in all HFC compared with their corresponding LFC pups, matched for age (Table 5.7&5.8). In liver CPG, the 15d HFC had lower proportions of LA and DGLA ($P<0.0001$) and higher AA ($P<0.005$), AA/LA and AA/DHA ($P<0.0001$) compared with the 15d LFC pups (Table 5.8).

5.3.2.1.2.2. Diabetic offspring (LFD vs. HFD)

At birth (1d), the HFD neonates had lower proportions of DPA n-3 ($P<0.005$), Σn-3 and Σn-6

($P<0.05$) and markedly higher DPA n-6 and DPA/DTA n-6 ($P<0.005$) compared with the LFD in liver CPG (Table 5.7). With regards to the suckling pups (15d), the trend of fatty acids (FA) showed that the HFD had low percent LA and DGLA and high AA, DTA, DPA n-6, AA/LA and DPA/DTA n-6 (Table 5.8). However, significance could not be denoted due to the high mortality rates and consequently, the small number of pups of the HFD dams that could be follewd up to 15d of postnatal life.

5.3.2.2. Liver ethanolamine phosphoglycerides

5.3.2.2.1. Offspring of the low-fat fed dams (LFC vs. LFD)

In liver EPG, the LFD newborn (1d) had higher percent AA, DPA n-6, Σn-6, AA/DHA, Σmet n-6/Σmet n-3 and Σn-6/Σn-3 ($P<0.05$) compared with their corresponding LFC newborn (Table 5.9). At 15d of life, percent LA ($P<0.005$) was markedly enhanced in liver EPG of the LFD suckling pups (Table 5.10). The proportions of AA, AA/LA, DHA and Σn-3 ($P<0.005$) were lower, whereas DGLA ($P<0.005$), Σn-6 and Σn-6/Σn-3 ($P<0.0001$) where higher in the 15d LFD compared with the 15d LFC pups (Table 5.10).

5.3.2.2.2. Offspring of the high-fat fed dams (HFC vs. HFD)

At 1d of life in liver EPG, the HFD had higher percent ALA ($P<0.05$) and lower DPA n-6 and DPA/DTA n-6 ($P<0.05$) compared with the HFC pups (Table 5.9). By contrast, at 15d of life the HFD had higher EPA (Table 5.10), however as stated above significance could not be stated.

5.3.2.2.3. Low-fat vs. high-fat group

5.3.2.2.3. 1. Control offspring (LFC vs. HFC)

In liver EPG, the HFC newborn (1d) and suckling pups (15d) had significantly higher percent AA ($P<0.0001$ at 1d; $P<0.005$ at 15d), DPA n-6, Σn-6 and DPA/DTAn-6 ($P<0.0001$) and substantially lower proportions of DHA and Σn-3 ($P<0.0001$) compared with the LFC pups, matched for age (Table 5.9&5.10). Hence, AA/DHA, Σmet n-6/Σmet n-3 and Σn-6/Σn-3 ($P<0.0001$) were enhanced in all HFC groups (1d and 15d) (Table 5.9&5.10). However, at 15d of life, the HFC had lower percentage of DPA n-3 and particularly EPA ($P<0.0001$) and

higher DTA ($P<0.005$) compared with their corresponding LFC group at 15d (Table 5.10). Moreover, the marked increase in LA ($P<0.005$) contributed to the lower AA/LA ratio ($P<0.005$) in the 15d HFC suckling pups (Table 5.10).

5.3.2.2.3. 2. Diabetic offspring (LFD vs. HFD)

In liver EPG at birth (1d), the HFD had lower percentage of DPA n-3 ($P<0.005$) and higher ALA ($P<0.01$) compared with their corresponding LFD neonates (Table 5.9). The enhanced proportions of AA ($P<0.05$) and DPA n-6 ($P<0.005$) contributed to the elevated DPA/DTA n-6 ($P<0.01$), AA/DHA, Σmet n-6/Σmet n-3 and Σn-6/Σn-3 ($P<0.05$) ratios in the HFD neonates (Table 5.9). On the other hand, the 15d HFD pups (Table 5.10) were characterized by reduced percent LA, EPA, DHA and Σn-3 and higher AA, DTA and DPA n-6, however, *P*-values could not be denoted due to the small sample size in HFD at 15d.

5.3.2.3. Liver free fatty acids

5.3.2.3.1. Offspring of the low-fat fed dams (LFC vs. LFD)

In liver FFA, percent Σn-6 ($P<0.05$) was higher in LFD at 1d and 15d of life compared with their corresponding LFC groups, matched for age (Table 5.11&5.12). Similarly, DPA/DTA n-6 ($P<0.05$) was elevated in the 1d LFD neonates (Table 5.11). Whilst, the 15d LFD pups, compared with the 15d LFC, had higer percent DHA and Σn-3 ($P<0.05$) and lower DTA ($P<0.005$) (Table 5.12).

5.3.2.3.2. Low-fat vs. high-fat group

5.3.2.3.2.1. Control offspring (LFC vs. HFC)

In liver FFA, compared with their corresponding LFC, ALA, DPA n-3 and especially EPA, DHA and Σn-3 were significantly lower in the HFC at 1d ($P<0.005$, $P<0.005$, $P<0.01$, $P<0.005$, $P<0.005$ respectively) and 15d pups ($P<0.0001$) (Table 5.11&5.12). Similarly, LA and DGLA were reduced in HFC at both 1d ($P<0.05$, $P<0.01$ respectively) and 15d ($P<0.0001$) (Table 5.11&5.12). By contrast, DPA n-6 ($P<0.005$ at 1d; $P<0.0001$ at 15d), DPA/DTA n-6 ($P<0.005$), Σmet n-6/Σmet n-3 and Σn-6/Σn-3 ($P<0.005$ at 1d; $P<0.0001$ at 15d) were elevated in liver FFA of all HFC groups (1d and 15d) (Table 5.11&5.12).

Moerovere, the 15d HFC had lower percentage of Σn-6 (P<0.005) and higher AA/LA (P<0.01) and AA/DHA (P<0.0001) compared with the 15d LFC pups (Table 5.12).

5.3.2.3.2.2. Diabetic offspring (LFD vs. HFD)

At birth (1d), the HFD had markedly higher proportions of DPA n-6 (P<0.05) and DPA/DTA n-6 (P<0.01), lower LA (P<0.01), DGLA and Σn-6 (P<0.05) and markedly lower ALA, EPA, DPA n-3, Σn-3 (P<0.005) and DHA (P<0.05) compared with their corresponding LFD in liver FFA (Table 5.11). Liver FFA of the HFD suckling pups (15d) was characterized by a substantially low percentage of ALA, EPA, DPA n-3, DHA, Σn-3, LA, and Σn-6 and a high DTA and DPA n-6 (Table 5.12), however, as above significance could not be denoted.

5.3.2.4. Liver triacylglycerols

5.3.2.4.1. Offspring of the low-fat fed dams (LFC vs. LFD)

In liver TG at 1d of life, the LFD had higher 18:3n-6, ALA, DPA n-3, DHA (P<0.05) and Σn-3 (P<0.01) and especially higher LA and Σn-6 (P<0.005) compared with their corresponding LFC neonates (Table 5.13). Whilst, at 15d of life, DTA (P<0.005) was lower and DPA/DTA n-6 (P<0.05) was higher in the LFD than the LFC group (Table 5.14).

5.3.2.4.2. Offspring of the high-fat fed dams (HFC vs. HFD)

At birth (1d), the HFD had lower proportions of DPA n-6, AA/DHA and Σmet n-6/Σmet n-3 (P<0.05) compared with the 1d HFC neonates in liver TG (Table 5.13). Whilst, the HFD suckling pups (15d) were characterized by enhanced proportions of DPA n-6, DHA and Σn-3 and reduced LA, Σn-6/Σn-3 (Table 5.14), however significance was not denoted due to the small sample size in HFD at 15d of postnatal life.

5.3.2.4.3. Low-fat vs. high-fat group

5.3.2.4.3.1. Control offspring (LFC vs. HFC)

In liver TG, all HFC had lower proportions of LA (P<0.05 at 1d; P<0.0001 at 15d) and higher DPA n-6 and DPA/DTA n-6 (P<0.005 at 1d; P<0.005 and P<0.05 at 15d, respectively)

compared with their corresponding LFC pups, matched for age (Table 5.13&5.14). The effect of a maternal high fat diet on the n-3 PUFA of the 1d and 15d offspring was also striking. ALA (P<0.01 at 1d; P<0.0001 at 15d), EPA (P<0.005), DPA n-3, DHA and Σn-3 (P<0.005 at 1d; P<0.0001 at 15d) were markedly lower while AA/DHA, Σmet n-6/Σmet n-3 and Σn-6/Σn-3 (P<0.005 at 1d; P<0.0001 at 15d) were significantly higher in the HFC vs. LFC pups (Table 5.13&5.14). Further more, the 15d HFC, compared with the LFC, had lower DGLA (P<0.005), and Σn-6 (P<0.0001) and higher AA (P<0.05), 18:3n-6 and AA/LA (P<0.005) in liver TG (Table 5.14).

5.3.2.4.3.2. Diabetic offspring (LFD vs. HFD)

In liver TG at birth (1d), the effect of maternal diabetes and a high-lard diet on the n-3 PUFA of the neonates was striking (Table 5.13). The proportions of ALA, EPA, DPA n-3, DHA and Σn-3 were substantially lower in the HFD compared with the LFD group (P<0.005). As a result, Σmet n-6/Σmet n-3 (P<0.05) and Σn-6/Σn-3 (P<0.005) were elevated in the 1d HFD neonates. In addition, liver TG of the 1d HFD had significantly higher proportions of DPA n-6 (P<0.05) and DPA/DTA n-6 (P<0.005) and lower LA, 18:3n-6 (P<0.05) and DGLA (P<0.01) compared with the 1d LFD neonates (Table 5.13). The 15d HFD showed a similar trend with regards to n-3 PUFA (Table 5.14), however significant differences could not be reported due to the small sample size. The 15d HFD group was also characterized by low percent LA and Σn-6 and high AA, DTA and DPA n-6 (Table 5.14).

5.3.2.2. Heart choline phosphoglycerides

5.3.2.2.1. Offspring of the low-fat fed dams (LFC vs. LFD)

In heart CPG at birth (1d), the LFD had higher DPA n-6 (P<0.005) and DPA/DTA n-6 (P<0.01) compared with their corresponding LFC neonates (Table 5.15). At 15d, 18:3n-6 (P<0.05) and EPA (P<0.05) were higher while DPA n-6 (P<0.005) and DTA (P<0.0001) were lower in heart CPG of the LFD vs. LFC (Table 5.16).

5.3.2.2.2. Offspring of the high-fat fed dams (HFC vs. HFD)

In heart CPG at birth (1d), the HFD had higher proportions of DHA (P<0.005) and Σn-3 (P<0.05) and lower DPA n-6 (P<0.05), AA/DHA, Σmet n-6/Σmet n-3 (P<0.05) and Σn-6/Σn-3

($P<0.005$) compared with the HFC neonates (Table 5.15).

5.3.2.2.3. Low-fat vs. high-fat group

5.3.2.2.3.1. Control offspring (LFC vs. HFC)

In heart CPG, EPA, DPA n-3, Σn-3 ($P<0.0001$ at 1d and 15d) and DHA ($P<0.005$ at 1d; $P<0.0001$ at 15d) were significantly lower in the HFC compared with the LFC pups, matched for age (Table 5.15&5.16). Consequently, AA/DHA ($P<0.005$), DPA n-6, DPA/DTA n-6, Σmet n-6/Σmet n-3 and Σn-6/Σn-3 ($P<0.0001$) were elevated markedly in the HFC at 1d and 15d (Table 5.15&5.16). In addition, the 15d HFC, compared with the 15d LFC pups had markedly higher proportions of AA and AA/LA ($P<0.0001$), higher DTA n-6 and Σn-6 ($P<0.005$) and lower ALA, LA ($P<0.0001$) and DGLA ($P<0.005$) in heart CPG (Table 5.16).

5.3.2.2.3.2. Diabetic offspring (LFD vs. HFD)

In heart CPG, the newborn (1d) of the HFD had lower proportions of 18:3n-6 ($P<0.005$), DGLA ($P<0.05$) EPA, DPA n-3 ($P<0.005$) and Σn-3 ($P<0.05$) and higher DPAn-6 ($P<0.005$), DPA/DTA n-6 ($P<0.05$) Σmet n-6/Σmet n-3 and Σn-6/Σn-3 ($P<0.01$) compared with their corresponding LFD newborn (Table 5.15). Although, significance could not be reported due to the small sample size in the 15d HFD, the effect of the combination of diabetes and a high in fat diet on the FA composition of the suckling pups was staggering. In heart CPG, the 15d HFD pups were characterized by greater proportions of AA, DTA, DPA n-6 and Σn-6 and substantially less DPA n-3, DHA and Σn-3 (Table 5.16). Hence, DPA/DTA n-6, AA/DHA and particularly AA/LA were elevated in the 15d HFD pups (Table 5.16).

5.3.2.3. Heart ethanolamine phosphoglycerides

5.3.2.3.1. Offspring of the low-fat fed dams (LFC vs. LFD)

In heart EPG, the LFD neonates (1d) had lower proportions of DHA and Σn-3 ($P<0.05$) and higher 18:3n-6, Σn-6 ($P<0.01$) and Σn-6/Σn-3 ($P<0.05$) compared with their corresponding LFC neonates (Table 5.17). On the other hand, the 15d LFD had lower percentage of DTA ($P<0.0001$) and DPA n-6 ($P<0.005$) and higer ALA and EPA ($P<0.05$) compared with their corresponding 15d LFC pups (Table 5.18).

5.3.2.3.2. Offspring of the High-fat fed dams (HFC vs. HFD)

At birth (1d) in heart EPG, the HFD had lower proportions of DGLA, DPA n-6 ($P<0.05$), Σn-6 ($P<0.005$) AA/DHA, Σmet n-6 /Σmet n-3 and Σn-6/Σn-3 ($P<0.005$) and higher AA/LA ($P<0.05$), DHA and Σn-3 ($P<0.005$) compared with the 1d HFC neonates (Table 5.17).

5.3.2.3.3. Low-fat vs. high-fat group

5.3.2.3.3.1. Control offspring (LFC vs. HFC)

In heart EPG, all HFC offspring had higher AA ($P<0.01$ at 1d; $P<0.0001$ at 15d), DTA ($P<0.05$ at 1d; $P<0.0001$ at 15d) and Σn-6 ($P<0.0001$) and markedly higher DPA n-6 and DPA/DTA n-6 ($P<0.0001$) compared with their corresponding LFC groups, matched for age (Table 5.17&5.18). Moreover, at 1d and 15d, a maternal high-fat diet significantly reduced the proportions of EPA ($P<0.0001$ at 1d and 15d) and DPA n-3 ($P<0.0001$ at 1d; $P<0.005$ at 15d) and markedly reduced DHA and Σn-3 ($P<0.0001$) in heart EPG of all HFC pups (Table 5.17&5.18). Thus, AA/DHA, Σmet n-6/Σmet n-3 and Σn-6/Σn-3 ($P<0.0001$) were elevated in the heart EPG of the HFC groups (1d and15d) (Table 5.17&5.18). Moreover, the 15d HFC had lower proportions of LA ($P<0.005$) and DGLA ($P<0.005$) and higher AA/LA ($P<0.0001$) compared with the 15d LFC suckling pups (Table 5.18).

5.3.2.3.3.2. Diabetic offspring (LFD vs. HFD)

In heart EPG at birth (1d), the HFD had lower proportions of EPA, DPA n-3 ($P<0.005$), DHA ($P<0.01$) and Σn-3 ($P<0.005$) compared with their corresponding LFD neonates (Table 5.17). Of the n-6, 18:3n-6 ($P<0.01$) and DGLA ($P<0.005$) were lower, whereas AA ($P<0.05$), DTA and DPA n-6 ($P<0.005$) were higher in the 1d HFD vs. 1d LFD in heart EPG. As a result, the ratios AA/LA, DPA/DTAn-6 ($P<0.05$), AA/DHA, Σmet n-6/Σmet n-3 and Σn-6 /Σn-3 ($P<0.005$) were elevated in the 1d HFD neonates (Table 5.17). In contrast, the15d HFD suckling pups were characterized by substantially low LA, EPA, DHA and Σn-3 and markedly greater proportions of AA, DPA n-6 and AA/LA in heart EPG (Table 5.18), however, as above *P*-values were not reported.

Nutrients	Breeding diet (4.28% corn oil)	High fat diet (32.9% fat, mainly lard)
Total lipids (g/kg)	428	329
Crude protein (g/kg)	223	180
Carbohydrate (g/kg)	510	385
Crude Fiber (g/Kg)	50	30
Gross Energy (KJ/kg)	1526	2277
Arginine (g/kg)	15.6	12.1
Cystine (g/kg)	3.6	2.6
Histidine (g/kg)	5.8	3.5
Methionine (g/kg)	4.7	3.5
Lysine (g/kg)	14.1	11.1
Isoleucine (g/kg)	10.5	8.3
Leucine (g/kg)	17.8	12.5
Threonine (g/kg)	9.2	6.4
Tryptophan (g/kg)	2.8	2.1
Valine (g/kg)	11.6	8.2
Choline (mg/kg)	1882	1317
Cyanocobalamin (μg/kg)	28.2	19.7
Folic acid (mg/kg)	2.7	1.9
Pyridoxine (mg/kg)	19.3	13.5
Thiamin (mg/kg)	25.7	19.1
Riboflavin (mg/kg)	10.9	7.6

Table 5.1. Nutrient composition of the experimental animal diets

	% Total fatty acids	
	Breeding (4.28% corn oil)	High fat diet (32.9% fat, mainly lard)
14:0	0.56	1.52
16:0	15.9	24.6
18:0	2.46	13.6
20:0	0.17	0.20
22:0	0.65	-
24:0	0.11	-
ΣSFA	19.9	40.0
16:1	0.71	2.27
18:1	18.6	38.3
20:1	0.90	0.84
22:1	0.11	-
Σmono	20.3	41.1
18:2n-6	51.6	15.5
20:2n-6	0.06	0.34
20:3n-6	-	0.03
20:4n-6	0.12	0.14
Σn-6	51.8	16.0
18:3n-3	5.73	1.66
22:5n-3	0.15	0.13
22:6n-3	0.09	0.06
Σn-3	5.97	1.85
PUFA/SFA	2.91	0.45
18:2n-6/18:3n-3	9.01	9.34
Σn-6/Σn-3	8.67	8.64

Table 5.2. Fatty acid composition of the experimental animal diets. ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σn-6; total n-6 PUFA, Σn-6/ Σn-3; total n-6 (PUFA)-to-total n-3 (PUFA) ratio.

% Total fatty acids

	LFC[a]	LFD[b]	HFC[c]	HFD[d]
16:0	18.6±2.31	17.5±3.63	18.1±4.50	19.0±3.72
18:0	27.9±3.39	28.7±3.11	28.8±4.46	29.5±2.76
20:0	0.04±0.01	0.06±0.02	0.04±0.02	Trace
22:0	Trace	Trace	0.09±0.07	Trace
24:0	0.04±0.01	Trace	0.05±0.03	0.05±0.03
ΣSFA	46.6±1.71[d]	46.3±1.25[d]	47.0±0.67[d]	48.7±2.56[a,b,c]
16:1	0.53±0.22[b,c,d]	0.36±0.17[a,c,d]	0.16±0.05[a,b]	0.18±0.06[a,b]
18:1	7.34±1.81[c,d]	6.50±2.68	5.70±0.93[a]	5.72±1.12[a]
20:1	0.07±0.02[d]	0.08±0.02[d]	0.07±0.03	0.06±0.01[a,b]
22:1	Trace	Trace	Trace	Trace
24:1	0.05±0.03	0.03±0.02	0.04±0.02	Trace
Σmono	7.99±1.98[c,d]	6.97±2.83	5.96±0.94[a]	6.00±1.14[a]
18:2n-6	15.7±1.99[c,d]	16.7±2.76[c,d]	11.0±1.70[a,b]	10.6±2.11[a,b]
18:3n-6	0.19±0.14[c,d]	0.17±0.11[c]	0.07±0.06[a,b]	0.08±0.06[a]
20:2n-6	0.22±0.06[d]	0.27±0.13[d]	0.20±0.04[d]	0.16±0.05[a,b,c]
20:3n-6	1.72±0.44[c,d]	1.63±0.49[c,d]	1.01±0.42[a,b]	0.75±0.37[a,b]
20:4n-6	15.3±2.49[c,d]	15.5±4.32[c,d]	22.1±3.93[a,b]	21.2±3.60[a,b]
22:4n-6	0.13±0.02[c,d]	0.15±0.07[c,d]	0.29±0.07[a,b,d]	0.24±0.03[a,b,c]
22:5n-6	0.16±0.05[c,d]	0.15±0.04[c,d]	0.69±0.16[a,b]	0.63±0.17[a,b]
Σn-6	33.4±2.07[c]	34.5±4.71	35.1±5.31[a]	33.6±5.87
18:3n-3	0.15±0.04[c,d]	0.16±0.06[c,d]	0.04±0.01[a,b]	0.03±0.01[a,b]
20:5n-3	1.69±0.26[c,d]	1.31±0.55[c,d]	0.09±0.04[a,b,d]	0.07±0.03[a,b,c]
22:5n-3	1.11±0.27[c,d]	1.31±0.46[c,d]	0.77±0.22[a,b]	0.89±0.24[a,b]
22:6n-3	7.21±0.92[c,d]	7.61±2.89[c,d]	8.96±3.22[a,b]	9.00±2.78[a,b]
Σn-3	9.51±1.46	9.93±3.22	9.83±3.33	9.98±2.90
AA/LA	0.99±0.25[c,d]	0.96±0.34[c,d]	2.03±0.42[a,b]	2.02±0.24[a,b]
AA/DHA	2.15±0.44[c,d]	2.21±0.79	2.77±0.92[a]	2.58±0.80[a]
DPA/DTA n-6	1.34±0.46[c,d]	1.22±0.61[c,d]	2.62±0.95[a,b]	2.64±0.62[a,b]
Σmetn-6/ Σmetn-3	1.91±0.37[c,d]	1.96 ±0.69[c]	2.73±0.90[a,b]	2.51±0.77[a]
Σn-6/ Σn-3	3.60±0.67	3.77±1.21	3.98±1.31	3.66±1.14

Table 5.3. Percent fatty acids in liver choline phosphoglycerides of the dams.
Values are means ± SD, n=15 (LFC: Low-fat control); n=10 (LFD: Low-fat diabetic); n=24 (HFC: High-fat control); n=12 (HFD: High-fat diabetic). ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3 ratio; total metabolites n-6 (PUFA)-to-total metabolites n-3 (PUFA) ratio; Σn-6/ Σn-3; total n-6 (PUFA)-to-total n-3 (PUFA) ratio. Groups that share a superscript character differ, $P<0.05$ (non-parametric tests).

% Total fatty acids

	LFC[a]	LFD[b]	HFC[c]	HFD[d]
16:0	14.8±1.22[c]	14.1±2.18	12.7±1.64[a]	14.1±2.55
18:0	22.0±1.76[c,d]	23.5±1.84[c,d]	27.2±1.64[a,b]	27.0±1.61[a,b]
20:0	Trace	Trace	Trace	Trace
22:0	Trace	Trace	Trace	Trace
24:0	0.04±0.02	Trace	Trace	Trace
ΣSFA	36.8±1.65[c,d]	37.7±0.85[c,d]	39.9±0.76[a,b]	41.1±2.05[a,b]
16:1	0.40±0.15[c,d]	0.32±0.18[c,d]	0.10±0.05[a,b]	0.10±0.06[a,b]
18:1	5.52±0.99[c,d]	5.44±1.01[c,d]	3.78±0.42[a,b,d]	4.35±0.51[a,b,c]
20:1	0.08±0.03	Trace	Trace	Trace
22:1	Trace	Trace	Trace	Trace
24:1	0.04±0.02	Trace	Trace	Trace
Σmono	6.00±1.04[c,d]	5.79±1.16[c,d]	3.88±0.44[a,b,d]	4.45±0.51[a,b,c]
18:2n-6	18.4±1.67[c,d]	19.6±2.27[c,d]	12.8±1.77[a,b,d]	14.1±1.87[a,b,c]
18:3n-6	0.06±0.02	Trace	Trace	Trace
20:2n-6	0.33±0.07	Trace	Trace	Trace
20:3n-6	1.11±0.18[c,d]	1.05±0.32[c,d]	0.59±0.20[a,b,d]	0.44±0.19[a,b,c]
20:4n-6	16.1±1.62[c]	14.4±2.51[c]	18.8±4.02[a,b,d]	16.5±3.50[c]
22:4n-6	0.34±0.06[c,d]	0.30±0.10[c,d]	1.00±0.50[a,b,d]	0.70±0.23[a,b,c]
22:5n-6	0.25±0.07[c,d]	0.19±0.08[c,d]	1.99±1.16[a,b,d]	1.13±0.35[a,b,c]
Σn-6	36.5±1.85	35.8±3.34	35.0±4.69	32.7±5.17
18:3n-3	0.23±0.06[c,d]	0.25±0.04[c,d]	0.05±0.02[a,b,d]	0.06±0.01[a,b,c]
20:5n-3	1.90±0.62[c,d]	1.89±1.12[c,d]	0.11±0.05[a,b]	0.09±0.05[a,b]
22:5n-3	2.40±0.51[c,d]	2.57±0.82[c,d]	1.38±0.24[a,b]	1.35±0.28[a,b]
22:6n-3	13.8±1.45[c,d]	13.4±3.25[c,d]	16.5±3.14[a,b]	17.0±2.80[a,b]
Σn-3	18.3±1.72	18.1±3.13	18.0±3.08	18.5±2.63
AA/LA	0.88±0.13[b,c,d]	0.74±0.16[a,c,d]	1.48±0.32[a,b,d]	1.17±0.22[a,b,c]
AA/DHA	1.18±0.19	1.13±0.30	1.21±0.39	1.01±0.35
DPA/DTA n-6	0.74±0.18[c,d]	0.69±0.32[c,d]	2.28±1.19[a,b]	1.75±0.46[a,b]
Σmet n-6/ Σmet n-3	1.01±0.16[c]	0.94±0.25[c]	1.29±0.34[a,b]	1.05±0.33
Σn-6/ Σn-3	2.02±0.28	2.04±0.46	2.03±0.50	1.83±0.48

Table 5.4. Percent fatty acids in liver ethanolamine phosphoglycerides of the dams.
Values are means ± SD, n=15 (LFC: Low-fat control); n=10 (LFD: Low-fat diabetic); n=24 (HFC: High-fat control); n=12 (HFD: High-fat diabetic). ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3 ratio; total metabolites n-6 (PUFA)-to-total metabolites n-3 (PUFA) ratio; Σn-6/ Σn-3; total n-6 (PUFA)-to-total n-3 (PUFA) ratio. Groups that share a superscript character differ, $P<0.05$ (non-parametric tests).

% Total fatty acids

	LFC[a]	LFD[b]	HFC[c]	HFD[d]
16:0	36.9±5.93[b,c,d]	31.3±3.13[a]	31.0±2.93[a]	29.4±1.58[a]
18:0	14.9±2.91[c,d]	13.3±1.69[c,d]	10.7±2.18[a,b]	11.4±2.69[a,b]
20:0	0.14±0.08	0.08±0.02	0.07±0.03	0.08±0.03
22:0	0.08±0.03	Trace	Trace	Trace
24:0	0.10±0.05	0.06±0.02	Trace	0.04±0.03
ΣSFA	53.0±7.61[b,c,d]	45.7±3.64[a,c,d]	42.3±3.27[a,b]	41.4±2.58[a,b]
16:1	1.79±0.37[b,c,d]	1.34±0.52[a]	1.04±0.18[a]	0.91±0.17[a]
18:1	16.2±3.80[c,d]	15.3±5.25[c,d]	25.7±1.87[a,b]	25.1±2.76[a,b]
20:1	0.19±0.07[c,d]	0.20±0.06[c,d]	0.36±0.05[a,b]	0.37±0.08[a,b]
22:1	0.14±0.07	0.07±0.02	Trace	0.06±0.03
24:1	Trace	0.05±0.01	0.08±0.06	0.05±0.04
Σmono	18.6±3.75[c,d]	16.9±5.77[c,d]	27.2±1.91[a,b]	26.4±2.90[a,b]
18:2n-6	11.1±3.21[b,c,d]	17.6±4.58[a]	16.5±1.77[a]	17.1±2.55[a]
18:3n-6	0.17±0.12[d]	0.24±0.06	0.22±0.07	0.30±0.11[a]
20:2n-6	0.41±0.07[c,d]	0.43±0.15[c,d]	0.33±0.06[a,b]	0.35±0.04[a,b]
20:3n-6	0.43±0.20[b]	0.70±0.16[a,c,d]	0.35±0.11[b]	0.41±0.10[b]
20:4n-6	3.58±1.54[b,c,d]	6.36±2.06[a]	6.05±1.33[a]	6.35±1.64[a]
22:4n-6	0.31±0.14[b,c,d]	0.48±0.13[a,c,d]	0.94±0.27[a,b]	0.87±0.18[a,b]
22:5n-6	0.50±0.30[c]	0.42±0.27[c]	0.76±0.34[a,b]	0.47±0.21
Σn-6	16.4±4.63[b,c,d]	26.0±5.46[a]	25.1±2.88[a]	25.7±3.36[a]
18:3n-3	0.38±0.18[b]	0.63±0.18[a,c,d]	0.34±0.12[b]	0.36±0.13[b]
20:5n-3	0.85±0.36[c,d]	1.02±0.09[c,d]	0.26±0.13[a,b]	0.21±0.05[a,b]
22:5n-3	0.51±0.22[b]	0.76±0.19[a,c]	0.41±0.19[b]	0.62±0.19
22:6n-3	1.72±0.71[b]	3.09±1.27[a,c]	1.60±0.70[b]	2.15±0.77
Σn-3	3.28±1.31[b,c]	4.81±1.41[a,c,d]	2.53±0.96[a,b]	3.28±1.01[b]
AA/LA	0.32±0.10	0.37±0.12	0.37±0.07	0.38±0.11
AA/DHA	2.25±0.81[c,d]	2.19±0.60[c,d]	4.32±1.45[a,b]	3.17±0.94[a,b]
DPA/DTA n-6	1.76±1.32[c,d]	0.80±0.53	0.87±0.42[a]	0.55±0.29[a]
Σmet n-6/ Σmet n-3	2.01±0.86[c,d]	2.02±0.39[c,d]	4.38±1.42[a,b]	3.16±0.87[a,b]
Σn-6/ Σn-3	5.10±1.15[c,d]	5.64±1.34[c,d]	10.9±3.09[a,b]	8.45±2.29[a,b]

Table 5.5. Percent fatty acids in liver free fatty acids of the dams.
Values are means ± SD, n=15 (LFC: Low-fat control); n=10 (LFD: Low-fat diabetic); n=24 (HFC: High-fat control); n=12 (HFD: High-fat diabetic). ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3 ratio; total metabolites n-6 (PUFA)-to-total metabolites n-3 (PUFA) ratio; Σn-6/ Σn-3; total n-6 (PUFA)-to-total n-3 (PUFA) ratio. Groups that share a superscript character differ, $P<0.05$ (non-parametric tests).

% Total fatty acids

	LFC[a]	LFD[b]	HFC[c]	HFD[d]
16:0	28.9±2.84[c,d]	25.7±3.69	26.2±1.37[a,d]	24.7±1.00[a,c]
18:0	5.31±0.88[b]	4.08±0.75[a,c,d]	5.21±0.71[b,d]	5.86±0.77[b,c]
20:0	0.09±0.02	0.09±0.03	0.06±0.01	0.10±0.03
22:0	0.20±0.08	Trace	0.11±0.03	0.13±0.02
24:0	0.03±0.02	Trace	0.01±0.01	Trace
ΣSFA	34.4±3.04[b,c,d]	29.9±4.00[a]	31.6±1.33[a]	30.7±1.13[a]
16:1	4.01±1.60[b,c,d]	2.25±0.91[a,c,d]	1.22±0.24[a,b,d]	0.93±0.14[a,b,c]
18:1	32.5±3.46[b]	24.7±7.71[a,c]	33.8±2.01[b,d]	30.9±2.90[c]
20:1	0.51±0.21[c,d]	0.42±0.15[c,d]	0.75±0.11[a,b]	0.74±0.17[a,b]
22:1	0.07±0.02[c,d]	0.05±0.02[c,d]	0.02±0.01[a,b]	0.03±0.01[a,b]
24:1	0.10±0.06	0.02±0.01	0.02±0.01	0.04±0.02
Σmono	37.1±3.71[b,d]	27.4±8.57[a,c]	35.7±1.98[b,d]	32.6±3.08[a,c]
18:2n-6	17.5±2.64[b,c,d]	26.8±7.90[a]	21.0±1.05[a]	21.6±1.67[a]
18:3n-6	0.24±0.10[c,d]	0.32±0.08[c,d]	0.44±0.16[a,b]	0.53±0.16[a,b]
20:2n-6	0.31±0.10	0.41±0.13	0.34±0.04	0.35±0.02
20:3n-6	0.27±0.12[b,c,d]	0.49±0.20[a]	0.43±0.09[a,d]	0.57±0.07[a,c]
20:4n-6	1.01±0.36[b,c,d]	1.66±0.75[a,c,d]	2.91±0.93[a,b,d]	3.77±1.07[a,b,c]
22:4n-6	0.32±0.16[c,d]	0.49±0.22[c,d]	1.23±0.46[a,b]	1.36±0.33[a,b]
22:5n-6	0.20±0.11[c,d]	0.16±0.06[c,d]	0.86±0.25[a,b]	0.78±0.13[a,b]
Σn-6	19.8±3.23[b,c,d]	30.3±9.14[a]	26.9±1.81[a]	28.5±3.07[a]
18:3n-3	0.65±0.15	Trace	0.61±0.08	0.64±0.14
20:5n-3	0.35±0.12[b,c]	1.07±0.33[a,c,d]	0.11±0.05[a,b,d]	0.27±0.11[b,c]
22:5n-3	0.56±0.18[b,c,d]	1.91±1.00[a,c]	0.84±0.23[a,b,d]	1.45±0.40[a,c]
22:6n-3	1.31±0.84[b,d]	3.27±1.89[a]	1.60±0.75[d]	2.97±0.92[a,c]
Σn-3	2.36±0.71[b,c,d]	5.98±2.67[a,c]	3.16±0.94[a,b,d]	5.30±1.36[a,c]
AA/LA	0.06±0.02[c,d]	0.06±0.01[c,d]	0.14±0.04[a,b,d]	0.17±0.04[a,b,c]
AA/DHA	0.88±0.30[b,c,d]	0.51±0.16[a,c,d]	2.12±0.81[a,b,d]	1.34±0.37[a,b,c]
DPA/DTA n-6	0.67±0.34[b]	0.31±0.09[a,c,d]	0.71±0.13[b]	0.61±0.14[b]
Σmet n-6/ Σmet n-3	1.15±0.25[b,c,d]	0.62±0.18[a,c,d]	2.55±0.70[a,b,d]	1.56±0.46[a,b,c]
Σn-6/ Σn-3	8.91±2.35[b,d]	5.63±2.06[a,c]	9.32±2.91[b,d]	5.70±1.57[a,c]

Table 5.6. Percent fatty acids in liver triacylglycerols of the dams.
Values are means ± SD, n=15 (LFC: Low-fat control); n=10 (LFD: Low-fat diabetic); n=24 (HFC: High-fat control); n=12 (HFD: High-fat diabetic). ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3 ratio; total metabolites n-6 (PUFA)-to-total metabolites n-3 (PUFA) ratio; Σn-6/ Σn-3; total n-6 (PUFA)-to-total n-3 (PUFA) ratio. Groups that share a superscript character differ, $P<0.05$ (non-parametric tests).

% Total fatty acids

	LFC[a]	LFD[b]	HFC[c]	HFD[d]
16:0	29.5±1.61	27.8±1.84	27.3±3.74	26.5±1.84
18:0	17.4±0.70[b,d]	19.2±1.66[a]	17.6±2.77	19.4±1.04[a]
20:0	Trace	Trace	Trace	Trace
22:0	0.12±0.04	0.15±0.04	0.19±0.09	Trace
24:0	Trace	Trace	Trace	Trace
ΣSFA	47.0±1.24	47.1±1.22	45.0±2.83	46.1±2.08
16:1	0.84±0.23[b]	0.52±0.22[a,c,d]	1.11±0.71[b]	1.14±0.59[b]
18:1	6.80±0.63[c,d]	6.57±0.33[c,d]	11.8±3.34[a,b]	13.0±3.44[a,b]
20:1	0.13±0.08	0.14±0.05	0.16±0.07	0.22±0.08
22:1	Trace	Trace	Trace	Trace
24:1	Trace	Trace	0.09±0.05	Trace
Σmono	7.59±0.81[c,d]	7.23±0.43[c,d]	13.0±3.95[a,b]	14.2±4.14[a,b]
18:2n-6	6.94±0.66	8.19±1.73	9.21±4.33	8.57±2.56
18:3n-6	0.13±0.03	Trace	0.15±0.06	Trace
20:2n-6	0.26±0.07	0.35±0.08[c]	0.22±0.07[b]	0.28±0.15
20:3n-6	0.84±0.13	0.81±0.15	0.86±0.28	0.97±0.38
20:4n-6	21.7±1.13[b,d]	20.3±1.29[a]	17.9±4.67	16.5±3.84[a]
22:4n-6	0.46±0.07[c]	0.43±0.07[c]	0.64±0.23[a,b]	0.47±0.14
22:5n-6	0.12±0.04[c,d]	0.13±0.07[c,d]	1.07±0.43[a,b]	0.68±0.23[a,b]
Σn-6	30.4±1.65[d]	30.2±2.01[d]	30.0±1.97[d]	27.6±1.37[a,b,c]
18:3n-3	0.05±0.01	0.09±0.04	0.09±0.03	Trace
20:5n-3	0.35±0.13	0.31±0.08	0.37±0.13	0.37±0.19
22:5n-3	1.20±0.18[c,d]	1.19±0.19[c,d]	0.60±0.32[a,b]	0.52±0.31[a,b]
22:6n-3	11.3±1.05[c,d]	11.2±1.35[c]	8.35±1.93[a,b]	8.56±2.55[a]
Σn-3	12.9±1.10[c,d]	12.8±1.50[c,d]	9.13±1.80[a,b]	9.45±2.75[a,b]
AA/LA	3.15±0.32	2.58±0.57	2.71±1.81	2.22±1.26
AA/DHA	1.94±0.25	1.84±0.28	2.17±0.40	2.04±0.64
DPA/DTA n-6	0.27±0.07[c,d]	0.28±0.14[c,d]	1.88±0.89[a,b]	1.58±0.69[a,b]
Σmet n-6/ Σmet n-3	1.84±0.24[c]	1.76±0.26[c]	2.30±0.35[a,b]	2.18±0.73
Σn-6/ Σn-3	2.38±0.31[c]	2.41±0.39[c]	3.41±0.70[a,b]	3.19±1.13

Table 5.7. Percent fatty acids in liver choline phosphoglycerides of the newborn pups (1 day of life). Values are means ± SD, n=10 (LFC: Low-fat control); n=9 (LFD: Low-fat diabetic); n=16 (HFC: High-fat control); n=6 (HFD: High-fat diabetic). ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3 ratio; total metabolites n-6 (PUFA)-to-total metabolites n-3 (PUFA) ratio; Σn-6/ Σn-3; total n-6 (PUFA)-to-total n-3 (PUFA) ratio. Groups that share a superscript character differ, $P<0.05$ (non-parametric tests).

% Total fatty acids

	LFC[a]	LFD[b]	HFC[c]	HFD[d]
16:0	25.3±1.60[b]	28.4±1.64[a,c]	25.2±1.53[b]	27.4±2.58
18:0	20.4±1.41[b,c]	18.8±0.74[a,c]	22.8±1.34[a,b]	20.5±2.65
20:0	Trace	Trace	Trace	Trace
22:0	Trace	Trace	Trace	Trace
24:0	Trace	Trace	Trace	Trace
ΣSFA	45.7±1.10[b,c]	47.2±1.26[a]	48.1±0.20[a]	48.0±0.31
16:1	0.19±0.06[c]	0.20±0.07[c]	0.34±0.09[a,b]	0.37±0.13
18:1	3.48±0.43[c]	3.60±0.61[c]	5.34±0.58[a,b]	5.60±0.93
20:1	0.13±0.04	0.15±0.03	0.15±0.06	Trace
22:1	Trace	Trace	Trace	Trace
24:1	Trace	Trace	Trace	Trace
Σmono	3.81±0.48[c]	3.94±0.66[c]	5.85±0.69[a,b]	6.12±1.04
18:2n-6	11.6±1.30[c]	12.4±1.23[c]	7.97±0.74[a,b]	7.13±0.78
18:3n-6	Trace	0.10±0.05	0.08±0.01	Trace
20:2n-6	0.34±0.08	0.40±0.08[c]	0.27±0.09[b]	0.19±0.03
20:3n-6	1.36±0.19[c]	1.34±0.36[c]	0.76±0.10[a,b]	0.67±0.06
20:4n-6	20.0±1.71[b,c]	17.0±1.27[a,c]	23.9±1.25[a,b]	21.9±2.64
22:4n-6	0.20±0.07[b,c]	0.14±0.04[a,c]	0.35±0.07[a,b]	0.32±0.05
22:5n-6	0.09±0.05[c]	0.06±0.04[c]	0.30±0.11[a,b]	0.28±0.09
Σn-6	33.5±0.83[b]	31.3±1.57[a,c]	33.6±0.80[b]	30.5±2.62
18:3n-3	0.07±0.02	0.09±0.04	Trace	Trace
20:5n-3	0.51±0.20	0.66±0.15	Trace	Trace
22:5n-3	1.80±0.23[c]	1.72±0.37[c]	0.98±0.11[a,b]	1.12±0.14
22:6n-3	13.3±1.21[c]	13.6±0.78[c]	10.2±0.27[a,b]	12.4±1.85
Σn-3	15.7±1.19[c]	15.9±0.84[c]	11.2±0.27[a,b]	13.6±1.97
AA/LA	1.76±0.34[b,c]	1.38±0.16[a,c]	3.03±0.42[a,b]	3.11±0.62
AA/DHA	1.51±0.17[b,c]	1.26±0.15[a,c]	2.35±0.16[a,b]	1.81±0.42
DPA/DTA n-6	0.39±0.21[c]	0.39±0.16[c]	0.87±0.24[a,b]	0.85±0.21
Σmet n-6/ Σmet n-3	1.42±0.16[b,c]	1.20±0.12[a,c]	2.30±0.16[a,b]	1.76±0.41
Σn-6/ Σn-3	2.16±0.20[b,c]	1.97±0.18[a,c]	3.01±0.13[a,b]	2.30±0.52

Table 5.8. Percent fatty acids in liver choline phosphoglycerides of the suckling pups (15 days of life). Values are means ± SD, n=16 (LFC: Low-fat control); n=8 (LFD: Low-fat diabetic); n=7 (HFC: High-fat control); n=4 (HFD: High-fat diabetic). ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3 ratio; total metabolites n-6 (PUFA)-to-total metabolites n-3 (PUFA) ratio; Σn-6/ Σn-3; total n-6 (PUFA)-to-total n-3 (PUFA) ratio. Groups that share a superscript character differ, $P<0.05$ (non-parametric tests).

% Total fatty acids

	LFC[a]	LFD[b]	HFC[c]	HFD[d]
16:0	21.1±1.46[b,c,d]	18.1±2.10[a]	17.1±4.26[a]	17.0±1.62[a]
18:0	22.5±1.11	24.5±1.81	23.1±2.61	23.6±1.86
20:0	Trace	Trace	0.06±0.03	0.10±0.09
22:0	0.11±0.07	Trace	0.21±0.13	0.29±0.20
24:0	Trace	Trace	Trace	Trace
ΣSFA	43.7±1.66[c]	42.9±1.40[c]	40.3±4.07[a,b]	40.9±3.20
16:1	Trace	Trace	0.26±0.13	Trace
18:1	2.90±0.57[c]	2.78±0.37[c,d]	4.62±1.65[a,b]	5.08±2.25[b]
20:1	0.11±0.06	0.10±0.04	0.11±0.04	0.13±0.03
22:1	0.06±0.03	0.07±0.03	0.07±0.04	Trace
24:1	Trace	Trace	0.08±0.06	0.10±0.05
Σmono	3.20±0.55[c]	2.98±0.41[c,d]	4.89±1.72[a,b]	5.31±2.32[b]
18:2n-6	1.75±0.36	2.28±0.44	3.64±2.53	3.29±2.07
18:3n-6	Trace	Trace	0.05±0.03	Trace
20:2n-6	0.21±0.19	0.17±0.07	0.11±0.05	Trace
20:3n-6	0.37±0.07	0.44±0.09	0.45±0.16	0.58±0.31
20:4n-6	16.8±0.67[b,c,d]	18.2±1.35[a,c,d]	22.2±3.06[a,b]	20.9±3.22[a,b]
22:4n-6	0.93±0.16	1.03±0.22	1.39±0.64	1.42±0.29
22:5n-6	0.22±0.07[b,c,d]	0.41±0.23[a,c,d]	1.75±0.54[a,b,d]	1.23±0.35[a,b,c]
Σn-6	20.3±0.92[b,c,d]	22.5±1.69[a,c]	29.6±3.81[a,b]	27.6±5.46[a]
18:3n-3	0.14±0.12	0.07±0.03[c,d]	0.11±0.05[b,d]	0.18±0.10[b,c]
20:5n-3	0.43±0.14	0.47±0.15	0.47±0.29	0.39±0.26
22:5n-3	1.67±0.17[d]	1.72±0.20[d]	1.22±0.75	0.78±0.40[a,b]
22:6n-3	25.4±2.47[c,d]	23.7±1.90[c]	16.7±4.46[a,b]	17.6±6.46[a]
Σn-3	27.7±2.45[c,d]	25.9±1.85[c]	18.4±4.83[a,b]	19.0±6.72[a]
AA/LA	9.98±2.06	8.18±1.50	10.6±7.40	8.94±5.11
AA/DHA	0.67±0.08[b,c,d]	0.78±0.11[a,c,d]	1.45±0.49[a,b]	1.42±0.81[a,b]
DPA/DTA n-6	0.25±0.08[c,d]	0.40±0.21[c,d]	1.39±0.43[a,b,d]	0.89±0.26[a,b,c]
Σmet n-6/ Σmet n-3	0.68±0.08[b,c,d]	0.79±0.10[a,c,d]	1.52±0.46[a,b]	1.54±0.87[a,b]
Σn-6/ Σn-3	0.74±0.09[b,c,d]	0.88±0.12[a,c,d]	1.75±0.59[a,b]	1.75±1.04[a,b]

Table 5.9. Percent fatty acids in liver ethanolamine phosphoglycerides of the newborn pups (1 day of life). Values are means ± SD, n=10 (LFC: Low-fat control); n=9 (LFD: Low-fat diabetic); n=16 (HFC: High-fat control); n=6 (HFD: High-fat diabetic). ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3 ratio; total metabolites n-6 (PUFA)-to-total metabolites n-3 (PUFA) ratio; Σn-6/ Σn-3; total n-6 (PUFA)-to-total n-3 (PUFA) ratio. Groups that share a superscript character differ, $P<0.05$ (non-parametric tests).

% Total fatty acids

	LFC[a]	LFD[b]	HFC[c]	HFD[d]
16:0	20.5±1.27[b,c]	17.1±1.68[a,c]	14.2±1.04[a,b]	15.7±1.87
18:0	24.0±1.42[b,c]	22.0±0.84[a,c]	25.8±1.22[a,b]	24.3±1.87
20:0	Trace	Trace	Trace	Trace
22:0	Trace	0.06±0.03	0.13±0.09	Trace
24:0	Trace	Trace	Trace	Trace
ΣSFA	44.5±2.13[b,c]	39.1±1.62[a]	40.2±1.00[a]	40.3±1.42
16:1	Trace	Trace	0.06±0.03	0.13±0.05
18:1	1.82±0.48[b,c]	3.41±0.28[a,c]	4.02±0.48[a,b]	4.17±0.42
20:1	0.09±0.04	0.08±0.01	0.08±0.05	0.09±0.03
22:1	Trace	Trace	Trace	Trace
24:1	0.04±0.02	Trace	Trace	Trace
Σmono	1.92±0.50[b,c]	3.53±0.26[a,c]	4.21±0.46[a,b]	4.43±0.43
18:2n-6	3.86±2.78[b,c]	11.8±0.84[a,c]	10.4±0.98[a,b]	9.84±0.67
18:3n-6	Trace	Trace	Trace	0.04±0.03
20:2n-6	0.18±0.17[b,c]	0.58±0.11[a,c]	0.45±0.13[a,b]	0.38±0.05
20:3n-6	0.55±0.09[b]	0.73±0.09[a,c]	0.49±0.06[b]	0.45±0.04
20:4n-6	18.3±1.59[b,c]	15.8±1.12[a,c]	20.6±1.05[a,b]	19.4±2.18
22:4n-6	0.57±0.15[c]	0.47±0.13[c]	0.82±0.16[a,b]	0.89±0.13
22:5n-6	0.14±0.06[c]	0.10±0.04[c]	0.53±0.13[a,b]	0.50±0.19
Σn-6	23.5±2.40[b,c]	29.5±1.74[a,c]	33.3±0.91[a,b]	31.5±2.02
18:3n-3	0.08±0.05	0.07±0.03	0.04±0.03	Trace
20:5n-3	0.84±0.31[c]	1.08±0.28[c]	0.09±0.04[a,b]	0.16±0.08
22:5n-3	2.66±0.33[c]	2.51±0.42[c]	1.26±0.12[a,b]	1.40±0.21
22:6n-3	23.6±1.59[b,c]	21.3±0.82[a,c]	17.7±1.04[a,b]	18.8±1.76
Σn-3	27.2±1.66[b,c]	25.0±1.07[a,c]	19.1±1.01[a,b]	20.4±1.76
AA/LA	5.73±1.74[b,c]	1.34±0.09[a,c]	2.01±0.25[a,b]	1.98±0.32
AA/DHA	0.78±0.09[c]	0.74±0.07[c]	1.17±0.12[a,b]	1.05±0.20
DPA/DTA n-6	0.24±0.08[c]	0.21±0.08[c]	0.65±0.10[a,b]	0.57±0.23
Σmet n-6/ Σmet n-3	0.73±0.08[c]	0.71±0.07[c]	1.21±0.12[a,b]	1.08±0.20
Σn-6/ Σn-3	0.87±0.12[b,c]	1.18±0.11[a,c]	1.75±0.14[a,b]	1.56±0.22

Table 5.10. Percent fatty acids in liver ethanolamine phosphoglycerides of the suckling pups (15 days of life). Values are means ± SD, n=16 (LFC: Low-fat control); n=8 (LFD: Low-fat diabetic); n=7 (HFC: High-fat control); n=4 (HFD: High-fat diabetic). ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3 ratio; total metabolites n-6 (PUFA)-to-total metabolites n-3 (PUFA) ratio; Σn-6/ Σn-3; total n-6 (PUFA)-to-total n-3 (PUFA) ratio. Groups that share a superscript character differ, $P<0.05$ (non-parametric tests).

% Total fatty acids

	LFC[a]	LFD[b]	HFC[c]	HFD[d]
16:0	29.4±3.94	26.1±2.18	27.4±2.03	26.3±3.38
18:0	7.92±1.63[b,c,d]	9.68±1.18[a,c,d]	12.0±2.41[a,b]	12.9±1.58[a,b]
20:0	Trace	Trace	0.10±0.04	Trace
22:0	0.06±0.02	0.09±0.06	0.07±0.05	0.05±0.03
24:0	Trace	Trace	0.05±0.02	Trace
ΣSFA	38.1±5.25	36.7±1.90	40.3±4.01	40.1±3.78
16:1	2.06±0.43[b]	1.16±0.27[a,c]	1.83±0.65[b]	1.49±0.36
18:1	14.0±1.49[b,c,d]	11.5±0.99[a,c,d]	21.1±5.12[a,b]	21.9±5.06[a,b]
20:1	0.19±0.07	0.17±0.06	0.28±0.15	0.32±0.23
22:1	Trace	Trace	Trace	Trace
24:1	Trace	Trace	0.05±0.02	Trace
Σmono	16.3±1.61[b,c,d]	12.9±1.27[a,c,d]	23.3±5.67[a,b]	23.7±5.54[a,b]
18:2n-6	13.1±1.24[c]	14.8±2.07[c,d]	10.5±2.19[a,b]	11.0±1.73[b]
18:3n-6	0.27±0.07	0.24±0.07	0.18±0.08	0.20±0.02
20:2n-6	0.32±0.10	0.40±0.09	0.17±0.04	0.17±0.05
20:3n-6	0.95±0.11[c,d]	1.01±0.17[c,d]	0.63±0.23[a,b]	0.72±0.20[a,b]
20:4n-6	13.0±1.64	15.4±1.69	13.2±6.10	12.4±4.59
22:4n-6	1.73±0.24	1.57±0.39	1.38±0.93	0.98±0.45
22:5n-6	0.26±0.07[c,d]	0.42±0.20[c,d]	1.28±0.31[a,b]	1.01±0.50[a,b]
Σn-6	29.6±2.75[b]	33.8±1.82[a,c,d]	27.2±5.15[b]	26.4±3.83[b]
18:3n-3	0.27±0.12[c,d]	0.37±0.15[c,d]	0.11±0.06[a,b]	0.09±0.05[a,b]
20:5n-3	1.74±0.52[c,d]	1.64±0.29[c,d]	0.27±0.11[a,b]	0.34±0.12[a,b]
22:5n-3	2.13±0.41[c,d]	2.35±0.44[c,d]	1.15±0.66[a,b]	0.56±0.42[a,b]
22:6n-3	9.05±1.87[c]	10.5±1.66[c,d]	5.86±1.38[a,b]	7.04±2.73[b]
Σn-3	13.2±2.73[c,d]	14.8±2.29[c,d]	7.07±2.02[a,b]	7.97±2.97[a,b]
AA/LA	1.00±0.14	1.07±0.23	1.41±0.86	1.20±0.64
AA/DHA	1.47±0.26	1.52±0.36	2.17±0.71	1.94±0.78
DPA/DTA n-6	0.15±0.03[b,c,d]	0.27±0.14[a,c,d]	1.62±1.32[a,b]	1.32±0.96[a,b]
Σmet n-6/ Σmet n-3	1.30±0.20[c]	1.35±0.31[c]	2.33±0.47[a,b]	2.13±0.71
Σn-6/ Σn-3	2.29±0.29[c]	2.33±0.41[c,d]	3.99±0.69[a,b]	3.63±1.04[b]

Table 5.11. Percent fatty acids in liver free fatty acids of the newborn pups (1 day of life).
Values are means ± SD, n=10 (LFC: Low-fat control); n=9 (LFD: Low-fat diabetic); n=16 (HFC: High-fat control); n=6 (HFD: High-fat diabetic). ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3 ratio; total metabolites n-6 (PUFA)-to-total metabolites n-3 (PUFA) ratio; Σn-6/ Σn-3; total n-6 (PUFA)-to-total n-3 (PUFA) ratio. Groups that share a superscript character differ, $P<0.05$ (non-parametric tests).

	% Total fatty acids			
	LFC[a]	LFD[b]	HFC[c]	HFD[d]
16:0	26.7±1.53[b]	23.6±2.53[a,c]	26.6±1.82[b]	26.1±1.17
18:0	8.60±0.78[c]	9.39±2.10	11.2±1.81[a]	11.3±2.42
20:0	0.05±0.02	Trace	0.08±0.03	Trace
22:0	Trace	Trace	Trace	Trace
24:0	0.05±0.02	Trace	0.04±0.02	Trace
ΣSFA	37.9±1.56[b]	35.4±2.58[a,c]	38.6±2.76[b]	37.9±3.26
16:1	0.61±0.08[b,c]	0.51±0.11[a,c]	0.84±0.05[a,b]	0.74±0.07
18:1	11.1±1.20[b,c]	9.54±1.38[a,c]	23.6±1.69[a,b]	21.6±1.92
20:1	0.19±0.05[b,c]	0.14±0.03[a,c]	0.38±0.07[a,b]	0.35±0.06
22:1	Trace	Trace	Trace	Trace
24:1	Trace	Trace	Trace	Trace
Σmono	11.9±1.25[b,c]	10.2±1.49[a,c]	24.9±1.64[a,b]	22.7±1.98
18:2n-6	22.4±1.79[c]	24.1±2.24[c]	17.5±1.68[a,b]	16.5±1.98
18:3n-6	0.21±0.07	0.24±0.05	0.24±0.06	0.19±0.06
20:2n-6	0.47±0.17	0.40±0.06	0.39±0.14	0.35±0.05
20:3n-6	0.95±0.07[c]	1.02±0.12[c]	0.55±0.14[a,b]	0.65±0.24
20:4n-6	9.06±1.63	10.2±2.15	8.81±1.21	10.2±1.51
22:4n-6	0.87±0.19[b]	0.58±0.17[a,c]	1.17±0.38[b]	1.24±0.13
22:5n-6	0.19±0.08[c]	0.16±0.04[c]	0.59±0.29[a,b]	0.50±0.20
Σn-6	34.1±1.90[b,c]	36.7±2.66[a,c]	29.3±2.66[a,b]	29.6±2.26
18:3n-3	0.98±0.20[c]	1.09±0.19[c]	0.44±0.10[a,b]	0.34±0.07
20:5n-3	1.87±0.70[c]	2.32±0.51[c]	0.34±0.12[a,b]	0.46±0.30
22:5n-3	3.22±0.71[c]	3.66±0.68[c]	1.05±0.36[a,b]	1.45±0.60
22:6n-3	8.06±1.08[b,c]	9.08±1.12[a,c]	2.89±0.40[a,b]	5.58±2.49
Σn-3	14.1±2.07[b,c]	16.1±1.44[a,c]	4.72±0.89[a,b]	7.83±3.16
AA/LA	0.41±0.10[c]	0.43±0.11	0.51±0.08[a]	0.63±0.15
AA/DHA	1.14±0.22[c]	1.13±0.24[c]	3.08±0.43[a,b]	2.21±1.20
DPA/DTA n-6	0.23±0.11[c]	0.31±0.13[c]	0.53±0.25[a,b]	0.41±0.17
Σmet n-6/ Σmet n-3	0.92±0.23[c]	0.85±0.20[c]	2.79±0.39[a,b]	2.09±1.12
Σn-6/ Σn-3	2.47±0.47[c]	2.29±0.28[c]	6.31±0.71[a,b]	4.34±1.89

Table 5.12. Percent fatty acids in liver free fatty acids of the suckling pups (15 days of life).
Values are means ± SD, n=16 (LFC: Low-fat control); n=8 (LFD: Low-fat diabetic); n=7 (HFC: High-fat control); n=4 (HFD: High-fat diabetic). ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3 ratio; total metabolites n-6 (PUFA)-to-total metabolites n-3 (PUFA) ratio; Σn-6/ Σn-3; total n-6 (PUFA)-to-total n-3 (PUFA) ratio. Groups that share a superscript character differ, $P<0.05$ (non-parametric tests).

% Total fatty acids

	LFC[a]	LFD[b]	HFC[c]	HFD[d]
16:0	33.7±1.98[b,c]	25.7±2.81[a]	25.4±3.90[a]	25.7±6.43
18:0	2.80±0.41[b,c,d]	3.38±0.37[a,c,d]	6.18±1.82[a,b]	7.11±1.47[a,b]
20:0	Trace	Trace	0.07±0.03	0.07±0.03
22:0	Trace	Trace	0.10±0.07	0.06±0.05
24:0	trace	Trace	0.05±0.03	Trace
ΣSFA	37.6±1.98[b,c]	30.1±2.78[a]	32.4±2.89[a]	33.8±5.19
16:1	2.36±0.36[b]	1.49±0.36[a]	1.95±0.78	1.89±0.55
18:1	18.7±1.43[b,c,d]	16.1±1.47[a,c,d]	31.2±9.69[a,b]	32.6±5.34[a,b]
20:1	0.25±0.07	0.22±0.03	0.37±0.15	0.36±0.17
22:1	Trace	0.07±0.03	0.06±0.03	Trace
24:1	Trace	Trace	Trace	Trace
Σmono	21.3±1.45[b,c,d]	17.9±1.79[a,c,d]	33.6±10.3[a,b]	34.9±5.76[a,b]
18:2n-6	13.6±0.45[b,c]	18.3±2.70[a,c,d]	11.8±3.59[a,b]	14.0±2.98[b]
18:3n-6	0.35±0.09[b]	0.49±0.06[a,c,d]	0.33±0.14[b]	0.36±0.12[b]
20:2n-6	0.38±0.07	0.46±0.16	0.32±0.15	Trace
20:3n-6	1.10±0.04	1.34±0.28[c,d]	0.75±0.48[b]	0.67±0.34[b]
20:4n-6	6.02±1.14	7.12±1.11	7.98±5.39	4.90±3.10
22:4n-6	2.37±0.37	2.65±0.69	2.68±1.99	1.45±1.15
22:5n-6	0.44±0.13[c,d]	0.63±0.21[c,d]	1.88±0.54[a,b,d]	1.20±0.55[a,b,c]
Σn-6	24.3±1.42[b]	31.0±2.34[a,d]	25.7±6.60	22.7±2.54[b]
18:3n-3	0.32±0.06[b,c,d]	0.55±0.16[a,c,d]	0.17±0.05[a,b]	0.20±0.08[a,b]
20:5n-3	1.40±0.35[c,d]	1.68±0.26[c,d]	0.30±0.20[a,b]	0.18±0.12[a,b]
22:5n-3	3.09±0.21[b,c,d]	3.77±0.57[a,c,d]	1.55±0.95[a,b]	0.74±0.59[a,b]
22:6n-3	10.7±0.98[b,c,d]	13.1±1.75[a,c,d]	3.92±1.87[a,b]	5.06±2.28[a,b]
Σn-3	15.5±1.15[b,c,d]	19.1±2.40[a,c,d]	5.69±2.75[a,b]	6.17±2.57[a,b]
AA/LA	0.44±0.09	0.40±0.10	0.78±0.58	0.40±0.32
AA/DHA	0.56±0.09[c]	0.56±0.15[c]	2.13±0.95[a,b,d]	1.12±0.63[c]
DPA/DTA n-6	0.18±0.04[c,d]	0.24±0.06[c,d]	0.74±0.54[a,b]	1.36±0.96[a,b]
Σmet n-6/ Σmet n-3	0.70±0.09[c]	0.70±0.17[c,d]	2.54±0.72[a,b,d]	1.62±0.70[b,c]
Σn-6/ Σn-3	1.57±0.10[c,d]	1.64±0.17[c,d]	5.02±2.18[a,b]	4.24±1.69[a,b]

Table 5.13. Percent fatty acids in liver triacylglycerols of the newborn pups (1 day of life).
Values are means ± SD, n=10 (LFC: Low-fat control); n=9 (LFD: Low-fat diabetic); n=16 (HFC: High-fat control); n=6 (HFD: High-fat diabetic). ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3 ratio; total metabolites n-6 (PUFA)-to-total metabolites n-3 (PUFA) ratio; Σn-6/ Σn-3; total n-6 (PUFA)-to-total n-3 (PUFA) ratio. Groups that share a superscript character differ, $P<0.05$ (non-parametric tests).

	% Total fatty acids			
	LFC[a]	LFD[b]	HFC[c]	HFD[d]
16:0	25.5±1.90[c]	23.9±3.02	23.0±1.85[a]	23.7±2.12
18:0	4.87±0.68[b,c]	6.27±1.37[a]	6.68±0.72[a]	6.89±0.60
20:0	0.08±0.03	0.09±0.03	0.05±0.01	0.07±0.01
22:0	0.05±0.05	0.04±0.02	Trace	Trace
24:0	0.03±0.02	Trace	Trace	Trace
ΣSFA	33.3±2.73	33.7±5.17	30.4±1.19	31.2±2.27
16:1	0.70±0.12[c]	0.76±0.15	0.85±0.06[a]	0.75±0.06
18:1	16.1±1.65[c]	15.7±2.34[c]	31.9±2.53[a,b]	29.4±3.89
20:1	0.34±0.12[c]	0.29±0.07[c]	0.46±0.11[a,b]	0.50±0.09
22:1	0.06±0.05	Trace	Trace	Trace
24:1	0.03±0.02	Trace	Trace	Trace
Σmono	17.2±1.78[c]	16.8±2.49[c]	33.2±2.50[a,b]	30.7±3.97
18:2n-6	26.7±1.21[c]	26.5±3.35[c]	21.3±0.65[a,b]	18.7±2.71
18:3n-6	0.24±0.04[c]	0.26±0.09	0.36±0.10[a]	0.30±0.12
20:2n-6	0.59±0.11[c]	0.50±0.13	0.46±0.10[a]	0.44±0.12
20:3n-6	0.91±0.10[c]	0.82±0.20	0.70±0.15[a]	0.76±0.18
20:4n-6	3.64±0.93[c]	2.97±0.47[c]	4.64±0.91[a,b]	5.03±0.58
22:4n-6	1.22±0.27[b]	0.85±0.18[a,c]	1.59±0.49[b]	1.72±0.29
22:5n-6	0.36±0.14[c]	0.37±0.16[c]	0.68±0.19[a,b]	1.19±0.63
Σn-6	33.7±1.45[c]	32.3±3.55	29.8±1.43[a]	28.2±2.41
18:3n-3	1.06±0.15[c]	1.01±0.23[c]	0.50±0.08[a,b]	0.40±0.11
20:5n-3	1.19±0.43[c]	1.32±0.65[c]	0.26±0.15[a,b]	0.35±0.27
22:5n-3	3.80±0.94[c]	3.32±1.32[c]	1.34±0.27[a,b]	1.94±0.70
22:6n-3	7.50±1.47[c]	7.98±2.74[c]	2.55±0.52[a,b]	5.15±2.88
Σn-3	13.6±2.75[c]	13.6±4.62[c]	4.62±0.91[a,b]	7.84±3.44
AA/LA	0.14±0.04[c]	0.11±0.02[c]	0.22±0.05[a,b]	0.28±0.07
AA/DHA	0.52±0.24[c]	0.42±0.15[c]	1.83±0.14[a,b]	1.28±0.74
DPA/DTA n-6	0.30±0.11[b,c]	0.43±0.15[a]	0.45±0.16[a]	0.66±0.26
Σmet n-6/ Σmet n-3	0.60±0.28[c]	0.51±0.19[c]	2.07±0.26[a,b]	1.53±0.75
Σn-6/ Σn-3	2.63±0.83[c]	2.66±1.08[c]	6.62±1.12[a,b]	4.43±2.61

Table 5.14. Percent fatty acids in liver triacylglycerols of the suckling pups (15 days of life). Values are means ± SD, n=16 (LFC: Low-fat control); n=8 (LFD: Low-fat diabetic); n=7 (HFC: High-fat control); n=4 (HFD: High-fat diabetic). ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3 ratio; total metabolites n-6 (PUFA)-to-total metabolites n-3 (PUFA) ratio; Σn-6/ Σn-3; total n-6 (PUFA)-to-total n-3 (PUFA) ratio. Groups that share a superscript character differ, $P<0.05$ (non-parametric tests).

	% Total fatty acids			
	LFC[a]	LFD[b]	HFC[c]	HFD[d]
16:0	25.2±1.22[c]	23.3±2.53	22.4±2.24[a]	23.4±2.34
18:0	16.7±0.73	17.7±1.21	16.8±1.19	17.7±1.04
20:0	0.09±0.01[b,c,d]	0.12±0.02[a]	0.11±0.02[a,d]	0.14±0.01[a,c]
22:0	0.05±0.02[b,c,d]	0.09±0.04[a]	0.10±0.04[a]	0.11±0.02[a]
24:0	0.04±0.02	0.04±0.02	0.03±0.02	Trace
ΣSFA	42.0±0.69[c]	41.2±1.80	39.4±2.85[a]	41.3±1.68
16:1	0.89±0.16[b]	0.72±0.20[a,c]	0.94±0.21[b]	0.78±0.14
18:1	16.1±2.46[c]	14.7±1.63[c,d]	20.0±2.04[a,b,d]	18.0±1.14[b,c]
20:1	0.26±0.05[c]	0.23±0.05[c,d]	0.34±0.10[a,b]	0.33±0.09[b]
22:1	0.06±0.03	0.10±0.04	0.09±0.05	Trace
24:1	0.06±0.02	0.06±0.03	0.08±0.03	0.06±0.03
Σmono	17.4±2.60[c]	15.8±1.81[c,d]	21.5±2.15[a,b,d]	19.2±1.17[b,c]
18:2n-6	5.43±1.50	6.03±0.94	7.28±3.26	5.62±1.57
18:3n-6	0.11±0.01[b,c,d]	0.13±0.02[a,c,d]	0.08±0.02[a,b]	0.07±0.02[a,b]
20:2n-6	0.59±0.08[b]	0.78±0.11[a,c,d]	0.56±0.07[b]	0.60±0.09[b]
20:3n-6	1.01±0.19	1.12±0.13[c,d]	0.97±0.17[b]	0.82±0.26[b]
20:4n-6	22.6±3.13	23.0±2.44	20.4±3.45	22.2±2.34
22:4n-6	1.37±0.22	1.48±0.20	1.67±0.46	1.62±0.29
22:5n-6	0.31±0.07[b,c,d]	0.49±0.13[a,c,d]	1.24±0.40[a,b,d]	0.85±0.14[a,b,c]
Σn-6	31.4±1.86	33.1±2.18	32.2±1.63	31.7±2.09
18:3n-3	0.06±0.03	0.08±0.02[c]	0.05±0.03[b]	0.04±0.03
20:5n-3	0.32±0.05[c,d]	0.32±0.03[c,d]	0.12±0.05[a,b]	0.12±0.02[a,b]
22:5n-3	1.43±0.30[c,d]	1.50±0.30[c,d]	0.69±0.23[a,b]	0.59±0.23[a,b]
22:6n-3	4.69±1.00[c]	4.83±0.77[c]	2.90±0.74[a,b,d]	4.19±0.40[c]
Σn-3	6.50±1.24[c,d]	6.72±1.04[c,d]	3.75±0.80[a,b,d]	4.94±0.38[a,b,c]
AA/LA	4.48±1.39	3.93±0.87	3.72±2.32	4.26±1.41
AA/DHA	4.91±0.55[c]	4.83±0.53[c]	7.54±2.59[a,b,d]	5.36±1.00[c]
DPA/DTA n-6	0.23±0.05[b,c,d]	0.33±0.10[a,c,d]	0.84±0.43[a,b]	0.54±0.14[a,b]
Σmet n-6/ Σmet n-3	4.10±0.41[c,d]	4.13±0.53[c,d]	7.02±1.82[a,b,d]	5.37±0.84[a,b,c]
Σn-6/ Σn-3	4.97±0.90[c,d]	5.02±0.82[c,d]	8.90±1.59[a,b,d]	6.46±0.74[a,b,c]

Table 5.15. Percent fatty acids in heart choline phosphoglycerides of the newborn pups (1 day of life). Values are means ± SD, n=10 (LFC: Low-fat control); n=9 (LFD: Low-fat diabetic); n=16 (HFC: High-fat control); n=6 (HFD: High-fat diabetic). ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3 ratio; total metabolites n-6 (PUFA)-to-total metabolites n-3 (PUFA) ratio; Σn-6/ Σn-3; total n-6 (PUFA)-to-total n-3 (PUFA) ratio. Groups that share a superscript character differ, $P<0.05$ (non-parametric tests).

% Total fatty acids

	LFC	LFD	HFC	HFD
16:0	21.9±1.59[b,c]	20.6±1.03[a,c]	18.3±1.54[a,b]	19.3±1.27
18:0	22.4±1.06[b,c]	23.4±1.04[a,c]	27.1±0.98[a,b]	26.3±1.10
20:0	0.07±0.02[b,c]	0.09±0.01[a]	0.09±0.01[a]	0.09±0.01
22:0	0.05±0.03	0.06±0.03	0.05±0.03	0.04±0.03
24:0	0.03±0.02	0.03±0.02	Trace	0.03±0.02
ΣSFA	44.4±1.14	44.1±0.53[c]	45.5±0.85[b]	45.7±0.91
16:1	0.17±0.05[c]	0.16±0.06[c]	0.23±0.02[a,b]	0.23±0.06
18:1	6.15±0.41[c]	6.02±0.46[c]	7.51±0.76[a,b]	6.79±2.11
20:1	0.11±0.05[c]	0.08±0.02[c]	0.16±0.04[a,b]	0.14±0.02
22:1	Trace	Trace	Trace	Trace
24:1	0.04±0.03	0.05±0.03	0.04±0.02	0.04±0.03
Σmono	6.50±0.45[c]	6.30±0.53[c]	7.93±0.78[a,b]	7.25±2.15
18:2n-6	5.65±0.61[c]	5.90±0.87[c]	3.51±0.35[a,b]	3.58±0.71
18:3n-6	0.16±0.02[b,c]	0.19±0.03[a,c]	0.08±0.02[a,b]	0.07±0.02
20:2n-6	0.45±0.06[c]	0.47±0.03[c]	0.39±0.04[a,b]	0.34±0.04
20:3n-6	0.80±0.12[c]	0.89±0.14[c]	0.65±0.07[a,b]	0.61±0.08
20:4n-6	23.0±1.61[c]	22.6±1.19[c]	28.0±1.22[a,b]	28.1±1.62
22:4n-6	0.99±0.26[b,c]	0.62±0.13[a,c]	1.51±0.19[a,b]	1.33±0.23
22:5n-6	0.23±0.08[b,c]	0.14±0.05[a,c]	0.67±0.12[a,b]	0.57±0.10
Σn-6	31.3±2.14[c]	30.8±0.92[c]	34.8±1.22[a,b]	34.6±1.57
18:3n-3	0.05±0.01[c]	0.05±0.02[c]	0.03±0.01[a,b]	Trace
20:5n-3	0.31±0.09[b,c]	0.46±0.13[a,c]	0.07±0.02[a,b]	Trace
22:5n-3	4.08±0.41[c]	4.11±0.66[c]	2.75±0.27[a,b]	2.64±0.71
22:6n-3	10.5±1.35[c]	10.9±0.55[c]	6.22±0.77[a,b]	7.10±1.39
Σn-3	14.9±1.63[c]	15.5±0.88[c]	9.07±1.04[a,b]	10.0±1.57
AA/LA	4.10±0.49[c]	3.92±0.67[c]	8.04±0.73[a,b]	8.14±2.04
AA/DHA	2.24±0.45[c]	2.08±0.15[c]	4.56±0.60[a,b]	4.05±0.62
DPA/DTA n-6	0.23±0.03[c]	0.23±0.08[c]	0.45±0.08[a,b]	0.43±0.07
Σmet n-6/ Σmet n-3	1.75±0.33[c]	1.62±0.13[c]	3.50±0.40[a,b]	3.14±0.36
Σn-6/ Σn-3	2.13±0.40[c]	2.00±0.15[c]	3.89±0.48[a,b]	3.50±0.48

Table 5.16. Percent fatty acids in heart choline phosphoglycerides of the suckling pups (15 days of life). Values are means ± SD, n=16 (LFC: Low-fat control); n=8 (LFD: Low-fat diabetic); n=7 (HFC: High-fat control); n=4 (HFD: High-fat diabetic). ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3 ratio; total metabolites n-6 (PUFA)-to-total metabolites n-3 (PUFA) ratio; Σn-6/ Σn-3; total n-6 (PUFA)-to-total n-3 (PUFA) ratio. Groups that share a superscript character differ, $P<0.05$ (non-parametric tests).

% Total fatty acids

	LFC	LFD	HFC	HFD
16:0	7.20±0.61^{c}	6.64±1.16	6.23±0.87^{a}	6.72±0.37
18:0	17.7±0.63^{c,d}	18.4±1.06^{d}	18.7±1.03^{a,d}	19.5±0.47^{a,b,c}
20:0	0.13±0.02^{d}	0.12±0.05^{d}	0.15±0.04^{d}	0.21±0.03^{a,b,c}
22:0	0.10±0.03	0.07±0.06	0.12±0.05	0.17±0.02
24:0	0.03±0.01	0.04±0.02	0.03±0.02	Trace
ΣSFA	25.2±0.94^{d}	25.3±1.83	25.2±1.48^{d}	26.6±0.53^{a,c}
16:1	0.42±0.13^{d}	0.35±0.09^{d}	0.35±0.11^{d}	0.23±0.04^{a,b,c}
18:1	6.24±1.01^{c}	5.81±1.62^{c}	7.66±1.53^{a,b}	6.82±0.72
20:1	0.07±0.03^{c}	0.08±0.07^{c,d}	0.12±0.04^{a,b}	0.10±0.03^{b}
22:1	0.05±0.03	0.07±0.06	0.07±0.05	Trace
24:1	0.06±0.03	0.07±0.03	0.08±0.04	0.07±0.02
Σmono	6.82±1.16^{c}	6.34±1.63^{c}	8.26±1.62^{a,b}	7.24±0.71
18:2n-6	6.75±2.10	8.29±2.72	7.37±2.54	5.25±0.49
18:3n-6	0.06±0.02^{b,c,d}	0.09±0.03^{a,c,d}	0.05±0.01^{a,b}	0.05±0.01^{a,b}
20:2n-6	0.33±0.09^{b}	0.53±0.16^{a,c,d}	0.30±0.10^{b}	0.33±0.03^{b}
20:3n-6	1.20±0.19^{d}	1.36±0.18^{c,d}	1.14±0.24^{b,d}	0.90±0.13^{a,b,c}
20:4n-6	27.0±1.48^{c,d}	26.9±1.85^{c,d}	29.4±2.14^{a,b}	29.3±0.88^{a,b}
22:4n-6	3.69±0.34^{c,d}	3.72±0.48^{c,d}	5.03±1.42^{a,b}	4.93±0.60^{a,b}
22:5n-6	0.75±0.13^{c,d}	0.96±0.33^{c,d}	2.49±0.47^{a,b,d}	1.99±0.40^{a,b,c}
Σn-6	39.7±1.18^{b,c,d}	41.8±1.89^{a,c}	45.8±2.32^{a,b,d}	42.8±0.83^{a,c}
18:3n-3	0.06±0.02	0.08±0.03	0.05±0.02	Trace
20:5n-3	0.55±0.15^{c,d}	0.51±0.07^{c,d}	0.17±0.08^{a,b}	0.14±0.02^{a,b}
22:5n-3	3.09±0.43^{c,d}	2.91±0.43^{c,d}	1.59±0.50^{a,b}	1.29±0.30^{a,b}
22:6n-3	11.0±1.24^{b,c,d}	9.82±0.71^{a,c,d}	5.25±1.16^{a,b,d}	8.03±0.88^{a,b,c}
Σn-3	14.7±1.50^{b,c,d}	13.3±1.03^{a,c,d}	7.04±1.34^{a,b,d}	9.48±0.70^{a,b,c}
AA/LA	4.44±1.63	3.74±1.87^{d}	4.59±2.18^{d}	5.64±0.68^{b,c}
AA/DHA	2.47±0.26^{c,d}	2.75±0.34^{c,d}	5.82±1.14^{a,b,d}	3.69±0.41^{a,b,c}
DPA/DTA n-6	0.20±0.02^{c,d}	0.25±0.07^{c,d}	0.53±0.16^{a,b}	0.41±0.11^{a,b}
Σmet n-6/ Σmet n-3	2.27±0.23^{c,d}	2.55±0.31^{c,d}	5.64±0.96^{a,b,d}	3.99±0.38^{a,b,c}
Σn-6/ Σn-3	2.73±0.33^{b,c,d}	3.16±0.34^{a,c,d}	6.72±1.23^{a,b,d}	4.54±0.40^{a,b,c}

Table 5.17. Percent fatty acids in heart ethanolamine phosphoglycerides of the newborn pups (1 day of life). Values are means ± SD, n=10 (LFC: Low-fat control); n=9 (LFD: Low-fat diabetic); n=16 (HFC: High-fat control); n=6 (HFD: High-fat diabetic). ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, Σmet n-6/Σmet n-3 ratio; total metabolites n-6 (PUFA)-to-total metabolites n-3 (PUFA) ratio; Σn-6/ Σn-3; total n-6 (PUFA)-to-total n-3 (PUFA) ratio. Groups that share a superscript character differ, $P<0.05$ (non-parametric tests).

% Total fatty acids

	LFC[a]	LFD[b]	HFC[c]	HFD[d]
16:0	8.46±0.55[c]	8.13±0.77[c]	7.04±0.13[a,b]	7.25±1.04
18:0	18.1±0.79[c]	18.3±0.48[c]	19.3±0.69[a,b]	19.7±1.08
20:0	0.11±0.03[b,c]	0.14±0.02[a]	0.14±0.02[a]	0.15±0.02
22:0	0.11±0.02	0.12±0.03	0.12±0.02	Trace
24:0	0.04±0.02	0.03±0.02	0.03±0.01	Trace
ΣSFA	26.8±1.15	26.7±0.75	26.6±0.76	27.2±2.04
16:1	0.10±0.02[c]	0.08±0.03[c]	0.12±0.02[a,b]	0.12±0.03
18:1	4.11±0.59	4.21±0.33	4.91±1.09	4.88±0.96
20:1	0.09±0.05	0.07±0.03	0.09±0.02	0.14±0.05
22:1	0.06±0.06	Trace	Trace	Trace
24:1	0.05±0.02	0.02±0.01	Trace	Trace
Σmono	4.37±0.65	4.38±0.34	5.15±1.12	5.22±0.93
18:2n-6	8.25±1.49[c]	8.74±1.11[c]	6.01±0.82[a,b]	4.96±0.41
18:3n-6	0.13±0.02[b,c]	0.15±0.02[a,c]	0.05±0.01[a,b]	0.06±0.03
20:2n-6	0.55±0.10[c]	0.57±0.07[c]	0.44±0.05[a,b]	0.36±0.15
20:3n-6	1.50±0.18[c]	1.50±0.11[c]	1.27±0.10[a,b]	1.02±0.24
20:4n-6	17.4±1.11[c]	16.9±0.85[c]	22.7±0.45[a,b]	22.0±0.40
22:4n-6	1.83±0.45[b,c]	1.22±0.17[a,c]	3.16±0.36[a,b]	2.51±0.27
22:5n-6	0.63±0.17[b,c]	0.42±0.07[a,c]	2.35±0.46[a,b]	1.68±0.16
Σn-6	30.3±2.77[c]	29.5±1.27[c]	36.0±0.65[a,b]	32.5±1.22
18:3n-3	0.05±0.02[b]	0.07±0.02[a,c]	0.04±0.01[b]	Trace
20:5n-3	0.34±0.11[b,c]	0.51±0.15[a,c]	0.10±0.02[a,b]	0.08±0.03
22:5n-3	4.86±0.44[c]	4.86±0.63[c]	4.05±0.29[a,b]	3.22±0.81
22:6n-3	22.5±2.15[c]	22.7±1.44[c]	15.5±0.91[a,b]	16.9±2.55
Σn-3	27.8±2.38[c]	28.1±1.43[c]	19.7±1.11[a,b]	20.2±3.18
AA/LA	2.17±0.39[c]	1.96±0.25[c]	3.84±0.54[a,b]	4.45±0.37
AA/DHA	0.78±0.13[c]	0.75±0.07[c]	1.47±0.11[a,b]	1.32±0.19
DPA/DTA n-6	0.35±0.04[c]	0.35±0.07[c]	0.75±0.15[a,b]	0.67±0.09
Σmet n-6/ Σmet n-3	0.81±0.14[c]	0.74±0.06[c]	1.53±0.09[a,b]	1.39±0.18
Σn-6/ Σn-3	1.11±0.21[c]	1.05±0.09[c]	1.83±0.12[a,b]	1.63±0.21

Table 5.18. Percent fatty acids in heart ethanolamine phosphoglycerides of the suckling pups (15 days of life). Values are means ± SD, n=16 (LFC: Low-fat control); n=8 (LFD: Low-fat diabetic); n=7 (HFC: High-fat control); n=4 (HFD: High-fat diabetic). ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-3; total n-3 polyunsaturated fatty acids (PUFA), Σn-6; total n-6 PUFA, AA/LA; arachidonic-to-linoleic acid ratio, AA/DHA; arachidonic-to-docosahexaenoic acid ratio, DPA/DTA n-6; docosapentaenoic-to-docosatetraenoic acid n-6 ratio, ΣΣmet n-6/Σmet n-3 ratio; total metabolites n-6 (PUFA)-to-total metabolites n-3 (PUFA) ratio; Σn-6/ Σn-3; total n-6 (PUFA)-to-total n-3 (PUFA) ratio. Groups that share a superscript character differ, $P<0.05$ (non-parametric tests).

5.4. Discussion

The unexpected findings, herein, may have a wider significance in relation to the understanding of the impact of an unfavorable uterine and postnatal nutritional environment in early development. Though the time lag between birth and tissue sampling, the liver and heart fatty acids (FA) of the newborn pups relate to the intrauterine accumulation and will be referred as fetal data. Both diabetes and the high-lard diet appeared to impair the metabolism of arachidonic (AA) and docosahexaenoic (DHA) acids in the pups (newborn and suckling) and their mothers. The effect of diabetes on the FA was more striking in the low-fat diet rats and pups than in their corresponding high-fat diet group. In the high-fat diabetics (HFD) the independent influence of diabetes appears to have been masked by the greater effect of the high-lard diet.

5.4.1. The effects of diabetes on tissue fatty acid composition

Diabetes altered hepatic arachidonic acid in the rat

Diabetes *per se* reduced the conversion of linoleic acid (LA) to AA in the liver of the dams and significantly more in their offspring. Brenner (2000) showed that Δ-6 desaturase activity, an enzyme that catalyses the rate-limiting first-step reaction in the conversion of LA to AA and α-linolenic acid (ALA) to DHA, is reduced in the diabetic rat. Detrimental factors contributing to the reduced desaturase activity in diabetics are insulin deficiency and/or aging (Rimoldi et al, 2001; Das, 2007). Several reports agreed that depression of Δ-6 desaturase activity altered polyunsaturated fatty acids (PUFA) composition of phospholipids (PL) in various tissues with a decrease in AA (Brenner et al, 1989, 2000; Takeda & Kitagawa, 1992; Kuwahara et al, 1997; Giron et al, 1999; McHowat et al, 2000; Rimoldi et al, 2001; Ovide-Bordeaux & Grynberg, 2004). Additionally, the insulin-deficient rats showed a decrease in the heart n-3 PUFA content, mainly DHA (known to affect oxygen consumption by heart) and a significant increase in (n-6)-to-(n-3) PUFA ratio (n-6/n-3) (Ovide-Bordeaux & Grynberg, 2004). The low arachidonic-to-linoleic acids ratio (AA/LA) in the mothers may suggest the impairment of Δ-6 desaturase (Table 5.4).

However, the reduction in AA and AA/LA was striking in the pups, particularly at 15days (d) of life despite that it was the mothers who were diabetics. Moreover, the low DHA in liver ethanolamine phosphoglycerides (EPG) of the suckling pups, but not in the newborn, could

be attributed to the long-term consequences of maternal diabetes affecting the n-3 PUFA of the growing pups or to the lower desaturase activity at 15d of age offspring. Fetal desaturase activity is increased dramatically at 3d prior to delivery (or 18d of gestation) up to 7d postnatally and is decreased by 44% from 7d up to weaning (Bourre et al, 1990). Additionally, preterm desaturase activity is lower in the preterm than term tissues of the offspring (Bourre et al, 1990) and the human placenta had a low membrane DHA due to the early delivery (Chapter 4). Consequently, metabolic effects which are difficult to detect in the mother may well expected to have a more pronounced impact on her offspring. Hence, in line with the "developmental origins of health and disease" (DOHaD) (Gluckman & Hanson, 2004; Langley-Evans, 2006; Silveira et al, 2007; Taylor & Poston, 2007), the fetus is less than a "perfect parasite" not to be affected by maternal conditions.

Hepatic triacylglycerols and free fatty acids of the mothers showed an atypical n-6 and n-3 pattern due to experimental diabetes

Usually, biosynthetic defects in PUFA are attributed to the inhibition of the desaturase activity. However, a reduction in the activity of long chain acyl-CoA synthetase, Acsls (formerly known as ACS) (Coleman et al, 2002; Marszalek et al, 2005) is also evident in diabetes (Igal et al, 1991). In disagreement with earlier reports (Holman et al, 1983; Huang et al, 1984; Kanazawa et al, 1993; Shin et al, 1995), the diabetic dams in the present study had higher proportions of AA and DHA in liver free fatty acids (FFA) and triaclyglycerols (TG) than the controls. This finding cannot be explained by the impairment of the activity of Acsls and/or desaturases. In fact, because Acsls catalyzes an essential step in the metabolism of FA, multiple isoforms exist which are tissue-specific and have different preferences for FA species (Coleman et al, 2002; Marszalek et al, 2005).

Moreover, TG mediate the uptake and transfer of FA to membrane PL in cell culture systems (Blank et al, 1993; Tsai et al, 1993). Garg et al (1989) observed that a consumption of fish oil rich in eicosapentaenoic acid (EPA) was associated with a shift in AA from liver PL to plasma TG and liver cholesterol esters (CE). These studies may indicate that TG, cholesterol and FFA play a pivotal role in the homeostasis of tissue PL fatty acids. Igal et al (2001) demonstrated that over expressed mitochondria glycerol-3-phosphate acyltransferase directs incorporation of exogenous fatty acid into TG rather than PL. Diabetic subjects also showed abnormally high concentrations of serum lipids mainly due to increased mobilization of FFA from the peripheral fat depots and excessive liver TG formation (Mir et al, 2008).

The data showed that diabetes enhanced AA and DHA in liver TG and FFA of the dams, suggesting that this effect was most likely due to an alteration in the regulatory mechanisms of the distribution of AA and DHA between neutral lipids (NL) and PL. Moreover, reduced AA, but increased DHA and oleic acid were reported in liver PL due to diabetes and these results were not correlated with the *in vitro* hepatic desaturase activity (Giron et al, 1999). All the above are consistent with the postulation that 'other factors independent of desaturation and elongation may play a pivotal role in altered lipid biochemistry in clinically induced and genetically diabetic rats (Poisson et al, 1993).

5.4.2. Feeding a high-fat diet to pregnant dams altered tissue fatty acid composition

The novelty of this study was to investigate the effect of increasing fat intake, mainly lard, during the periconceptual, gestation and lactation periods on the PUFA composition of the offspring from diabetic and non-diabetic dams. The data showed that the high-fat diet altered percent AA and DHA and their intermediate metabolites in the mothers and their offspring.

Liver fatty acid composition of the dams fed on the high-fat diet

All dams, control and diabetic had enhanced DHA, AA, docosapentaenoic acid n-6 (DPA n-6), docosapentaenoic-to-docosatetraenoic acid (DPA/DTA n-6) and AA/LA ratios, significantly reduced LA, di-hommo-γ-linolenic acid (DGLA), and markedly reduced ALA and EPA in liver choline (CPG) phosphoglycerides and EPG at 16d postdelivery, reflecting high-fat intakes. In addition, AA and AA/LA were elevated in liver TG of all lard-fed dams. The presence of AA in the liver requires conversion of LA to AA (Wang et al, 2006; Ciapaite et al, 2007) since the AA content in the experimental diets was <0.2% (this study). Similarly, elevated AA due to high-fat intakes has been reported in other tissues, such as the EPG fraction of the rat rod outer segments (ROS) (Suh et al, 1994) or mice adipose tissue PL (Tallman & Taylor, 2003). Moreover, coronary artery disease (CAD) patients, from a population of the same geographical area and socioeconomical status, who may follow a Western diet, had low red blood cell (RBC) LA, high RBC and serum PL AA/LA ratio, and high RBC eicosapentaenoic-to- α-linolenic acids ratio (EPA/ALA) (Martinelli et al, 2008).

Moreover, the combination of a high-fat diet (mainly lard) and diabetes (HFD), compared with the high-fat diet *per se* (HFC), increased maternal hepatic LA and reduced DGLA and AA in

liver EPG of the HFD dams. In contrast, diabetic rats fed on 20% fat diet, mainly as beef tallow had reduced LA, but unaltered AA in PL compared with their corresponding controls fed on the same high-fat diet (Venkatraman et al, 1991). Therefore, the depressed desaturase activity in the diabetic state is influenced by the content and composition of the fat in the diet (Field et al, 1990; Venkatraman et al, 1991; Giron et al, 1999). Similarly, olive oil fed diabetic rats had low LA and AA in liver microsomal PL (Giron et al, 1999). Hence, other factors that could contribute to the changes in membrane PUFA composition of the high-fat fed and diabetic rats include fatty acid chain elongation (Venkatraman et al, 1991; Wang et al, 2006) and membrane lipid turnover or FA oxidation (Venkatraman et al, 1991).

However, AA was elevated in liver TG of the HFD (this study) and in muscle TG metabolites, ceramides and diacylglycerol (Smith et al, 2007). Additionally, feeding a high-fat diet to diabetic dams evoked vascular dysfunction in the mesenteric arteries at 16d postnatally (Koukkou et al, 1998). Several reports suggested that defetcs in vascular tissue FA metabolism due to high-fat diet and/or diabetes were associated with insulin resistance (IR) and vascular dysfunction and appeared to involve increased tissue FA uptake and increased accumulation of TG and ceramide and diacylglycerols, but reduced FA oxidation (Luiken et al, 2002; Ouwens et al, 2007; Smith et al, 2007; Chabowski et al, 2008).

Liver and heart fatty acid composition of the offspring born to high-fat fed dams

The effect of the high-fat diet on membrane AA and DHA was more pronounced in the offspring, neonates in particular, than the mothers. Since, the growing fetus is reliant on direct placental transfer of these polyenoic FA from the maternal circulation (Crawford et al, 1976, 1998) and there was an increase in maternal PL DHA, this finding suggests impairment in the transfer mechanism by the high-fat diet. The neonates of the diabetic and non-diabetic dams fed on the lard diet had low DHA (Figure 5.1) in liver and heart PL (CPG and EPG). Consequently AA/DHA and n-6/n-3 PUFA (Figure 5.2) was increased in liver and more prominently in the heart PL tissue at birth, reflecting impaired intrauterine lipid environment and maternal FA metabolism.

It is also conceivable that some of the diabetes effects have been masked by the high-fat diet. Chronic high–fat diet consumption, independent of maternal obesity and/or diabetes, significantly increased the risk of NAFLD in the developing nonhuman primates due to a 3-fold increase in liver TG levels, activation of several markers of oxidative stress and

premature activation of genes in the gluconeogenic pathway (McCurdy et al, 2009). Also, herein, the rodent data showed that high-fat feeding during pregnancy and lactation markedly reduced ALA, EPA, DHA, and total n-3 PUFA (Figure 5.3A&B) in liver TG and FFA at birth. The marked reduction in DHA and the concomitant increase in DPA n-6 is a substitution observed in n-3 deficiency state (Holman et al, 1982; Crawford et al, 1990; Coti Bertrand et al, 2006; Levant et al, 2007; Ozias et al, 2007).

In the suckling pups, in contrast to the fetus, there was a greater influence on the intermediate metabolites, DGLA and EPA in PL, reflecting post-delivery milk intakes. Hence, the reduction in the intermediate metabolites indicates that the process of synthesis was adversely influenced by the high-fat diet. The suckling pups can synthesise AA and DHA, whereas the fetus receives them preformed via the placenta. The human and rat milk contains <1% DHA (Innis, 2005; Tam & Innis, 2006) which would yield a different FA composition of plasma PL during the postnatal life, reaching a pattern similar to that found in the adults (Rum & Honstra, 2002; Decsi et al, 1995; Crawford, 2000). The latter could explain why neonatal plasma DHA is lower than fetal plasma level (Innis, 2005; Tam & Innis, 2006), even though plasma lipid TG concentration of the 10d suckling pups were increased due to maternal high-lard diet (Nakashima, 2007) and the lipid content in the milk reported to be higher in the high-fat fed dams (Trottier et al, 1998). However, FFA level in the milk was not affected by dietary fat manipulation (Trottier et al, 1998).

Crawford (2000) demonstrated that the placenta preferentially transfers DHA from the maternal to fetal circulation and Tam & Innis (2006) that the increase in EPG and DHA in the rat brain cortex between 19d of gestation and 20d postnatally, emphasizes the enrichment of DHA during early development. Herein, PUFA uptake was compromised in the vascular tissues of neonates and the low DHA level persisted in the suckling pups of the high-fat fed dams (Figure 5.1). Moreover, the marked reduction in ALA, EPA, DHA and total n-3 in liver NL (Figure 5.3A&B), similarly to the neonates, may suggest that adipose tissue depots were poor or that adipose tissue lipolysis was reduced in the pups due to maternal high-fat diet.

The reduction in DHA in membrane PL and NL of the suckling pups by maternal high-fat diet or diabetes and high-fat diet, similar to the neonates, was confirmed by the rise in AA (Figure 5.4) and DPA n-6 (Figure 5.5). The increase in DPA n-6 was particularly striking in the EPG lipid fraction of all high-fat groups, a well established compensatory substitution (Ozias et al, 2007; Igarashi et al, 2007). An increase in membrane long-chain PUFA, AA and DPA n-6 and DTA when DHA is marginal or deficient has been reported in rats and humans (Holman et al,

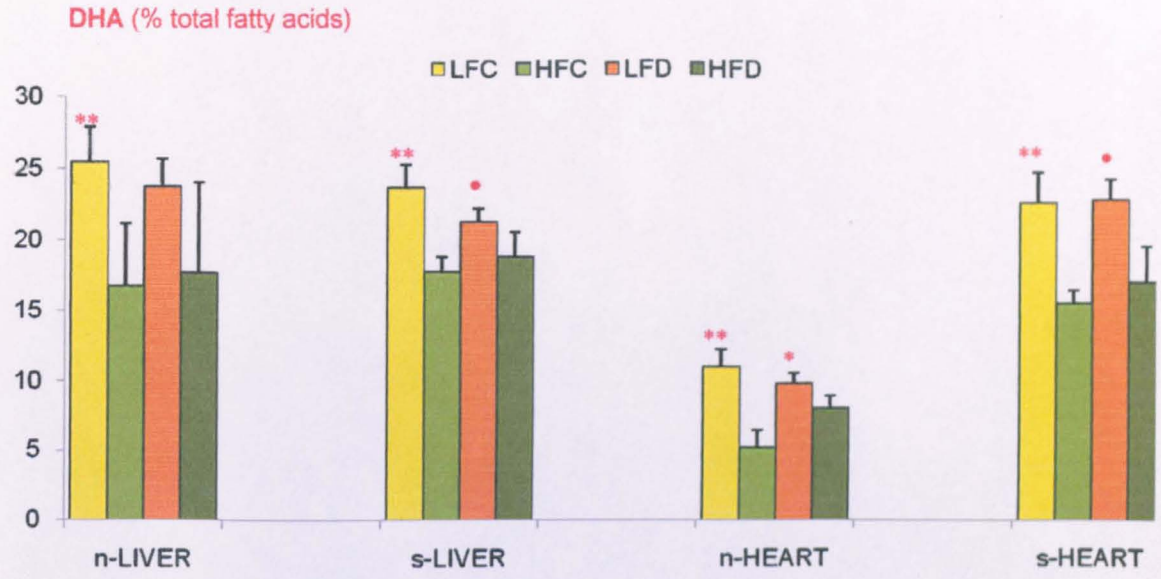


Figure 5.1. Docosahexaenoic acid (DHA) in liver and heart ethanolamine phosphoglycerides (EPG) of neonates (n-liver and n-heart) and suckling pups (s-liver and s-heart) from control and diabetic dams fed on low or high-fat diets. Values are means ± SD; Neonates (1d): n=10 (Low-fat control, LFC); n =9 (Low-fat diabetic, LFD); n=16 (High-fat control, HFC); n=6 (High-fat diabetic, HFD), Suckling pups (15d): n=16 (LFC); n =8 (LFD); n=7 (HFC); n=4 (HFD). Differed from their corresponding high-fat groups, *P<0.01; **P<0.0001 (non-parametric tests). •Due to the deleterious effect of high fat diet and diabetes only a small number of pups could be followed up the suckling period in the HFD group, however, the trait in fatty acid differences with their corresponding low fat group was similar to that of the control groups (high-fat vs. low-fat).

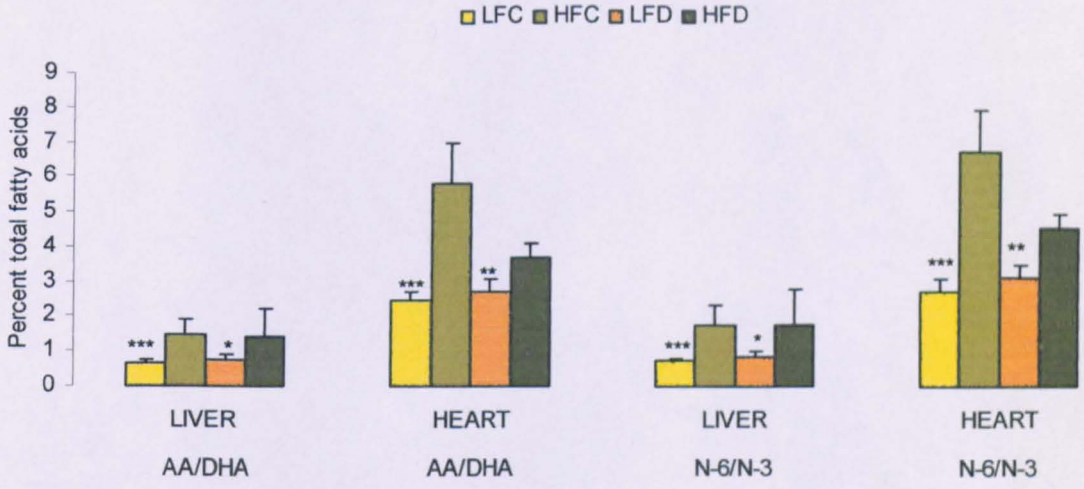
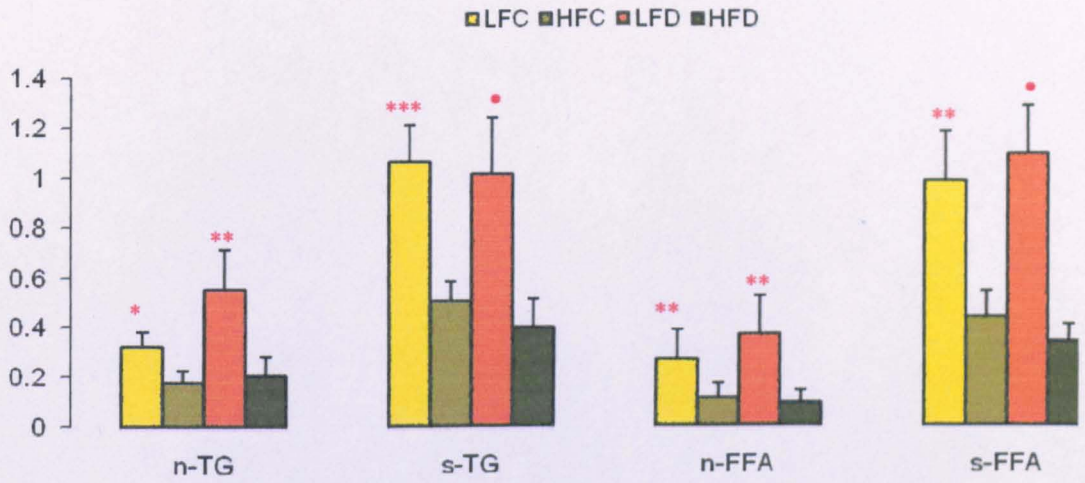


Figure 5.2. Neonatal arachidonic-to docosahexaenoic acid (AA/DHA) and total n-6 polyunsaturated fatty acid (PUFA) -to-total n-3 (PUFA) (n-6/n-3) ratios in liver and heart ethanolamine phosphoglycerides (EPG). Values are means ± SD; Neonates (day 1): n=10 (Low-fat control, LFC); n =9 (Low-fat diabetic, LFD); n=16 (high-fat control, HFC); n=6 (High-fat diabetic, HFD). Differed from their corresponding high-fat groups, *P<0.05; **P<0.005, ***P<0.0001 (non-parametric tests).

(I) **ALA** (% Total fatty acids)



(II) **EPA** (% Total fatty acids)

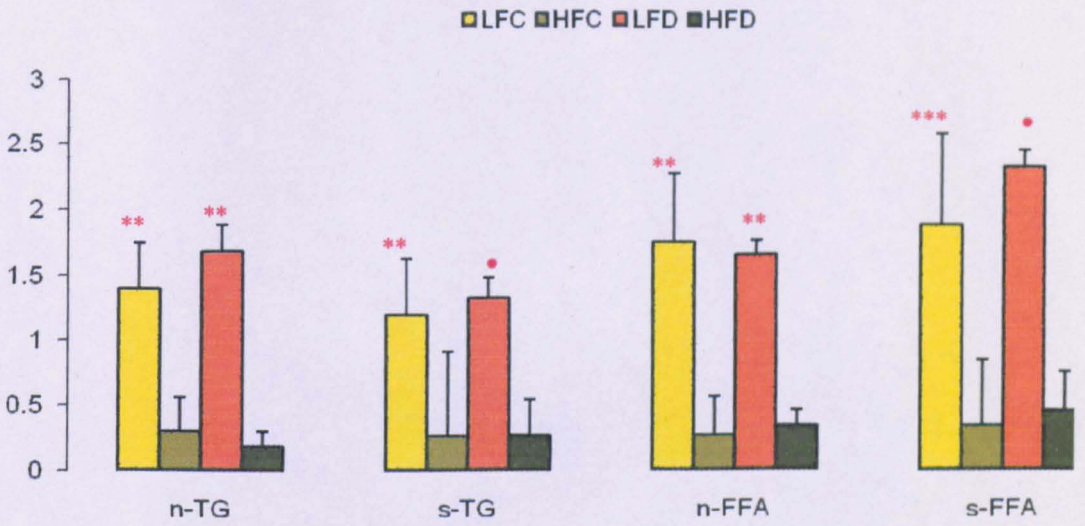
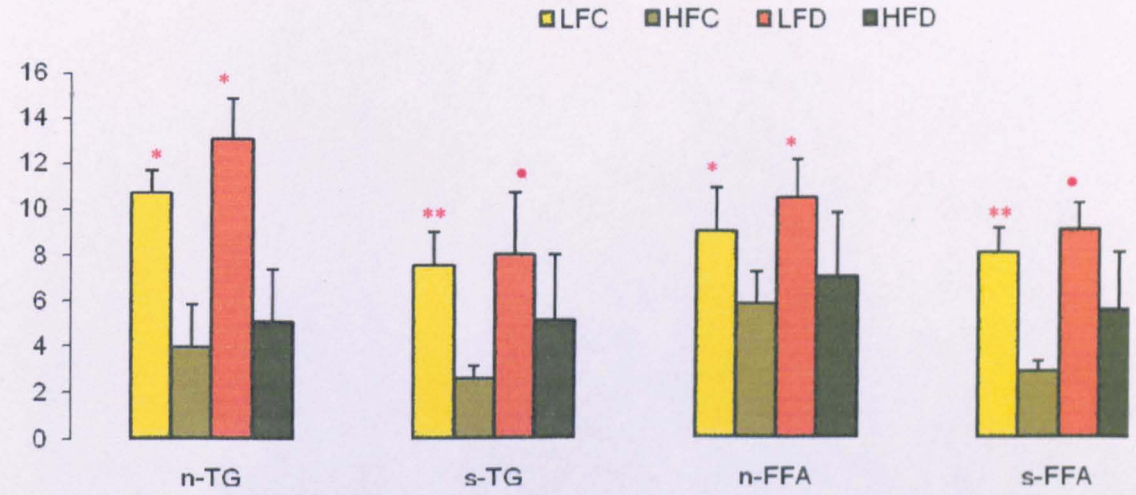


Figure 5.3A. a-Linolenic (ALA) (I) and eicosapentaenoic (EPA) (II) acids in liver triacylglycerlos (TG) and free fatty acids (FFA) of neonates (n-TG, n-FFA) and suckling pups (s-TG, s-FFA) from control and the diabetic rats fed on low or high-fat diets. Values are means ± SD; Newborn (day 1): n=10 (Low-fat control, LFC); n =9 (Low-fat diabetic, LFD); n=16 (High-fat control, HFC); n=6 (High-fat diabetic, HFD), Suckling pups (day 15): n=16 (LFC); n =8 (LFD); n=7 (HFC); n=4 (HFD). Differed from their corresponding high-fat groups, * $P<0.01$, ** $P<0.005$; *** $P<0.0001$ (non-parametric tests). •Due to the deleterious effect of high fat diet and diabetes only a small number of pups could be followed up the suckling period in the HFD group, however the trait in fatty acid differences with their corresponding low fat group was similar to that of the control groups (high-fat vs. low-fat).

(I) DHA (% Total fatty acids)



(II) Σn-3 (% Total faty acids)

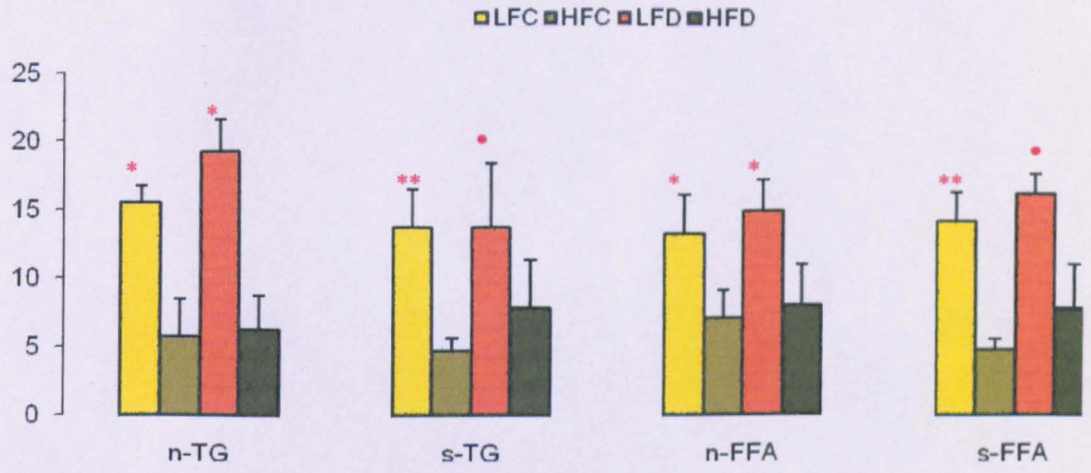


Figure 5.3B. Docasahexaenoic acid (DHA) (I) and total n-3 (Σn-3) polyunsaturated fatty acids (II) in liver triacylglycerols (TG) and free fatty acids (FFA) of neonates (n-TG, n-FFA) and suckling pups (s-TG, s-FFA) from control and diabetic rats fed on low or high-fat diets. Values are means ± SD; Newborn (day 1): n=10 (Low-fat control, LFC); n =9 (Low-fat diabetic, LFD); n=16 (High-fat control, HFC); n=6 (High-fat diabetic, HFD), Suckling pups (day 15): n=16 (LFC); n =8 (LFD); n=7 (HFC); n=4 (HFD). Differed from their corresponding high-fat groups, *P<0.005; **P<0.0001 (non-parametric tests). •Due to the deleterious effect of high fat diet and diabetes only a small number of pups could be followed up the suckling period in the HFD group, however the trait in fatty acid differences with their corresponding low fat group was similar to that of the control groups (high-fat vs. low-fat).

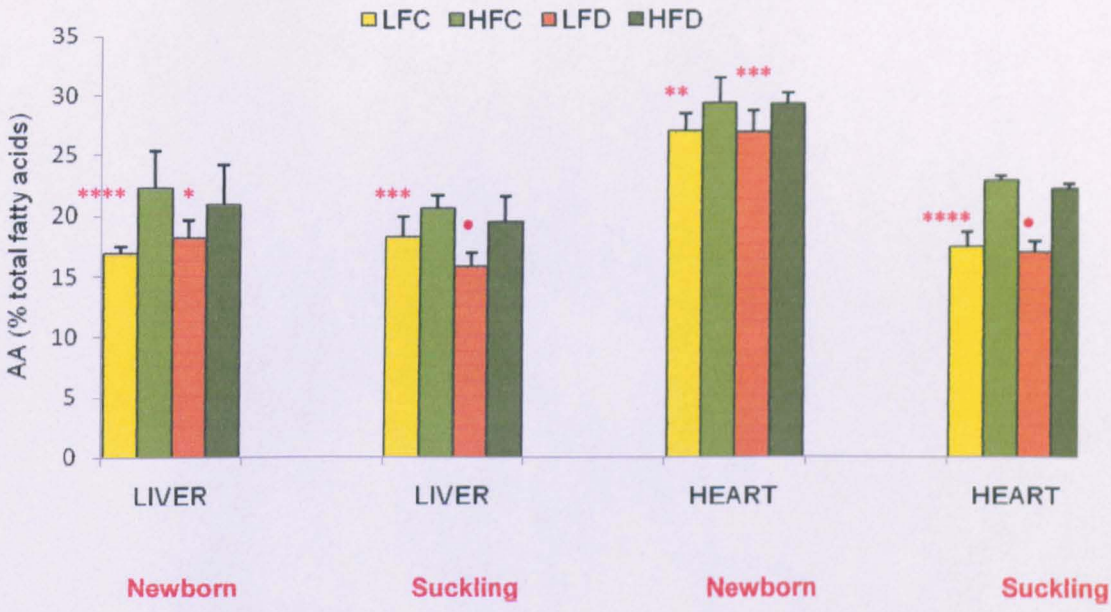


Figure 5.4. Arachidonic acid (AA) in liver and heart ethanolamine phosphoglycerides (EPG) of newborns and suckling pups from control and diabetic dams fed on low or high-fat diets. Values are means ± SD; Newborn (1d): n=10 (Low-fat control, LFC); n =9 (Low-fat diabetic, LFD); n=16 (High-fat control, HFC); n=6 (High-fat diabetic, HFD), Suckling pups (15d): n=16 (LFC); n =8 (LFD); n=7 (HFC); n=4 (HFD). Differed from their corresponding high-fat groups, *P<0.05; **P<0.01; ***P<0.005; **** P<0.0001 (non-parametric tests). •Due to the deleterious effect of high fat diet and diabetes only a small number of pups could be followed up the suckling period in the HFD group, however, the trait in fatty acid differences with their corresponding low fat group was similar to that of the control groups (high-fat vs. low-fat).

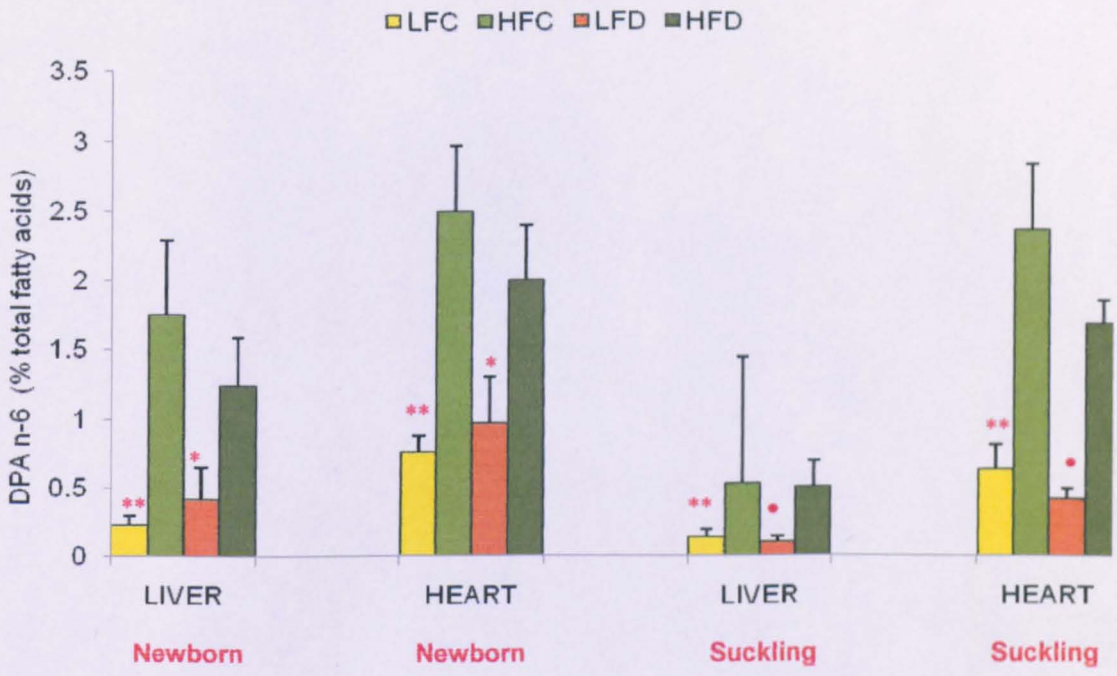


Figure 5.5. Docasapentaenoic acid (DPA n-6) in liver and heart ethanolamine phosphoglycerides (EPG) of the offspring. Values are means ± SD; Newborn (day 1): n=10 (Low-fat control, LFC); n =9 (Low-fat diabetic, LFD); n=16 (High-fat control, HFC); n=6 (High-fat diabetic, HFD), Suckling pups (day 15): n=16 (LFC); n =8 (LFD); n=7 (HFC); n=4 (HFD). Differed from their corresponding low fat groups, *P<0.05; **P<0.0001 (non-parametric tests). •Due to the deleterious effect of high fat diet and diabetes only a small number of pups could be followed up the suckling period in the HFD group, however the trait in fatty acid differences with their corresponding low fat group was similar to that of the control groups (high-fat vs. low-fat).

1982; Crawford et al, 1990). Interestingly, in previous studies of placental transfer of FA, Kuhn et al (1990) found that the placental uptake and fetal effluent of radiolabelled AA from the placentas of pregnancies complicated by insulin-dependent diabetes mellitus (IDDM) was significantly increased. The Kuhn et al (1990) data are consistent with the details reported herein, where the proportions of AA were increased in the EPG of in the newborn and in both EPG and CPG of the suckling pups, reflecting a change in the AA/DHA ratio in membrane PL. It is also in agreement with the data in IDDM, where there was a specific reduction in the proportions of DHA in plasma CPG in mother and fetus (Ghebremeskel et al, 1997).

The increased AA uptake in the vascular tissues of the offspring may suggest a reservoir of this important effector molecule that in disease state could down-regulate expression of the insulin- responsive glucose transporter and maybe related to the development of IR. *In vitro* studies have demonstrated that chronic exposure of murine adipocyte cells to AA altered glucose transporter gene expression (Tebbey et al, 1994). AA induced total loss of GLUT4 protein in adipocytes, in a similar manner to insulin itself, raising the speculation that AA regulates the insulin-induced intracellular signaling pathway or that the signaling pathways of AA and insulin converge at some common downstream intermediate (Tebbey et al, 1994).

Therefore, the reduction in DHA and EPA and increase in AA in offspring induced by high-fat maternal diet would be expected to compromise vascular integrity. The additional impact of diabetes is difficult to be detected from the data, which suggests that the high-fat diet overwhelmed the metabolic effect of the diabetes. Nonetheless, the data did indicate an additive effect would be expected on the metabolic intermediates. The impact of a high-fat diet on the expression of diabetes is suggested by companion papers (Koukkou et al, 1998), where it has been shown that the high-fat diet in these animals induced vascular dysfunction in the rat pups measured at 15d. The relaxation dysfunction was worsened in the presence of diabetes. In the absence of high-fat, the diabetes itself had little effect on vascular relaxation (Koukkou et al, 1998).

5.5. Conclusion

The high-fat diet had a major effect in reducing the cardio- and vascular-protective DHA, thus changing the DHA:AA value quite strikingly, in a manner expected to up-regulate thrombogenic potential. In view of interest in the early effects of nutrition on risk for cardiovascular disease later in life, the present data here clearly demonstrated that a high-fat diet during pregnancy changes the PUFA profile of fetal liver and heart membrane lipids in a manner which would be expected to pose a risk to vascular development.

CHAPTER 6: THE EFFECT OF A HIGH- FAT DIET ON AORTIC FATTY ACID COMPOSITION OF THE ADULT OFFSPRING

6.1. Introduction

Obesity is increasing in epidemic proportions globally. The cluster of metabolic and cardiovascular disorders, which is termed collectively as "metabolic syndrome" due to its highly atherogenic profile, includes hyperglycaemia, hyperlipidemia and obesity and hypertension (Cersosimo & DeFronzo, 2006; Gao et al, 2007). Insulin resistance (IR) and endothelium dysfunction may also play a central role in the pathogenesis of atherosclerosis (Cersosimo & DeFronzo, 2006; Hartge et al, 2007).

The endothelium is the innermost layer of blood vessels and, thus, the largest organ in the body (Hartge et al, 2007). One of the key functions of the endothelium is to control vascular tone and subsequently to regulate blood pressure and flow to organs and tissues via the secretion of diverse substances. These include the two main vasodilators, prostacyclin (PGI_2) and nitric oxide, involved in the inhibition of platelet aggregation and thrombosis, and the so-called vasoconstrictors, endothelin-1 (ET-1), angiotensin II (AngII), thromboxane A_2 and reactive oxygen species (Verma, 2002; Verma & Anderson, 2002; Gao et al, 2007; Hartge et al, 2007; Sarafidis & Bakris, 2007; Gryglewski, 2008). However, a third group of major vasodilators, the endothelial-derived hyperpolarization factors (EDHF) has also been described (Campbell & Harder, 1999; Gauthier et al, 2008; Chawengsub et al, 2008).

Arachidonic acid (AA; 20:4n-6) is the major essential fatty acid component of the inner cell membrane lipid in human vascular endothelium (Crawford et al, 1997) and AA and AA metabolites are among the major EDHF mediators (Pfister & Campbell, 1992; Barlow et al, 2000; Sandow et al, 2002; Chauhan et al, 2003; Miura et al, 2003; Gauthier et al, 2004, 2008; Herradon et al, 2007; Chawengsub et al, 2008). On the other hand, docosahexaenoic acid (DHA; 22:6n-3) and other n-3 polyunsaturated fatty acids (PUFA) are cardioprotective and antiarrhythmic agents (Mori et al, 2000; Wijendran & Hayes, 2004). Moreover, PUFA may influence the activity of membrane-bound protein, such as enzymes and ion channels (Asano et al, 1997; Cui et al, 2003; Zheng et al, 2005) and are known to modulate inflammatory response.

Cardiovascular diseases may have origins in fetal life and the period of maternal high-fat

feeding before and after gestation might be a determinant of the long-term effects in the adult offspring (Ferezou-Viala et al, 2007). It is evident that the neonates and the suckling offspring of dams fed on a 32.9% fat diet, resembling the high-fat intakes in the Western societies, exhibited profound irregularities in the PUFA composition of the liver and heart which could undermine many aspects of vascular function. In addition, the 15 days (d) and 60d old offspring of the high-fat fed mothers demonstrated vascular dysfunction together with plasma lipid abnormalities (Koukkou et al, 1998). Because of the detrimental effects of the combination of maternal diabetes and a high-fat diet (Chapter 5), resulting in greater mortality rates, this study was designed to investigate the effect of a moderately maternal high-fat diet (20% fat, mainly lard) on aortic fatty acid composition of the 160d old offspring.

6.2. Methods

Animal growth and husbandry were kindly performed by our collaborators, Prof. Poston's group from St. Thomas' Hospital (Ethical approval see section 2.2.2.).

6.2.1. Animals and diets

Similar to the study described in Chapter5, female Sprague -Dawley rats (12 - 14 weeks old) were fed either a low- fat diet (4.28% fat) or a high-fat diet (final analysis: 20% fat) for 10 days (d) prior to mating, through pregnancy and lactation (21d, postpartum). The high-fat diet was made up of the control diet premix supplemented with 16% (w/w) animal lard (final analysis; 20% fat) and has been previously described by our collaborators (Gerber et al, 1999; Armitage et al, 2007). To offset the dilution effect of the lard, the levels of protein and essential micronutrients in the high fat diet were adjusted. After weaning, the female offspring were fed ad libidum the standard chow diet (fat 3%, protein 15%, carbohydrate 62%, fibre 5%; Rat and Mouse Diet No:1 [RM1], SDS) up to 160d of life. The composition of fatty acid and other essential nutrients of the two experimental diets are shown in Table 6.1A&B.

At delivery, a proportion of each litter was killed in order that five to six pups were left with the mother in both groups, a common standardized procedure reported in all experimental protocols. Animals were weighed every 2d until 20-21d of gestation, then weekly until the time of culling. At 160d of life all offspring were killed by CO_2 inhalation and cervical dislocation. Fresh toracic aortas (n=11 control and n=12 high-fat group) were dissected free

of adventitia and analysed immediately or stored at -70 °C for subsequent fatty acid (FA) analysis (section 2.2.2).

6.2.2. Sample size and statistics

These are described at sections 2.4.1. & 2.4.2.

6.3. Results

6.3.1. Aortic choline and ethanolamine phosphoglycerides

In choline (CPG) and ethanolamine (EPG) phosphoglycerides (Table 6.2), the proportions of stearate ($P<0.0001$ in CPG and EPG) and total saturated fatty acids (ΣSFA) ($P<0.01$ in CPG, $P<0.05$ in EPG) were lower while palmitate ($P<0.05$, $P<0.0001$), C20:1 ($P<0.05$, $P<0.005$) and palmitoleate ($P<0.0001$) were higher in the high-fat compared with the control group. The proportions of oleate and total monounsaturated fatty acids (Σmono) in both lipid components and C20 in CPG were markedly enhanced in the high-fat group, $P<0.0001$.

Moreover, the percentage di-hommo-γ-linolenic acid (DGLA) in CPG and docosapentaenoic of the n-3 (DPA n-3) and docosapentaenoic acid of the n-6 series (DPA n-6) in EPG was lower in the high-fat compared with the control group ($P<0.0001$). By contrast, a-linolenic (ALA) and eicosapentaenoic (EPA) acids were elevated in the former group in EPG ($P<0.0001$ and $P<0.01$, respectively). In both CPG and EPG, the high-fat had higher proportions of linoleic acid, LA ($P<0.05$, $P<0.0001$ respectively) and lower docosahexaenoic, DHA ($P<0.0001$, $P<0.01$), docosatetraenoic, DTA ($P<0.01$, $P<0.0001$) acids, total n-6 (Σn-6) polyunsaturated fatty acids, (PUFA) ($P<0.0001$, $P<0.005$) and total n-3 PUFA, Σn-3 ($P<0.005$, CPG and EPG) compared with the control group (Table 6.2).

This altered DTA and LA profile between the two dietary groups was striking in EPG (Figure 6.1). Moreover, the percentage AA ($P<0.0001$, CPG and EPG) was substantially lower in the high-fat compared with the low-fat group and, hence, arachidonic-to-linoleic acid ratio, AA/LA ($P<0.005$ in CPG and $P<0.0001$ in EPG) was reduced in the 160d offspring of the high-fat group (Figure 6.1).

Nutrients	Breeding diet (4.28% corn oil)	High fat diet (20% fat, mainly lard)
Total lipids (g/kg)	428	200
Crude protein (g/kg)	223	180
Carbohydrate (g/kg)	510	410
Crude Fiber (g/Kg)	50	30
Gross Energy (KJ/kg)	1526	1862
Arginine (g/kg)	15.6	15.0
Cystine (g/kg)	3.6	3.4
Histidine (g/kg)	5.8	5.1
Methionine (g/kg)	4.7	3.5
Lysine (g/kg)	14.1	14.1
Isoleucine (g/kg)	10.5	10.3
Leucine (g/kg)	17.8	16.6
Threonine (g/kg)	9.2	8.6
Tryptophan (g/kg)	2.8	2.7
Valine (g/kg)	11.6	11.1
Choline (mg/kg)	1882	1655
Cyanocobalamin (μg/kg)	28.2	21.5
Folic acid (mg/kg)	2.7	2.1
Pyridoxine (mg/kg)	19.3	18.9
Thiamin (mg/kg)	25.7	25.6
Riboflavin (mg/kg)	10.9	10.6

Table 6.1A. Nutrient composition of the experimental animal diets

	% Total fatty acids	
	Breeding (4.28% corn oil)	High fat diet (20% fat, mainly lard)
14:0	0.71	1.54
16:0	15.5	24.7
18:0	2.90	13.8
20:0	0.24	0.21
ΣSFA	19.64	40.2
16:1	0.75	2.55
18:1	19.3	38.4
20:1	0.95	0.94
Σmono	22.1	42.3
18:2n-6	49.0	13.8
20:2n-6	0.09	0.37
20:3n-6	-	-
20:4n-6	0.09	0.16
Σn-6	49.0	14.5
18:3n-3	6.00	1.39
22:5n-3	0.16	0.14
22:6n-3	1.27	0.18
Σn-3	7.43	1.85
18:2n-6/18:3n-3	8.17	9.92
Σn-6/Σn-3	6.60	7.84

Table 6.1B. Fatty acid composition of the experimental animal diets. ΣSFA; total saturated fatty acids, Σmono; total monounsaturated fatty acids, Σn-6; total n-6 polyunsaturated fatty acids (PUFA), Σn-3; total n-3 PUFA, Σn-6/ Σn-3; total n-6 (PUFA)-to-total n-3 (PUFA) ratio.

	CPG		EPG	
	% Total fatty acids			
	Control	High-fat	Control	High-fat
16:0	25.6±1.58	27.1±1.26[a]	6.58±1.39	9.03±1.51[d]
18:0	23.0±1.65	19.1±1.59[d]	18.7±1.97	13.4±0.81[d]
20:0	0.06±0.02	0.21±0.07[d]	0.06±0.01	0.07±0.03
22:0	Trace	0.31±0.11	Trace	0.07±0.02
24:0	Trace	0.20±0.10	Trace	0.04±0.02
∑SFA	48.9±0.79	47.2±1.73[b]	25.4±2.99	22.7±1.61[a]
16:1	1.05±0.32	1.95±0.38[d]	0.53±0.18	1.69±0.38[d]
18:1	13.1±1.79	19.5±2.09[d]	7.26±1.58	17.2±2.57[d]
20:1	0.12±0.02	0.17±0.06[a]	0.10±0.02	0.14±0.03[c]
22:1	Trace	Trace	Trace	Trace
24:1	Trace	0.16±0.04	Trace	0.15±0.03
∑mono	14.3±2.06	21.7±2.21[d]	7.95±1.93	19.1±2.91[d]
18:2n-6	7.88±2.53	10.3±1.37[a]	2.83±0.96	11.8±2.16[d]
18:3n-6	0.07±0.02	0.09±0.01	0.04±0.01	0.03±0.01
20:2n-6	0.37±0.08	0.38±0.11	0.15±0.04	0.18±0.06
20:3n-6	1.28±0.14	0.96±0.11[d]	0.78±0.11	0.75±0.08
20:4n-6	20.1±2.85	13.2±2.05[d]	29.2±3.16	20.5±1.88[d]
22:4n-6	1.90±0.55	1.36±0.20[b]	6.93±1.60	3.41±0.75[d]
22:5n-6	0.63±0.22	0.46±0.11	1.60±0.24	1.18±0.20[d]
∑n-6	32.2±2.53	26.7±2.20[d]	42.0±3.41	37.8±2.65[c]
18:3n-3	0.08±0.03	0.10±0.02	0.06±0.02	0.15±0.03[d]
20:5n-3	0.15±0.02	0.15±0.03	0.18±0.02	0.23±0.04[b]
22:5n-3	0.60±0.07	0.52±0.16	1.90±0.16	1.30±0.37[d]
22:6n-3	1.98±0.14	1.35±0.39[d]	5.91±1.32	4.16±1.27[b]
∑n-3	2.77±0.15	2.12±0.53[c]	8.04±1.40	5.83±1.57[b]

Table 6.2. Percent fatty acids in aorta choline (CPG) and ethanolamine (EPG) phosphoglycerides of the offspring (160 days of life).
Values are means ± SD, n=11; control, n=12; high-fat. ∑SFA; total saturated fatty acids, ∑mono; total monounsaturated fatty acids, ∑n-6; total n-6 polyunsaturated fatty acids (PUFA), ∑n-3; total n-3 PUFA. Differed from control, [a]$P<0.05$; [b]$P<0.01$; [c]$P<0.005$; [d]$P<0.0001$ (Student's unpaired t-test).

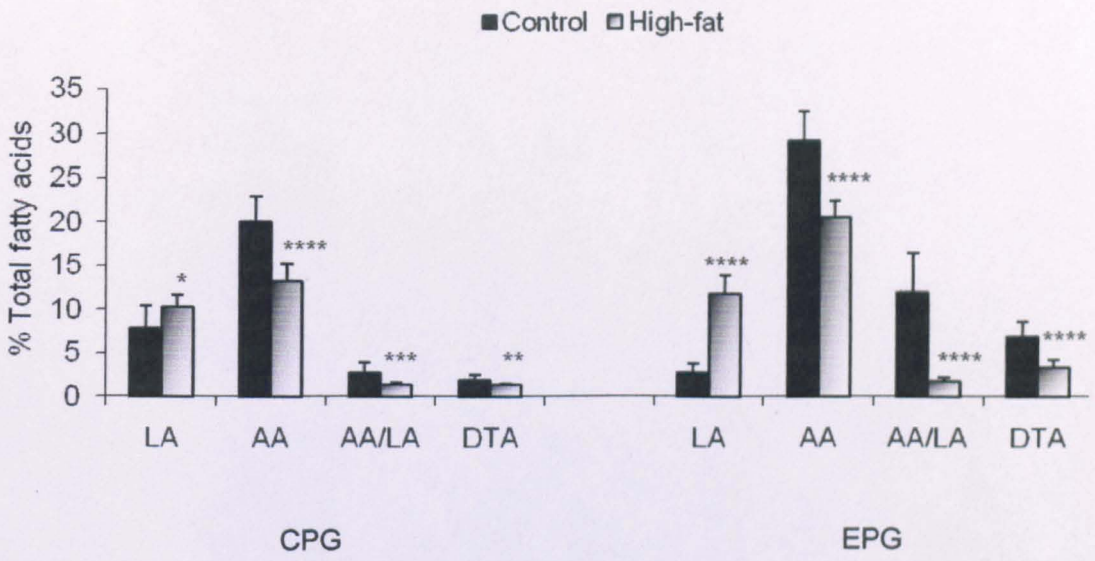


Figure 6.1. Percent linoleic (LA), arachidonic (AA) acids, arachidonic-to-linoleic acid ratio (AA/LA) and docosatetraenoic acid (DTA) in aorta choline (CPG) and ethanolamine (EPG) phosphoglycerides of the 160 days old offspring. Data are expressed as means ± SD, (n=11; control, n=12 high-fat). Differed from control, **P*<0.05; ***P*<0.01; ****P*<0.005; *****P*<0.0001 (Student's unpaired t-test).

6.4. Discussion

This study was focused on maternal overnutrition in pregnancy and in the offspring and the unexpected findings, herein, may be applicable to the dietary habits in the Western world. The previous study (Chapter 5) showed that a diet rich in fat, mainly animal lard, fed to pregnant dams, reduced docosahexaenoic acid (DHA) in the liver and heart of their offspring, neonates and suckling pups. The suckling/weaning transition in the rat is accompanied by marked dietary changes, since the very high fat-low carbohydrate diet (31% fat in maternal milk on a dry weight basis) feeding of the suckling pups is relatively quickly replaced by a low-fat high carbohydrate diet at weaning (Brooks & Lampi, 1999). Hence, the effects of the maternal diet during pregnancy and suckling period were also discriminated, herein, as most protocols include provision of the diet to the dam until weaning (Armitage et al, 2005).

The major finding of this study was that a maternal high-fat diet fed to rats during pregnancy and weaning markedly reduced aortic membrane arachidonic acid (AA) and DHA of their 160 days (d) old female offspring. These defects occurred despite the offspring being reared on a normal diet. In the aorta, these polyunsaturated fatty acids (PUFA) should originate from the predominant type of cell, vascular smooth muscle cells (SMC), but there would be a contribution of the endothelium (Lu et al, 2005). In fact, AA is the major essential fatty acid component of the inner cell membrane lipid in human vascular endothelium (Crawford et al, 1997).

6.4.1. The aorta of pups born to dams fed high- fat diet in pregnancy and normal diet while nursing contain high saturated and monounsaturated fatty acids

The offspring of the 20%-fat fed dams had elevated palmitic, palmitoleic and oleic acids and total monounsaturated fatty acids (MONO) in aorta choline (CPG) and ethanolamine (EPG) phosphoglycerides. The raised palmitic and oleic acids may contribute to cardiovascular risk since high concentrations of both fatty acids are reported to inhibit endothelial nitric oxide synthesis (Davda et al, 1995; Moers & Schrezenmeir, 1997).

Rat diets high in saturated fatty acids (SFA, mainly palmitic acid) and MONO (i.e. oleic acid) are reported to induced hypertension, in a similar manner, in association with reduced endothelium-dependent vasorelaxation, dyslipidemia and insulin resistance, IR (Gao et al, 2007). Moreover, Adan et al (1999) reported that exogenously hypercholesterolemic (ExHC)

rats which were treated initially with hypervitamin D and subsequently fed on an atherogenic diet containing 10% olive oil exhibited severe advanced atherosclerotic lesions. Also, feeding apoB only, LDLr $^{+/-}$, mice on MONO or SFA diets had increased cholesterol esters (CE) MONO content within low-density lipoprotein (LDL) particles which is highly associated with an increase in atherosclerosis (Bell et al, 2007a&b). However, the marked increase in total MONO, particularly in EPG of the arteries was the result of the high-fat diet fed to the mothers during pregnancy and lactation (this study). This suggests that the normal diet fed to the pups from weaning to adulthood does not seem to modulate the composition of the aorta, but the aorta seems to have the finger print of the high fat diet fed to the mothers.

6.4.2. The aorta of pups born to dams fed fed high-fat diet in pregnancy and normal diet while nursing contain reduced arachidonic and docosahexaenoic acids

In the 160d aorta of the high-fat group, linoleic acid (LA) was markedly increased and AA was reduced (Figure 6.1). Dietary LA, derived most likely by hydrolysis of triacylglycerols (TG)-rich lipoproteins, can elicit endothelial dysfunction, a characteristic feature of early-stage of atherosclerosis (Hennig et al, 2001; Maingrette & Renier, 2005), and potentiate tumor necrosis factor (TNF)-mediated endothelial cell injury (Hennig et al, 2001). This could be due to the excess dietary LA in the Western type diets (Spector et al, 1981; Kritchevsky et al, 2003) resulting in increased LA uptake and cellular accumulation within the endothelial cells, to the exclusion of AA (Spector et al, 1981; Hennig et al, 2001). Similarly, the human data reported increased concentrations of LA/ (total n-6 PUFA) in the phospholipid (PL) fractions of human coronary arteries in cases of sudden cardiac death (Luostarinen et al, 1993) or aorta and/or adipose tissue of coronary artery disease (CAD) patients (Bahrami et al, 2006, 2009).

In disagreement with Bell et al (2007b), who suggested that replacing MONO with PUFA will benefit CAD patients, Kritchevsky et al (2003) proposed that oleic-rich diets (avocado and olive oil) and PUFA n-6 (mainly LA)-rich diets are of the same order of atherogenicity. ExHC rats also fed on an atherogenic diet supplemented with 1% safflower oil (SO) exhibited advanced lesions (Adan et al, 1999). MONO are more protective in early stages of atherogenic processes which involve inflammatory processes and LDL oxidation while PUFA n-6 (mainly LA) are more effective in later stages when they do not provide aortic cholesterol with its preferred substrate (oleic acid) for esterification (Kritchevsky et al, 2003). Hence, maternal fat intakes (this study) may also modulate the aortic n-6 PUFA composition of their

offspring, predisposing the offspring to high risk for the development of cardiovascular disorders.

Furthermore, feeding pregnant dams on a high-fat diet, mainly animal lard (16% wt/wt) resulted in a reduced DHA and total n-3 PUFA profile in aortic CPG and EPG of their 160d offspring (this study). Vascular dysfunction in association with elevated plasma TG and reduced concentrations of plasma high-density lipoprotein cholesterol was also evident in the 160d offspring of the dam fed the 20%- fat diet as assessed by our collaborators in another report. Endothelium-dependent relaxation induced by acetylcholine was blunted in isolated branches of the femoral artery of the adult female offspring. Hence, the low aortic DHA is a profile expected to be seen in parallel with enhanced vascular endothelial dysfunction and increased serum TG level (Wijendran & Hayes, 2004).

Several mechanisms exist to explain the low aorta AA and DHA content of the offspring. Firstly, placental transfer of DHA and AA may be compromised due to exposure of the fetus to an abnormal *in utero* lipid environment. The developing fetus is dependent on maternal AA and DHA and the neonates and suckling pups born to high-fat fed dams had reduced liver and heart DHA (Chapter 5). Secondly, it is conceivable that a maternal high-fat feeding may 'programme' the activity of enzymes involved in fatty acid metabolism and/or transport. Das (2007) proposed that the low DGLA, AA, EPA and DHA due to a defect in Δ-5 and -6 desaturase activities, would lead to inadequate formation of anti-inflammatory and platelet anti-aggregatory agents, such as prostaglandin E2 (PGE_2), prostacyclin (PGI_2), prostaglandin I3 (PGI_3), lipoxins, resolving, neuroprotectine D1 (NPD1), nitric oxide and nitrolipids, promoting respiratory uncoupling and atherosclerosis. Hence, the desaturase may be susceptible to 'fetal programming' since reduced Δ-5 desaturase activity was associated with deficiencies of DHA in the offspring of rats fed a protein restricted diet (Ozanne et al, 1998).

In addition, the high LA and low AA and DHA in the aorta, resembles the n-6 and n-3 PUFA profile reported in human coronary arteries of patients with ischaemic heart disease. The coronary arteries from thirty cases of sudden cardiac death due to ischaemic heart disease showed greater formation of atherosclerotic lesions together with lower n-6 PUFA, with exception of LA, and lower n-3 PUFA compared with the controls (mostly traffic accident victims) (Luostarinen et al, 1993), indicating impaired LA metabolism, probably due to reduced Δ-6 desaturase activity. Similarly, the low AA/LA in liver of the high-fat dams and their offspring (Chapter 5) and aorta (figure 6.1) of the offspring may suggest impaired Δ-6

desaturase activity. Reduced AA (20%) in PL and neutral lipids (NL) and enhanced LA in PL of the aorta were also reported in the diabetic patients undergoing arterio-venous shunt surgery before renal dialysis (Lecomte et al, 1998). Alternatively, the process of aging could also contribute to the reduced desaturase activity in the older rats (Bourre et al, 1990).

Thirdly, the loss of AA in the aorta of the 160d old offspring could suggest increased peroxide formation as seen in the vascular wall of small diabetic vessels (Lecomte et al, 1998). Pregnant rats fed on the same high-fat diet described herein (20% lard and corn oil wt/wt) demonstrated oxidative stress by the measurement of the 8-Epi-prostaglandin F2 alpha, 8-epi $PGF_{2\alpha}$ (Gerber et al, 1999). Similarly, human plasma 9-hydroxyeicosatetraenoic acid (9-HETE) and F_2-isoprostane were strongly associated with angiographic evidence of CAD (Shishehbor et al, 2006) and more currently, it was reported that 8-iso-PGF2α increased parathyroid hormone-related peptide (PTHrP) and reduced PTH/PTHrP receptor (PTH1r) expression in rat cultured aortic SMC (Meziani et al, 2008).

Harats et al (2000) also showed that of the lipoxygenase (LO) enzymes, 15-LO, in endothelial cells of the vessel wall of LDL receptor-deficient mice fed on high cholesterol and high-fat diet, via preproendothelin promoter resulted in acceleration of early atherosclerotic lesions. In addition, 12/15-HETE produced by local monocyte-macrophage activation could serve as a ligand for peroxisome proliferator-activated receptor gamma (PPARγ), resulting in CD36 upregulation (George et al, 2000), an oxidized LDL receptor expressed in many cells (monocytes/macrophages, endothelial cells, adipocytes, platelets), which consequently will enhance plaque formation (Nozaki et al, 1995; Nakagawa et al, 1998; George et al, 2000).

Given the heterogeneity of plaque formation and progression mechanisms (deposit of lipids, cholesterol, calcium and cellular debris that accumulates in the lining of the artery wall), (Felton et al, 1997; Kyselovic et al, 2005), the lipid content and distribution within the plaque may influence the propensity of atherosclerotic plaques to disrupt (Felton et al, 1997). Ii et al (2008) also demonstrated that Group IVA phospholipase A_2 (IVA PLA_2), an enzyme catalyzing the initial step of AA cascade, partly is involved in the oxLDL –induced production of the metalloproteinases (MMP) isoenzyme, MMP-9 in mouse peritoneal macrophages, leading to migration of the vascular SMC into the intima and the rapture of the atherosclerotic plaques. The reduced proportions of n-6 PUFA and total n-3 and n-6 PUFA at the edge vs. the center of disrupted plaques could reflect oxidative damage (Felton et al, 1997). Likewise, the atherosclerotic ascending aorta had a lower n-3 PUFA content compared with the internal mammary arteries obtained from human individuals with CAD who underwent open heart

surgery (Bahrami et al, 2006). In addition, the human aorta and adipose tissue of the CAD who underwent coronary aorta artery bypass grafting had lower n-3 PUFA composition than the non-CAD patients (matched for age, sex and BMI) who underwent aortic valve replacement surgery (Bahrami et al, 2009).

The loss in arachidonic and docosahexaenoic acids in the aorta

The major finding of this study was that a maternal high-fat diet during pregnancy and weaning reduced aortic membrane AA and DHA (>30% in CPG and >29.5% in EPG) of the offspring. Thereby, the loss of AA and DHA in the aorta due to an abnormal *in utero* environment may suggest an atherogenic profile that would favor inappropriate inflammatory processes, impaired endothelial function or SMC proliferation, leading to the initiation of atherosclerosis. Supportive data on the association of low AA and DHA with endothelial dysfunction showed that the AA metabolites acting as endothelial-derived hyperpolarization factors (EDHF) have had their greatest dilation effects in small arteries and arterioles while the greatest endothelium-dependent dilation to nitric oxide (NO) occurred in large arteries (Illiano et al, 1992; Nishikawa et al, 1999; Campbell & Falck, 2007). Likewise, Golfetto et al (2001) reported the first case of a 65 years old man who after treatment of heamorrhagic stroke with AA improved recovery and the AA effect may be related to the enhanced proliferation of the micro-vessels around the affected area.

The AA-induced endothelium-dependent relaxation of the rabbit aorta (Pfister & Campbell, 1992; Campbell et al, 2003) showed to be the LO polar metabolite, 11,12,15-trihydroxyeicostrienoic acid (11,12,15-THETA) (Campbell et al, 2003). Furthermore, the CYP 450 metabolites of AA that function as EDHF have been identified in human, bovine and the rat coronary arteries (Miura & Gutterman, 1998; Lu et al, 2001; Gauthier et al, 2002, 2003; Larsen et al, 2006; Campbell & Falck, 2007). In human and bovine coronary arteries, AA and epoxyeicosatrienoic acids (EETs) activated K_{Ca} channels (Miura & Gutterman, 1998; Larsen et al, 2006; Campbell & Falck, 2007). Consequently, the hyperpolariased membranes inhibited the activation of voltage-activated calcium channels, reducing the calcium entry, causing relaxation (Campbell & Harder, 1999; Miura & Gutterman, 1998; Larsen et al, 2006; Campbell & Falck, 2007).

Mamas & Terrar (2001) was the first study to report that extracellular AA (10μM) reduced calcium currents, transients and contraction in guinea-pig isolated ventricular in association with suppressed spontaneous electrical activity induced by β-adrenergic agonist isoprenaline

or the Na+ pump inhibitor. Similarly, extracellular AA inhibited contractile function of adult rat isolated ventricular myocytes (ARVM), suppressed cell shortening and calcium transients of intact ARVM (Liu, 2007). These negative inotropic actions of AA appeared not to be mediated through the AA metabolites, suggesting that a direct effect may be involved (Mamas& Terrar, 2001; Liu, 2007) at the extracellular side of the cell membrane (Liu, 2007), causing a reduction in Ca entry through L-type calcium channels. Moreover, Nakano et al (2007) was the first study to show that vascular AA is related to endothelium-dependent vasorelaxation, hence, age-related endothelial dysfunction would be improved by supplementation with AA.

Thus, AA may be a cardioprotective and an antiarrhythmic agent (McHowat et al, 1998; Liu & McHowat, 1998; Mamas & Terrar, 2001; Liu, 2007). During hypoxia or ischaemia, the release of AA occurs in parallel with increased accumulation of lysoplasmenylcholine (LPlasC). It is likely that the direct inhibitory effect of free AA on L-type Ca^{2+} channel current can counterbalance the deleterious effects of LPlasC to prevent intracellular Ca^{2+} overload (Liu & McHowat, 1998; McHowat et al, 1998) and, hence, to protect the cell from injury (Liu, 2007). In contrast, as LPasC accumulation increase with AA release under hypoxia or cytokine release (Liu & McHowat, 1998; McHowat et al, 1998), resulting in an increased L-type Ca^{2+} channel current and sarcoplasmic reticulum (SR) function and inducing arrhythmias in ventricular myocytes (Liu et al, 2003), in a similar manner, high concentrations of free AA ($\geq\mu M$) become cytotoxic because of disruption of membrane integrity (Liu, 2007).

Likewise, DHA and other n-3 PUFA are cardioprotective, anti-arrhythmic and potent regulators of blood vessel function (Ye et al, 2002; Wijendran & Hayes, 2004). Substituting an atherogenic diet supplemented with 1% safflower oil (SO) fed to ExHC rats with 1% of ethyl ester DHA or EPA, resulted in less-developed lesions mainly composed of thin-layered foam cells (Adan et al, 1999). Moreover, Bousserouel et al (2003) suggested that the anti-inflammatory effect of EPA and DHA on rat SMC was attributed to the reduced secreted PLA_2 gene expression (Bousserouel et al, 2003). Moreover, an important role of the n-3 PUFA in regulating vascular tone and cardiac excitability has been proposed (Asano et al, 1997; Engler & Engler, 2000; Engler et al, 2000).

However, the present data showed that DHA was the main reduced n-3 PUFA in the aorta of the adult offspring, suggesting that the loss of DHA most likely would influence the constrictor/vasodilator profile and contribute to the vascular abnormalities in the small arteries. Otsuka et al (2005) was the first study to show that DHA strongly diminishes Thromboxane A_2 (TXA_2)-induced increase in vascular tone in guinea-pig conduit artery. This

novel action for DHA to inhibit TXA_2 receptor (TP receptor)-mediated vascular contraction may partly contribute to the circulatory-protective effect of DHA. Moreover, Mori et al (2000) demonstrated that in overweigth, mildly hyperlipidemic men, DHA, but no EPA, enhanced vasodilator responses relatively to olive oil (placebo) on the microcirculation of the forearm. There is also evidence that DHA was a more powerful inhibitor of platelet TXA_2 production and markedly reduced plasma and lipoprotein cholesterol content in the rat, including high-density lipoprotein (HDL) and liver cholesterol (Adan et al, 1999). In addition, the elevated LA and reduced AA in liver, aorta and platelet PL (CPG and EPG) fatty acid composition was more prominent in the ethyl-ester DHA group than ethyl-ester EPA, suggesting that DHA may be a more powerful inhibitor of Δ-6 desaturase in liver than dietary ethyl-ester EPA (Adan et al, 1999).

Furthermore, dietary DHA, but not EPA, enhanced blood flow in human arterioles after acetylcholine infusions in the presence of an inhibitor of NO synthesis, suggesting that DHA may enhance release of an EDHF, perhaps providing an EDHF precursor (Mori et al, 2000). The CYP epoxygenase metabolites of DHA (epoxydocosapentaenoic acids, EPDs) are of the most potent dilators of coronary microvessels and the most potent fatty acid epoxides known to activate large-conductance Ca^{2+}-activated K+ (BK_{Ca}) channels (Ye et al, 2002). A more current report also showed that DHA can activate large-conductance Ca^{2+}-activated K+ (BK_{Ca}) channels and block voltage-dependent K+ (K(v)) channels in rat coronary artery SMC (Lai et al, 2009). However, due to the well recognized heterogeneity of the endothelium between vascular beds and arteries of different sizes, this statement requires further investigation. In, addition synthesis of EDHF was inhibited by NO (Illiano et al, 1992; Campbell et al, 2003), suggesting that the different biochemical and functional interrelationships between NO and EDHF need to be defined.

6.5. Conclusion

The present study showed that feeding pregnant dams to high-lard diet reduced AA and DHA in the aorta of the adult offspring. These data strongly support the hypothesis that disturbance *in utero* environment through maternal lipid nutrition, impaired synthesis of the cardioprotective PUFA and, hence, may set the offspring at risk for the development of cardiovascular disorders, however, further investigations are required to elucidate the mechanism by which an altered n6 and n-3 PUFA may partly predispose the offspring to vascular dysfunction in adulthood.

CHAPTER 7.0. GENERAL DISCUSSION AND FUTURE STUDIES

7.1. Overview

The trophoblast is a fetally-derived placental tissue that is interimposed between the fetus and the mother in direct contact with maternal blood in the hemochorial placentae. The unique anatomy of the human placenta is basically due to differentiation of its epithelial stem cells, the cytotrophoblast cells. Placental complex villous structure greatly increases the surface area of contact between the fetal circulation in the placenta and the maternal circulation to meet the high demands of the growing fetus. If the blood flow to the placental bed through the uterine spiral arteries is compromised, it could result in preterm birth and fetal loss. Human fetal growth and development has a unique requirement for the supply of dietary lipids because of the extensive involvement of cell membrane growth during early development. Similarly, the placenta to support adequate transplacental exchange and prolonged development within the uterus, resulting in a highly developed newborn offspring is expected to exert a high demand for essential fatty acids (EFA).

The polyunsaturated fatty acids (PUFA), linoleic (LA) and α-linolenic (ALA) acids, are EFA because they can be provided only by the diet. Ingested LA is metabolized to di-hommo-γ-linolenic (DGLA), arachidonic (AA) and docosapentaenoic (DPA n-6) acids and ALA to eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids. Δ-6 desaturase is a rate limiting enzyme in the EFA synthesis pathway. DHA requires 2 rate-limiting Δ-6 desaturasions while AA synthesis requires one, and both AA and DHA are important structural components of the cell membranes. AA is the major essential fatty acid component of the inner cell membrane lipid in human vascular endothelium and AA and its metabolites are among the major endothelial-derived hyperpolarization mediators. DHA has a primary role in the function of the retina and the brain. Moreover, PUFA may influence the activity of membrane-bound protein, such as enzymes and ion channels and are known to modulate inflammatory response.

It is also evident that during intrauterine life the human placenta selectively transfers AA and DHA from the maternal circulation to the fetus, a process known as biomagnifications. Because the placenta lacks desaturase activity, the supply of AA and DHA to the fetus, by the placenta, depends on the maternal diet, circulating lipids and the length of gestation. Thereby, maternal nutrition and metabolism in early pregnancy may provide an intrauterine environment to which the placenta responds in order to match fetal growth rate in late pregnancy. On the other hand, with the increases in maternal BMI during pregnancy, the risk of preterm delivery and other maternal complications increases.

Additionally, gestational age at delivery and obesity are risk factors for delaying the onset of lactogenesis II, suggesting that the obesity-prematurity relationship is complex. It is also evident that in human (Type 1 and 2) and experimental diabetes, the activity of Δ-6 and -5 desaturases, vital for the synthesis of AA and DHA are impaired.

The aims of the studies reported in this thesis were to investigate the effects of (A) maternal diabetes and preterm delivery, independently, on placental fatty acid composition and (B) maternal diabetes and/or high-fat intakes during pregnancy on the fatty acids of the vascular tissues of the offspring in laboratory animal model.

CHAPTER 3 (Study 1): Investigated fatty acid composition of the placentae obtained from healthy women who underwent elective abortion between gestational ages (GA), 8 weeks (wk) and 14 wk or delivered a healthy baby at term (38-41 wk). Analysis of the individual phospholipid (PL) fractions demonstrated that AA is quantitatively an important placental membrane constituent, ranged 17-35.9% in choone (CPG), ethanolamine (EPG) and Inositol (IPG) phosphoglycerides. At term, AA was reduced in CPG, EPG and IPG and DHA in triacylglycerols (TG) while DGLA was markedly increased in all PL. The high dominance of AA, particularly in early gestation, is evident, suggesting an important role for AA in maintaining membrane structure, function and integrity. This finding is consistent with several studies supporting an important role for AA and AA metabolites in the mediation of metabolic and endocrine function of ovarian and placental cell membranes, organogenesis and in the establishment and maintenance of pregnancy. The reduction in AA and enrichment of term membranes with DGLA in normal pregnancies could imply a role for the n-6 PUFA favoring blood flow. On the other hand, the neutral lipid (NL) composition strongly suggested that DHA was preferentially incorporated in placental TG, supporting an important role in providing DHA to the fetus.

CHAPTER 4 (Study 2): Examined fatty acid composition of the placenta from women with GDM and mothers who delivered preterm babies, and compared with the healthy term pregnancies. GDM elevated AA and DHA in the placental CPG and EPG phosphoglycerides, suggesting increased uptake and esterification mainly in placental PL, instead of being transferred to the fetus. This finding is in line with previous studies showing that the neonates of the GDM women were born with low plasma and red blood cells (RBC) AA and DHA. On the other hand, preterm birth resulted in a reduced DHA content and increased AA/DHA and DPAn-6/DHA in the placental CPG and serine phosphoglycerides (SPG) and AA in SPG, implying a reduced DHA bioavailability to the fetus.

CHAPTER 5 (Study 3): Studied the effects of maternal diabetes and/or high-fat intakes during the periconceptual, gestation and lactation periods on liver and heart fatty acid composition of the offspring. The marked reduction in liver AA and AA/LA in the neonates and suckling pups may suggest impaired desaturase activity in the rats due to maternal diabetes. In contrast, feeding a 32.9%-fat diet (mainly lard) to pregnant rats reduced DHA in liver and heart CPG and EPG of all offspring, particularly in the neonates, reflecting impaired intrauterine lipid environment and maternal fatty acid metabolism. In the suckling pups, maternal high-fat feeding had a greater influence on the intermediate metabolites, DGLA and EPA, reflecting post-delivery milk intakes. Moreover, the marked reduction in ALA, EPA and DHA in liver TG and free fatty acids (FFA) may suggest that adipose tissue depots were poor or that adipose tissue lipolysis was reduced in these animals due to maternal high-fat diet. Vascular dysfunction in the offspring born to high-fat or diabetic and high-fat fed dams was evident, as assessed by our collaborators in another study.

CHAPTER 6 (Study 4): Studied the effect of a high in fat diet during pregnancy on the aorta fatty acids of the adult offspring. Feeding dams a 20%-fat (mainly lard) diet during pregnancy and lactation reduced AA and DHA in the aorta of the 160d old offspring. These maternal effects occurred despite that the offspring being reared in a normal breeding diet. It is conceivable that the reduced AA and DHA in the aorta may partly contribute to the vascular dysfunction in the adult offspring as assessed by our collaborators.

7.2. Future research

A) Support for a role of AA in endothelial driven organogenesis comes from studies describing a highly selective activation of protein kinase C by arachidonoyl diacylglycerol itself derived from the phosphatidylinositol-4-phosphate (PIP) cycle. Still, the suggestion that AA may participate in early developmental processes such as implantation, vascular growth and hence organogenesis (Chapter 3), requires further investigations. Hence, it will be interesting to study whether AA stimulates expression of cell signaling factors responsible for vascularisation and angiogenesis.

B) The enhanced uptake of AA and DHA by the placenta due to maternal diabetes suggested that the high accumulation of these PUFA in the placenta may be due to impaired supply or uptake or both, since it is evident that the RBC and plasma of the GDM

mothers at diagnosis and their neonates at birth had an abnormal fatty acid composition. Actually, AA and DHA were reduced in the newborn offspring of women with GDM. A question which arose from this study and other related study and must be elucidated is, does supplementing pregnant women at high risk for GDM with AA and DHA will restore the RBC lipid abnormality and normalise placental and neonatal PUFA?

C) The role for AA and DHA in placental apoptosis due to preterm delivery should further be investigated, since AA and DHA were reduced in SPG (a marker for apoptotic cells) in human preterm pregnancies. A question which arose from this study and other related study was whether supplementing mothers at risk for preterm delivery with DGLA or AA and eicosapentaenoic acid (EPA) and DHA will prevent PUFA deficiency in the placenta and neonates and will prolong gestation? Additional supplementation studies may assist to re-establish the n-6/n-3 PUFA ratio in pregnancy and the AA/DHA ratio in milk.

D) The experimental studies clearly demonstrated that maternal high-fat diet during pregnancy had a profound effect on vascular fatty composition of the offspring (neonates, suckling pups and adult offspring) and this effect was independent the diet fed to the offspring after weaning (160d old). However, from these studies, it is not possible to infer whether other types of diets also will have a similar impact. Hence, there is a need to elucidate whether the observed effect is a reflection of the diet or the developmental time (prenatal) the diet was fed to the mother, by feeding rats during princely diets with different fatty acid composition (high n-6 PUFA, low n-6 PUFA, high n-3 PUFA, low n-3 PUFA, high n-9 or low n-9 monounsaturated fatty acids).

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APPENDIX A

PAGE NUMBERING AS ORIGINAL

	Low-fat control (LFC; n=15)		Low-fat diabetic (LFD; n=10)		High-fat control (HFC; n=24)		High-fat diabetic (HFD; n=12)	
	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR
16:0	18.3	16.3-19.8	16.3	14.3-21.8	16.7	15.0-19.0	18.2	17.4-19.7
18:0	28.5	26.5-30.6	28.5	25.7-31.5	29.7	27.3-32.1	29.3	28.0-31.7
20:0	0.04	0.03-0.05	0.06	0.04-0.07	0.04	0.02-0.05	-	-
22:0	-	-	-	-	0.07	0.03-0.13	-	-
24:0	0.04	0.03-0.05	-	-	0.04	0.03-0.06	0.04	0.03-0.06
ΣSFA	47.1	45.5-47.7	46.3	45.1-47.7	47.0	46.6-47.6	47.7	47.2-49.8
16:1	0.44	0.34-0.67	0.29	0.22-0.52	0.16	0.13-0.18	0.17	0.13-0.21
18:1	7.14	5.80-8.17	5.66	4.60-8.26	5.43	5.09-6.32	5.37	5.30-5.89
20:1	0.06	0.06-0.07	0.08	0.07-0.09	0.07	0.04-0.09	0.05	0.05-0.06
22:1	-	-	-	-	-	-	-	-
24:1	0.03	0.03-0.08	0.03	0.02-0.04	0.03	0.02-0.05	-	-
Σmono	7.73	6.33-9.06	6.02	4.92-8.91	5.78	5.30-6.59	5.63	5.50-6.18
18:2n-6	15.6	14.4-16.5	16.4	14.1-19.1	10.6	10.2-12.1	11.2	9.69-11.9
18:3n-6	0.13	0.10-0.27	0.18	0.06-0.22	0.06	0.03-0.11	0.06	0.05-0.10
20:2n-6	0.23	0.20-0.25	0.23	0.19-0.39	0.20	0.19-0.22	0.17	0.14-0.20
20:3n-6	1.67	1.38-2.12	1.76	1.18-2.00	1.07	0.91-1.22	0.71	0.39-1.15
20:4n-6	15.1	13.0-17.0	16.1	11.8-19.9	23.6	20.9-24.5	22.6	20.6-23.0
22:4n-6	0.12	0.11-0.15	0.13	0.10-0.17	0.27	0.24-0.34	0.23	0.23-0.25
22:5n-6	0.15	0.14-0.17	0.16	0.12-0.18	0.69	0.59-0.77	0.65	0.55-0.72
Σn-6	33.7	31.5-34.7	34.3	29.3-39.7	37.2	35.9-37.9	35.0	33.6-36.7
18:3n-3	0.15	0.11-0.19	0.16	0.10-0.22	0.04	0.03-0.04	0.04	0.02-0.04
20:5n-3	1.73	1.41-1.95	1.30	0.61-1.88	0.09	0.06-0.12	0.06	0.05-0.08
22:5n-3	1.05	0.91-1.32	1.22	0.98-1.57	0.74	0.60-0.92	0.84	0.72-1.04
22:6n-3	7.02	6.61-8.15	6.76	6.32-7.51	8.21	6.86-9.09	8.51	7.21-10.1
Σn-3	9.35	8.44-11.0	9.23	8.14-10.9	8.94	7.65-9.84	9.38	8.12-11.3
AA/LA	0.92	0.84-1.20	0.89	0.69-1.29	2.15	1.62-2.31	2.02	1.89-2.13
AA/DHA	2.11	1.76-2.46	2.42	1.56-2.90	2.89	2.63-3.56	2.59	2.19-3.03
DPA/DTA	1.22	1.00-1.71	1.10	0.77-1.71	2.34	1.83-3.15	2.69	2.30-3.02
Σmetn-6/ Σmetn-3	1.87	1.70-2.04	2.07	1.36-2.69	2.90	2.70-3.48	2.52	2.15-2.90
Σn-6/ Σn-3	3.71	3.09-3.89	3.53	3.11-4.98	4.21	3.70-4.91	3.72	3.08-4.23

Table 5.3A. Fatty acids in liver choline phosphoglycerides of the dams. Values are medians with interquartile range (IQR).

	Low-fat control (LFC; n=15)		Low-fat diabetic (LFD; n=10)		High-fat control (HFC; n=24)		High-fat diabetic (HFD; n=12)	
	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR
16:0	14.7	13.8-15.8	13.2	12.6-16.4	12.2	11.8-13.5	13.2	12.2-15.3
18:0	22.5	21.1-23.1	23.7	21.9-24.8	27.7	26.2-28.5	27.4	25.7-28.0
20:0	-	-	-	-	-	-	-	-
22:0	-	-	-	-	-	-	-	-
24:0	0.03	0.02-0.04	-	-	-	-	-	-
ΣSFA	37.4	36.0-38.1	37.5	37.1-38.6	39.9	39.4-40.5	40.9	39.5-43.1
16:1	0.38	0.29-0.47	0.31	0.12-0.49	0.10	0.06-0.14	0.10	0.06-0.13
18:1	5.60	4.83-5.89	5.30	4.91-6.18	3.75	3.49-4.13	4.42	4.17-4.75
20:1	0.08	0.06-0.09	-	-	-	-	-	-
22:1	-	-	-	-	-	-	-	-
24:1	0.04	0.02-0.06	-	-	-	-	-	-
Σmono	6.19	5.16-6.37	5.64	5.15-6.74	3.87	3.61-4.28	4.53	4.28-4.77
18:2n-6	18.0	17.2-18.6	19.0	18.3-21.9	13.4	11.9-13.9	14.3	13.3-15.5
18:3n-6	0.05	0.04-0.07	-	-	-	-	-	-
20:2n-6	0.34	0.28-0.38	-	-	-	-	-	-
20:3n-6	1.15	0.99-1.23	1.14	0.79-1.32	0.63	0.56-0.70	0.44	0.28-0.63
20:4n-6	16.4	14.7-16.9	14.6	12.8-16.7	20.6	17.2-21.7	16.5	15.3-18.8
22:4n-6	0.36	0.29-0.38	0.27	0.25-0.32	0.80	0.72-0.95	0.66	0.57-0.77
22:5n-6	0.25	0.20-0.29	0.17	0.13-0.25	1.62	1.08-2.39	1.15	0.84-1.38
Σn-6	36.6	34.3-38.0	36.3	32.5-38.6	37.2	34.5-37.7	33.8	30.7-35.4
18:3n-3	0.22	0.17-0.26	0.24	0.22-0.27	0.04	0.03-0.06	0.07	0.05-0.08
20:5n-3	1.85	1.39-2.47	1.67	0.86-2.78	0.11	0.09-0.14	0.09	0.06-0.14
22:5n-3	2.34	1.96-2.80	2.41	2.00-2.94	1.36	1.19-1.54	1.36	1.09-1.60
22:6n-3	13.5	12.8-14.9	12.7	11.7-13.4	15.2	14.3-18.4	16.8	15.1-18.2
Σn-3	18.7	16.8-19.7	16.8	16.0-20.1	16.8	15.8-19.8	18.1	16.8-19.8
AA/LA	0.89	0.77-0.96	0.74	0.58-0.86	1.53	1.25-1.62	1.13	1.03-1.28
AA/DHA	1.16	1.06-1.29	1.23	0.97-1.34	1.31	0.97-1.50	0.98	0.81-1.25
DPA/DTA	0.72	0.61-0.88	0.60	0.42-1.03	1.41	1.36-2.77	1.61	1.49-2.17
Σmetn-6/ Σmetn-3	0.97	0.92-1.15	1.01	0.77-1.15	1.42	1.08-1.51	1.01	0.85-1.27
Σn-6/ Σn-3	1.96	1.80-2.26	2.21	1.62-2.34	2.22	1.75-2.38	1.80	1.58-2.10

Table 5.4A. Fatty acids in liver ethanolamine phosphoglycerides of the dams. Values are medians with interquartile range (IQR).

	Low-fat control (LFC; n=15)		Low-fat diabetic (LFD; n=10)		High-fat control (HFC; n=24)		High-fat diabetic (HFD; n=12)	
	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR
16:0	36.5	32.8-41.1	31.5	29.0-33.7	31.1	30.2-32.9	29.4	28.3-30.8
18:0	15.8	12.5-17.0	13.1	11.4-15.0	11.0	9.38-12.3	10.4	9.64-12.9
20:0	0.12	0.07-0.17	0.07	0.06-0.10	0.07	0.06-0.10	0.07	0.05-0.11
22:0	0.07	0.06-0.11	-	-	-	-	-	-
24:0	0.09	0.06-0.13	0.05	0.03-0.08	-	-	0.03	0.03-0.07
ΣSFA	51.2	48.7-58.2	45.7	43.6-47.6	42.7	41.7-44.8	41.7	39.2-44.0
16:1	1.79	1.55-2.06	1.39	0.72-1.82	0.98	0.93-1.13	0.89	0.85-0.93
18:1	15.8	13.3-19.5	15.0	10.4-19.7	26.0	23.8-27.1	26.1	22.9-27.3
20:1	0.16	0.14-0.23	0.18	0.16-0.24	0.34	0.33-0.38	0.36	0.32-0.40
22:1	0.12	0.07-0.20	0.06	0.05-0.08	-	-	0.06	0.04-0.08
24:1	-	-	0.05	0.04-0.06	0.08	0.03-0.14	0.03	0.02-0.06
Σmono	18.6	16.1-21.7	16.6	11.4-21.9	27.3	25.4-28.5	27.5	24.1-28.9
18:2n-6	10.7	9.49-12.9	16.6	14.1-22.2	16.7	14.8-17.9	17.6	14.6-19.4
18:3n-6	0.16	0.08-0.26	0.24	0.22-0.28	0.20	0.17-0.26	0.27	0.24-0.35
20:2n-6	0.42	0.35-0.45	0.41	0.39-0.54	0.32	0.28-0.35	0.34	0.32-0.37
20:3n-6	0.43	0.25-0.52	0.69	0.56-0.78	0.35	0.30-0.41	0.38	0.34-0.50
20:4n-6	3.24	2.42-5.37	6.33	4.59-8.31	5.90	5.30-6.50	5.93	5.23-7.81
22:4n-6	0.29	0.21-0.40	0.44	0.39-0.52	0.88	0.76-1.06	0.83	0.72-1.00
22:5n-6	0.47	0.18-0.83	0.35	0.22-0.65	0.67	0.52-1.00	0.45	0.29-0.57
Σn-6	15.9	13.6-19.0	25.5	22.0-31.9	25.0	23.0-27.0	26.4	22.8-28.3
18:3n-3	0.34	0.22-0.61	0.58	0.45-0.78	0.34	0.25-0.44	0.37	0.22-0.46
20:5n-3	0.72	0.55-1.25	1.03	0.93-1.11	0.23	0.14-0.35	0.22	0.18-0.26
22:5n-3	0.44	0.35-0.70	0.77	0.62-0.94	0.35	0.28-0.52	0.67	0.51-0.76
22:6n-3	1.81	1.15-2.12	2.96	2.14-3.95	1.42	1.01-2.07	2.07	1.41-2.89
Σn-3	3.31	2.36-4.36	4.72	3.86-5.37	2.28	1.75-3.24	3.38	2.26-4.30
AA/LA	0.29	0.26-0.37	0.39	0.26-0.47	0.37	0.31-0.42	0.36	0.30-0.42
AA/DHA	2.06	1.65-2.52	2.34	1.66-2.55	4.51	3.22-5.57	3.12	2.35-3.52
DPA/DTA	1.29	0.69-3.00	0.64	0.35-1.24	0.76	0.52-1.06	0.46	0.35-0.66
Σmetn-6/ Σmetn-3	1.72	1.41-2.42	2.04	1.78-2.15	4.54	3.26-5.21	3.04	2.42-3.72
Σn-6/ Σn-3	4.80	4.32-5.75	5.87	4.67-6.39	11.2	8.12-12.8	8.15	6.63-9.84

Table 5.5A. Fatty acids in liver free fatty acids of the dams. Values are medians with interquartile range (IQR).

	Low-fat control (LFC; n=15)		Low-fat diabetic (LFD; n=10)		High-fat control (HFC; n=24)		High-fat diabetic (HFD; n=12)	
	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR
16:0	29.8	25.7-31.6	26.9	21.6-28.8	26.4	25.8-27.0	24.5	23.5-25.6
18:0	5.38	4.58-5.87	3.89	3.57-4.26	5.15	4.56-5.91	5.73	5.47-6.11
20:0	0.09	0.08-0.10	0.08	0.06-0.12	0.06	0.05-0.07	0.09	0.08-0.11
22:0	0.19	0.14-0.24	-	-	0.11	0.09-0.13	0.13	0.12-0.14
24:0	0.03	0.02-0.03	-	-	0.02	0.01-0.02	-	-
ΣSFA	35.5	31.6-36.5	31.2	25.6-33.1	31.5	30.9-32.1	31.2	29.7-31.7
16:1	3.59	2.51-5.44	2.45	1.29-3.18	1.17	1.04-1.35	0.91	0.79-1.04
18:1	32.6	30.2-35.4	23.8	17.2-31.7	33.6	32.3-35.3	29.5	28.7-33.8
20:1	0.47	0.38-0.67	0.38	0.30-0.59	0.79	0.70-0.82	0.72	0.60-0.90
22:1	0.07	0.06-0.10	0.05	0.04-0.07	0.02	0.01-0.02	0.03	0.02-0.04
24:1	0.10	0.05-0.15	0.02	0.01-0.03	0.01	0.01-0.02	0.04	0.04-0.05
Σmono	37.5	34.6-39.9	26.7	18.9-35.7	35.5	34.3-37.2	31.4	30.1-35.6
18:2n-6	16.6	15.6-18.8	26.2	20.1-35.1	20.9	20.3-21.5	21.1	20.1-23.1
18:3n-6	0.27	0.14-0.32	0.31	0.26-0.38	0.40	0.34-0.57	0.54	0.42-0.60
20:2n-6	0.29	0.24-0.38	0.40	0.30-0.53	0.34	0.32-0.35	0.35	0.33-0.36
20:3n-6	0.23	0.21-0.33	0.45	0.31-0.70	0.45	0.36-0.51	0.54	0.52-0.62
20:4n-6	1.00	0.75-1.18	1.49	1.11-2.48	2.80	2.29-3.43	3.75	3.00-4.78
22:4n-6	0.32	0.23-0.37	0.36	0.32-0.71	1.32	0.93-1.54	1.33	1.15-1.55
22:5n-6	0.17	0.11-0.29	0.14	0.12-0.22	0.88	0.70-1.03	0.79	0.65-0.90
Σn-6	18.7	17.6-21.4	29.4	22.6-39.9	27.2	25.4-28.4	28.5	25.5-31.7
18:3n-3	0.65	0.55-0.76	-	-	0.61	0.56-0.66	0.68	0.53-0.77
20:5n-3	0.36	0.24-0.43	1.11	0.83-1.34	0.11	0.07-0.14	0.25	0.16-0.38
22:5n-3	0.50	0.45-0.65	1.63	1.09-2.71	0.86	0.71-0.96	1.33	1.17-1.95
22:6n-3	1.12	0.84-1.59	3.09	1.58-5.20	1.73	0.88-2.17	3.19	2.19-3.66
Σn-3	2.38	1.79-2.83	5.70	3.34-8.75	3.42	2.45-3.85	5.55	4.12-6.32
AA/LA	0.06	0.04-0.07	0.06	0.05-0.08	0.13	0.11-0.17	0.16	0.14-0.22
AA/DHA	0.88	0.63-1.10	0.49	0.38-0.68	1.81	1.45-2.71	1.31	0.99-1.64
DPA/DTA	0.70	0.37-0.97	0.32	0.22-0.39	0.66	0.63-0.82	0.68	0.44-0.73
Σmetn-6/ Σmetn-3	1.11	0.92-1.35	0.62	0.46-0.75	2.38	2.02-3.02	1.47	1.13-1.77
Σn-6/ Σn-3	8.12	7.54-10.1	4.84	4.05-7.19	7.98	7.13-11.2	5.07	4.78-6.29

Table 5.6A. Fatty acids in liver tiracylglycerols of the dams. Values are medians with interquartile range (IQR).

	Low-fat control (LFC; n=10)		Low-fat diabetic (LFD; n=9)		High-fat control (HFC; n=16)		High-fat diabetic (HFD; n=6)	
	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR
16:0	29.5	28.3-30.4	27.7	26.7-29.1	26.0	24.2-30.3	26.4	25.2-28.2
18:0	17.3	16.8-18.0	18.9	18.0-20.4	17.3	16.1-20.0	19.4	18.3-20.4
20:0	-	-	-	-	-	-	-	-
22:0	0.15	0.07-0.15	0.14	0.13-0.15	0.16	0.11-0.27	-	-
24:0	-	-	-	-	-	-	-	-
ΣSFA	47.0	45.8-47.8	46.7	46.2-48.2	44.3	43.0-47.7	46.9	44.0-47.6
16:1	0.87	0.60-1.05	0.45	0.35-0.78	0.90	0.45-1.74	1.15	0.59-1.69
18:1	6.81	6.21-7.20	6.59	6.32-6.76	11.9	9.18-13.6	13.3	9.44-15.8
20:1	0.11	0.08-0.18	0.13	0.10-0.15	0.13	0.12-0.21	0.18	0.16-0.30
22:1	-	-	-	-	-	-	-	-
24:1	-	-	-	-	0.08	0.04-0.14	-	-
Σmono	7.19	7.05-8.23	7.20	6.85-7.45	12.9	9.75-15.3	14.2	10.2-17.6
18:2n-6	7.06	6.38-7.42	7.75	6.66-10.0	9.07	4.70-13.8	8.89	5.96-10.6
18:3n-6	0.12	0.11-0.17	-	-	0.13	0.12-0.18	-	-
20:2n-6	0.26	0.19-0.35	0.34	0.28-0.41	0.21	0.18-0.28	0.24	0.15-0.44
20:3n-6	0.85	0.71-0.95	0.86	0.65-0.94	0.89	0.59-1.05	0.92	0.66-1.26
20:4n-6	22.0	20.8-22.7	20.4	19.1-21.5	18.6	15.7-21.9	15.2	13.2-21.0
22:4n-6	0.45	0.42-0.51	0.44	0.38-0.49	0.61	0.50-0.83	0.45	0.38-0.60
22:5n-6	0.12	0.10-0.15	0.10	0.07-0.16	0.98	0.70-1.36	0.64	0.53-0.89
Σn-6	30.8	29.1-31.5	30.4	28.3-32.1	30.4	28.5-31.0	27.9	26.2-28.9
18:3n-3	0.05	0.04-0.06	0.08	0.06-0.12	0.09	0.07-0.12	-	-
20:5n-3	0.34	0.24-0.45	0.28	0.24-0.34	0.35	0.25-0.50	0.31	0.26-0.52
22:5n-3	1.15	1.04-1.43	1.20	1.05-1.36	0.50	0.38-0.84	0.35	0.32-0.85
22:6n-3	11.5	10.5-12.3	11.5	9.97-12.4	8.77	7.52-9.79	9.53	5.72-10.7
Σn-3	13.0	12.0-14.0	13.3	11.5-13.9	9.12	8.54-10.1	10.5	6.40-11.8
AA/LA	3.08	2.90-3.51	2.48	2.09-3.19	2.05	1.14-4.47	1.71	1.22-3.55
AA/DHA	1.92	1.72-2.16	1.88	1.59-2.08	2.08	1.77-2.53	1.98	1.50-2.38
DPA/DTA	0.27	0.23-0.31	0.27	0.20-0.31	1.93	1.01-2.67	1.42	0.99-2.36
Σmetn-6/ Σmetn-3	1.84	1.65-2.08	1.77	1.53-2.02	2.18	2.06-2.63	1.98	1.58-2.72
Σn-6/ Σn-3	2.33	2.12-2.73	2.28	2.03-2.79	3.32	3.04-3.82	2.63	2.40-4.23

Table 5.7A. Fatty acids in liver choline phosphoglycerides of the newborn pups (1 day of life). Values are medians with interquartile range (IQR).

	Low-fat control (LFC; n=16)		Low-fat diabetic (LFD; n=8)		High-fat control (HFC; n=7)		High-fat diabetic (HFD; n=4)	
	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR
16:0	25.1	24.2-25.8	28.7	26.8-30.0	24.9	23.8-25.8	26.6	25.6-30.1
18:0	20.3	19.4-21.4	18.5	18.3-19.6	22.9	22.3-24.0	21.6	17.8-22.0
20:0	-	-	-	-	-	-	-	-
22:0	-	-	-	-	-	-	-	-
24:0	-	-	-	-	-	-	-	-
ΣSFA	46.1	45.0-46.4	47.0	46.2-48.4	48.0	47.9-48.3	47.9	47.7-48.3
16:1	0.18	0.14-0.25	0.23	0.12-0.25	0.35	0.27-0.41	0.32	0.29-0.50
18:1	3.37	3.22-3.60	3.40	3.21-3.69	5.59	4.92-5.78	5.62	4.71-6.48
20:1	0.13	0.11-0.16	0.16	0.13-0.17	0.15	0.10-0.19	-	-
22:1	-	-	-	-	-	-	-	-
24:1	-	-	-	-	-	-	-	-
Σmono	3.67	3.46-3.99	3.64	3.54-4.12	6.16	5.37-6.32	6.05	5.16-7.15
18:2n-6	11.6	10.7-12.4	12.0	11.3-13.5	8.06	7.29-8.72	7.08	6.43-7.88
18:3n-6	-	-	0.08	0.06-0.15	0.08	0.07-0.09	-	-
20:2n-6	0.32	0.29-0.39	0.39	0.35-0.44	0.27	0.19-0.35	0.19	0.16-0.21
20:3n-6	1.32	1.24-1.47	1.25	1.14-1.35	0.80	0.69-0.84	0.68	0.62-0.73
20:4n-6	20.1	18.8-21.1	16.8	16.0-17.7	23.4	22.8-25.2	22.1	19.3-24.2
22:4n-6	0.21	0.14-0.25	0.14	0.11-0.16	0.36	0.32-0.38	0.32	0.28-0.38
22:5n-6	0.07	0.04-0.13	0.05	0.04-0.07	0.30	0.18-0.40	0.29	0.19-0.36
Σn-6	33.7	32.9-34.1	31.1	30.0-32.6	33.4	33.0-34.2	31.3	27.7-32.5
18:3n-3	0.06	0.05-0.08	0.10	0.06-0.12	-	-	-	-
20:5n-3	0.50	0.33-0.69	0.61	0.58-0.81	-	-	-	-
22:5n-3	1.74	1.64-1.93	1.73	1.36-1.95	0.96	0.93-1.08	1.34	1.01-1.27
22:6n-3	13.1	12.3-14.3	13.6	13.2-14.1	10.2	9.82-10.4	12.8	10.4-13.9
Σn-3	15.2	14.8-16.7	15.8	15.3-16.8	11.2	10.9-11.3	14.2	11.5-15.1
AA/LA	1.74	1.54-1.96	1.45	1.25-1.50	2.90	2.61-3.49	2.91	2.65-3.76
AA/DHA	1.50	1.37-1.63	1.21	1.13-1.43	2.32	2.22-2.48	1.83	1.39-2.21
DPA/DTA	0.30	0.21-0.52	0.34	0.26-0.58	0.82	0.72-1.06	0.88	0.63-1.04
Σmetn-6/ Σmetn-3	1.43	1.28-1.54	1.18	1.10-1.33	2.25	2.16-2.44	1.76	1.37-2.15
Σn-6/ Σn-3	2.20	1.99-2.31	1.93	1.83-2.17	2.96	2.92-3.08	2.23	1.83-2.83

Table 5.8A. Fatty acids in liver choline phosphoglycerides of the suckling pups (15 days of life). Values are medians with interquartile range (IQR).

	Low-fat control (LFC; n=10)		Low-fat diabetic (LFD; n=9)		High-fat control (HFC; n=16)		High-fat diabetic (HFD; n=6)	
	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR
16:0	20.8	19.8-22.5	19.2	15.9-19.9	18.0	16.1-19.6	17.1	15.4-18.2
18:0	22.5	21.4-23.6	23.7	23.5-25.7	22.8	21.0-25.0	23.5	21.9-25.2
20:0	-	-	-	-	0.06	0.04-0.08	0.08	0.04-0.17
22:0	0.10	0.05-0.14	-	-	0.21	0.11-0.25	0.28	0.10-0.48
24:0	-	-	-	-	-	-	-	-
ΣSFA	43.7	42.8-44.6	43.1	41.8-44.1	42.1	40.0-42.3	41.2	37.7-43.1
16:1	-	-	-	-	0.25	0.14-0.36	-	-
18:1	2.74	2.66-2.84	2.64	2.56-2.97	4.69	2.98-6.08	5.02	2.78-7.50
20:1	0.08	0.08-0.17	0.10	0.08-0.12	0.10	0.07-0.14	0.12	0.11-0.16
22:1	0.06	0.03-0.09	0.08	0.04-0.09	0.07	0.03-0.12	-	-
24:1	-	-	-	-	0.07	0.04-0.10	0.10	0.05-0.15
Σmono	2.98	2.81-3.50	2.86	2.74-3.13	4.95	3.20-6.29	5.22	2.99-7.76
18:2n-6	1.72	1.46-2.04	2.13	1.97-2.55	3.03	1.10-6.06	3.31	1.26-5.11
18:3n-6	-	-	-	-	0.04	0.03-0.07	-	-
20:2n-6	0.16	0.08-0.28	0.15	0.13-0.24	0.09	0.07-0.14	-	-
20:3n-6	0.35	0.32-0.41	0.45	0.36-0.51	0.43	0.31-0.59	0.52	0.28-0.91
20:4n-6	16.8	16.4-17.4	18.6	16.8-19.3	22.3	19.8-25.0	20.3	18.6-24.3
22:4n-6	0.87	0.80-1.10	1.00	0.90-1.09	1.14	0.88-1.94	1.42	1.20-1.70
22:5n-6	0.23	0.18-0.28	0.34	0.26-0.47	1.58	1.32-2.26	1.09	0.99-1.65
Σn-6	20.6	19.5-21.2	22.5	20.9-24.2	30.9	26.0-32.5	26.2	23.3-34.0
18:3n-3	0.11	0.06-0.21	0.07	0.04-0.10	0.10	0.07-0.12	0.15	0.12-0.24
20:5n-3	0.39	0.31-0.57	0.46	0.37-0.57	0.41	0.23-0.63	0.36	0.16-0.61
22:5n-3	1.71	1.51-1.83	1.70	1.59-1.82	0.88	0.60-1.91	0.61	0.49-1.25
22:6n-3	25.6	25.3-26.9	23.4	21.8-25.6	15.8	12.8-20.3	17.3	11.7-24.0
Σn-3	28.0	27.3-29.0	25.6	24.2-27.6	17.4	14.3-22.6	18.8	12.7-25.6
AA/LA	9.49	8.44-11.5	8.18	7.10-8.91	8.60	3.98-18.0	8.06	4.23-13.8
AA/DHA	0.65	0.64-0.68	0.78	0.68-0.89	1.61	0.99-1.83	1.15	0.81-2.13
DPA/DTA	0.27	0.21-0.30	0.32	0.26-0.47	1.31	1.04-1.63	0.88	0.61-1.14
Σmetn-6/ Σmetn-3	0.67	0.65-0.71	0.80	0.69-0.89	1.64	1.12-1.88	1.22	0.89-2.33
Σn-6/ Σn-3	0.73	0.71-0.77	0.88	0.76-1.00	1.83	1.15-2.29	1.38	0.93-2.73

Table 5.9A. Fatty acids in liver ethanolamine phosphoglycerides of the newborn pups (1 day of life).Values are medians with interquartile range (IQR).

	Low-fat control (LFC; n=16)		Low-fat diabetic (LFD; n=8)		High-fat control (HFC; n=7)		High-fat diabetic (HFD; n=4)	
	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR
16:0	20.5	19.5-21.4	17.3	15.5-18.3	14.1	13.5-14.6	15.2	14.2-17.6
18:0	24.1	23.6-25.0	22.3	21.1-22.6	25.5	25.2-27.0	24.2	22.5-26.1
20:0	-	-	-	-	-	-	-	-
22:0	-	-	0.06	0.03-0.08	0.10	0.05-0.23	-	-
24:0	-	-	-	-	-	-	-	-
ΣSFA	45.1	43.8-45.8	39.1	37.7-40.2	40.5	39.2-40.8	40.5	38.9-41.5
16:1	-	-	-	-	0.05	0.05-0.07	0.13	0.08-0.16
18:1	1.68	1.55-1.85	3.29	3.18-3.65	4.32	3.59-4.35	4.28	3.73-4.51
20:1	0.07	0.07-0.12	0.08	0.07-0.09	0.08	0.05-0.12	0.09	0.07-0.12
22:1	-	-	-	-	-	-	-	-
24:1	0.03	0.02-0.06	-	-	-	-	-	-
Σmono	1.74	1.64-2.01	3.59	3.27-3.71	4.43	3.70-4.60	4.50	3.98-4.79
18:2n-6	3.05	2.59-3.81	11.8	11.1-12.6	10.5	9.70-11.1	9.94	9.16-10.4
18:3n-6	-	-	-	-	-	-	0.03	0.02-0.08
20:2n-6	0.14	0.10-0.15	0.59	0.46-0.67	0.40	0.34-0.59	0.37	0.34-0.43
20:3n-6	0.53	0.48-0.61	0.73	0.65-0.76	0.50	0.42-0.53	0.45	0.41-0.48
20:4n-6	18.3	17.7-19.3	15.9	14.9-16.4	20.7	20.2-21.6	20.0	17.1-21.0
22:4n-6	0.52	0.48-0.62	0.48	0.33-0.59	0.88	0.68-0.94	0.91	0.76-1.01
22:5n-6	0.11	0.10-0.19	0.09	0.07-0.11	0.53	0.40-0.67	0.48	0.33-0.68
Σn-6	23.6	22.3-24.5	29.5	28.5-30.7	33.5	32.2-34.0	32.3	29.4-32.8
18:3n-3	0.06	0.04-0.13	0.08	0.05-0.10	0.03	0.03-0.07	-	-
20:5n-3	0.74	0.59-1.15	1.01	0.88-1.38	0.09	0.06-0.13	0.13	0.10-0.24
22:5n-3	2.67	2.38-2.90	2.46	2.16-2.84	1.31	1.13-1.37	1.39	1.20-1.61
22:6n-3	23.7	22.6-24.5	21.5	20.7-22.1	17.8	17.4-18.4	18.9	17.0-20.5
Σn-3	27.0	26.3-28.0	24.5	24.1-26.1	19.2	18.9-19.9	20.6	18.6-21.9
AA/LA	5.92	4.87-7.04	1.32	1.26-1.43	2.08	1.81-2.22	1.97	1.68-2.30
AA/DHA	0.79	0.72-0.85	0.73	0.67-0.82	1.18	1.13-1.20	1.06	0.85-1.23
DPA/DTA	0.22	0.19-0.30	0.20	0.16-0.22	0.65	0.57-0.74	0.56	0.35-0.79
Σmetn-6/ Σmetn-3	0.74	0.68-0.78	0.72	0.64-0.76	1.21	1.17-1.25	1.08	0.89-1.26
Σn-6/ Σn-3	0.87	0.83-0.92	1.19	1.09-1.27	1.75	1.66-1.77	1.58	1.34-1.76

Table 5.10A. Percent fatty acids in liver ethanolamine phosphoglycerides suckling pups (15 days of life).Values are medians with interquartile range (IQR).

	Low-fat control (LFC; n=10)		Low-fat diabetic (LFD; n=9)		High-fat control (HFC; n=16)		High-fat diabetic (HFD; n=6)	
	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR
16:0	30.1	25.4-33.0	26.5	24.4-28.0	27.5	25.7-29.2	25.1	24.4-28.1
18:0	7.26	6.67-9.97	10.2	8.51-10.6	11.3	10.2-13.5	12.7	11.7-13.7
20:0	-	-	-	-	0.10	0.07-0.10	-	-
22:0	0.06	0.03-0.08	0.05	0.04-0.14	0.04	0.04-0.10	0.06	0.02-0.08
24:0	-	-	-	-	0.06	0.03-0.07	-	-
ΣSFA	39.3	32.6-42.9	36.6	35.2-38.0	39.6	37.1-43.4	38.7	37.8-43.3
16:1	2.10	1.70-2.39	1.19	0.87-1.40	1.64	1.27-2.50	1.52	1.22-1.83
18:1	14.1	13.1-15.1	11.9	10.5-12.4	20.0	16.5-24.7	20.4	18.3-27.2
20:1	0.16	0.15-0.23	0.19	0.11-0.22	0.29	0.16-0.38	0.27	0.16-0.44
22:1	-	-	-	-	-	-	-	-
24:1	-	-	-	-	0.04	0.03-0.08	-	-
Σmono	16.4	14.9-17.8	13.2	11.5-14.1	22.1	18.3-27.5	22.1	19.7-29.5
18:2n-6	13.0	12.0-13.7	14.4	13.1-16.3	9.80	8.47-12.6	10.7	9.52-12.9
18:3n-6	0.27	0.21-0.34	0.26	0.18-0.31	0.16	0.12-0.25	0.20	0.18-0.22
20:2n-6	0.35	0.21-0.41	0.40	0.36-0.47	0.17	0.14-0.21	0.18	0.12-0.21
20:3n-6	0.93	0.85-1.04	0.99	0.86-1.15	0.57	0.49-0.78	0.70	0.62-0.83
20:4n-6	13.2	11.2-14.3	15.3	13.8-16.6	12.1	7.53-19.7	10.9	9.53-15.0
22:4n-6	1.79	1.46-1.95	1.58	1.18-1.87	1.34	0.49-2.14	0.82	0.62-1.40
22:5n-6	0.26	0.20-0.32	0.36	0.23-0.65	1.31	1.17-1.42	1.01	0.53-1.48
Σn-6	29.7	26.9-32.5	33.5	32.1-35.1	26.8	21.9-32.7	25.4	24.2-28.6
18:3n-3	0.25	0.17-0.38	0.33	0.25-0.53	0.09	0.05-0.15	0.06	0.05-0.14
20:5n-3	1.59	1.30-2.29	1.53	1.41-1.96	0.29	0.19-0.35	0.33	0.23-0.45
22:5n-3	2.04	1.87-2.33	2.33	2.05-2.65	1.12	0.44-1.74	0.42	0.31-0.72
22:6n-3	8.37	7.77-10.2	10.4	9.23-11.2	5.85	4.83-6.80	6.49	4.56-9.60
Σn-3	12.4	11.3-14.7	14.8	12.7-16.3	7.11	5.24-9.21	7.69	5.27-10.6
AA/LA	0.96	0.87-1.16	1.09	0.86-1.28	1.29	0.64-2.28	0.92	0.82-1.60
AA/DHA	1.47	1.28-1.73	1.34	1.27-1.79	1.98	1.58-2.81	1.98	1.06-2.78
DPA/DTA	0.15	0.13-0.17	0.24	0.17-0.33	1.12	0.49-2.77	1.22	0.36-2.38
Σmetn-6/ Σmetn-3	1.31	1.17-1.49	1.26	1.09-1.56	2.33	1.92-2.69	2.45	1.24-2.66
Σn-6/ Σn-3	2.30	2.15-2.47	2.29	1.99-2.54	3.84	3.42-2.27	3.90	2.47-4.58

Table 5.11A. Fatty acids in liver free fatty acids of the newborn pups (1 day of life). Values are medians with interquartile range (IQR).

	Low-fat control (LFC; n=16)		Low-fat diabetic (LFD; n=8)		High-fat control (HFC; n=7)		High-fat diabetic (HFD; n=4)	
	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR
16:0	27.0	25.6-28.0	24.6	21.0-25.5	26.7	26.1-27.3	26.3	24.9-27.2
18:0	8.52	8.07-9.13	9.72	7.51-10.8	11.4	9.93-12.7	10.3	9.78-13.9
20:0	0.05	0.03-0.07	-	-	0.07	0.06-0.10	-	-
22:0	-	-	-	-	-	-	-	-
24:0	0.06	0.04-0.07	-	-	0.05	0.03-0.07	-	-
ΣSFA	38.2	36.7-39.2	35.5	34.1-37.7	38.5	35.9-39.6	36.6	35.8-41.4
16:1	0.61	0.57-0.65	0.47	0.45-0.56	0.84	0.79-0.87	0.73	0.69-0.81
18:1	10.9	10.3-12.1	9.12	8.58-10.0	22.7	22.5-24.7	20.8	20.3-23.6
20:1	0.20	0.16-0.22	0.16	0.12-0.16	0.36	0.34-0.46	0.35	0.31-0.41
22:1	-	-	-	-	-	-	-	-
24:1	-	-	-	-	-	-	-	-
Σmono	11.6	11.1-13.0	9.75	9.19-10.7	24.0	23.9-25.9	21.9	21.4-24.8
18:2n-6	22.3	21.1-23.0	24.7	21.6-25.8	17.4	17.0-18.6	16.8	14.4-18.1
18:3n-6	0.18	0.17-0.22	0.24	0.19-0.29	0.22	0.20-0.31	0.21	0.13-0.24
20:2n-6	0.48	0.44-0.56	0.38	0.37-0.43	0.40	0.26-0.48	0.34	0.32-0.41
20:3n-6	0.96	0.90-1.00	1.03	0.94-1.09	0.56	0.41-0.67	0.65	0.42-0.88
20:4n-6	8.88	7.96-9.86	9.77	8.92-12.2	8.37	8.26-9.27	10.3	8.65-11.5
22:4n-6	0.83	0.70-1.00	0.58	0.43-0.74	1.11	0.82-1.56	1.20	1.13-1.38
22:5n-6	0.17	0.13-0.23	0.17	0.12-0.20	0.49	0.38-0.69	0.50	0.31-0.70
Σn-6	34.0	33.0-35.5	36.2	34.5-39.6	27.9	27.7-32.6	29.9	27.3-31.5
18:3n-3	0.99	0.89-1.10	1.08	0.99-1.26	0.42	0.38-0.56	0.35	0.27-0.40
20:5n-3	1.77	1.24-2.29	2.49	2.08-2.68	0.33	0.22-0.44	0.38	0.22-0.77
22:5n-3	3.25	2.55-3.61	3.63	3.07-4.34	1.05	0.70-1.31	1.50	0.85-1.99
22:6n-3	7.94	7.19-8.87	9.06	8.05-9.66	2.67	2.52-3.24	5.29	3.37-8.09
Σn-3	13.7	13.0-15.3	16.2	15.2-17.5	4.50	3.96-5.85	8.05	4.75-10.7
AA/LA	0.39	0.36-0.44	0.41	0.37-0.49	0.48	0.44-0.60	0.57	0.53-0.78
AA/DHA	1.16	0.94-1.23	1.07	0.93-1.35	3.14	2.77-3.33	2.08	1.12-3.42
DPA/DTA	0.21	0.13-0.29	0.31	0.20-0.37	0.48	0.36-0.56	0.43	0.24-0.57
Σmetn-6/ Σmetn-3	0.89	0.74-1.06	0.81	0.71-0.97	2.77	2.37-2.97	1.87	1.14-3.25
Σn-6/ Σn-3	2.43	2.19-2.75	2.27	2.14-2.49	6.52	5.60-7.06	4.16	2.63-6.24

Table 5.12A. Percent fatty acids in liver free fatty acids of the suckling pups (15 days of life). Values are medians with interquartile range (IQR).

	Low-fat control (LFC; n=10)		Low-fat diabetic (LFD; n=9)		High-fat control (HFC; n=16)		High-fat diabetic (HFD; n=6)	
	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR
16:0	33.9	31.9-35.3	25.9	23.9-26.6	24.6	21.9-27.8	22.8	21.6-30.3
18:0	2.63	2.51-3.21	3.27	3.05-3.65	5.80	4.72-7.46	7.72	5.43-8.32
20:0	-	-	-	-	0.07	0.05-0.10	0.07	0.05-0.09
22:0	-	-	-	-	0.11	0.03-0.17	0.05	0.02-0.10
24:0	-	-	-	-	0.04	0.02-0.07	-	-
ΣSFA	37.6	35.9-39.3	30.4	28.2-31.1	31.8	30.4-34.5	32.4	30.4-36.5
16:1	2.42	2.04-2.59	1.55	1.11-1.85	1.62	1.39-2.89	1.78	1.53-2.35
18:1	18.9	17.2-20.1	15.8	14.8-17.6	25.8	24.2-42.7	34.2	26.1-37.0
20:1	0.28	0.17-0.30	0.22	0.19-0.25	0.40	0.20-0.48	0.33	0.21-0.55
22:1	-	-	0.07	0.04-0.09	0.05	0.04-0.09	-	-
24:1	-	-	-	-	-	-	-	-
Σmono	21.4	19.9-22.6	17.7	16.3-19.7	27.8	26.3-45.2	36.4	28.0-40.0
18:2n-6	13.6	13.2-13.9	17.3	16.0-21.3	11.0	9.49-12.4	14.6	11.0-16.6
18:3n-6	0.35	0.27-0.43	0.48	0.44-0.54	0.31	0.20-0.46	0.34	0.26-0.44
20:2n-6	0.40	0.31-0.44	0.50	0.34-0.61	0.30	0.17-0.46	-	-
20:3n-6	1.10	1.06-1.13	1.27	1.10-1.59	0.67	0.28-1.10	0.61	0.36-0.97
20:4n-6	5.89	5.05-6.91	7.25	5.99-7.76	10.4	1.71-12.03	3.90	2.51-7.41
22:4n-6	2.31	2.08-2.67	2.85	1.92-3.14	3.41	0.40-4.36	1.12	0.55-2.48
22:5n-6	0.44	0.33-0.55	0.54	0.46-0.79	1.90	1.41-2.34	1.07	0.81-1.65
Σn-6	24.4	22.8-25.4	31.6	28.9-33.3	28.6	17.8-30.3	22.2	21.1-24.0
18:3n-3	0.34	0.26-0.36	0.50	0.47-0.70	0.16	0.13-0.21	0.22	0.10-0.27
20:5n-3	1.54	1.07-1.67	1.68	1.43-1.93	0.29	0.11-0.51	0.19	0.05-0.30
22:5n-3	3.09	2.96-3.20	3.84	3.44-4.16	1.66	0.56-2.43	0.59	0.27-1.18
22:6n-3	11.0	9.87-11.4	12.6	11.8-15.0	4.36	1.97-5.34	4.88	3.15-6.91
Σn-3	15.5	14.5-16.4	18.6	16.8-21.7	6.61	2.44-7.84	5.85	3.95-8.83
AA/LA	0.43	0.38-0.49	0.42	0.29-0.48	1.01	0.14-1.22	0.28	0.15-0.72
AA/DHA	0.56	0.50-0.61	0.56	0.43-0.63	1.98	1.42-3.07	1.32	0.39-1.65
DPA/DTA	0.19	0.16-0.21	0.25	0.19-0.29	0.59	0.45-0.97	1.44	0.41-2.22
Σmetn-6/ Σmetn-3	0.71	0.61-0.78	0.70	0.55-0.79	2.99	2.23-3.08	1.84	0.87-2.18
Σn-6/ Σn-3	1.62	1.44-1.64	1.61	1.48-1.67	4.38	3.71-5.36	3.83	3.00-5.61

Table 5.13A. Fatty acids in liver triacylglycerols of the newborn pups (1 day of life). Values are medians with interquartile range (IQR).

	Low-fat control (LFC; n=16)		Low-fat diabetic (LFD; n=8)		High-fat control (HFC; n=7)		High-fat diabetic (HFD; n=4)	
	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR
16:0	25.2	23.7-26.8	23.3	21.6-25.9	22.6	21.7-24.1	23.6	21.7-25.7
18:0	4.81	4.30-5.49	6.23	5.59-7.21	6.56	6.08-7.39	7.01	6.27-7.40
20:0	0.08	0.06-0.10	0.09	0.07-0.13	0.05	0.04-0.07	0.07	0.06-0.08
22:0	0.02	0.02-0.06	0.05	0.02-0.06	-	-	-	-
24:0	0.02	0.02-0.04	-	-	-	-	-	-
ΣSFA	33.0	31.3-35.8	33.0	29.7-36.8	30.4	29.3-30.6	30.6	29.3-33.5
16:1	0.63	0.60-0.79	0.71	0.66-0.90	0.85	0.79-0.91	0.76	0.69-0.79
18:1	16.3	14.6-17.2	15.5	13.9-16.4	33.1	29.5-34.0	29.0	26.1-33.3
20:1	0.30	0.26-0.40	0.28	0.22-0.37	0.42	0.37-0.57	0.49	0.42-0.58
22:1	0.03	0.02-0.10	-	-	-	-	-	-
24:1	0.02	0.02-0.04	-	-	-	-	-	-
Σmono	17.4	15.6-18.1	16.7	14.8-17.7	34.3	30.9-35.3	37.0	27.3-34.7
18:2n-6	26.5	25.8-27.4	27.0	23.8-29.6	21.4	20.9-21.6	19.2	16.0-21.0
18:3n-6	0.25	0.21-0.27	0.27	0.18-0.34	0.32	0.27-0.47	0.35	0.18-0.38
20:2n-6	0.58	0.52-0.69	0.52	0.45-0.59	0.45	0.37-0.47	0.42	0.33-0.56
20:3n-6	0.92	0.81-0.96	0.85	0.65-0.97	0.69	0.67-0.77	1.05	0.60-0.94
20:4n-6	3.45	2.97-4.13	2.98	2.53-3.24	4.21	3.89-5.73	5.24	4.42-5.42
22:4n-6	1.20	1.00-1.34	0.84	0.68-0.95	1.39	1.21-1.95	1.74	1.44-1.98
22:5n-6	0.37	0.23-0.48	0.41	0.21-0.46	0.74	0.46-0.81	1.10	0.64-1.83
Σn-6	34.1	32.5-34.8	32.6	28.8-35.0	29.6	28.2-30.8	28.5	25.7-30.3
18:3n-3	1.10	1.01-1.13	1.01	0.81-1.22	0.51	0.43-0.53	0.42	0.29-0.49
20:5n-3	1.18	0.94-1.46	1.16	0.95-1.87	0.22	0.18-0.33	0.23	0.19-0.64
22:5n-3	3.92	3.26-4.26	3.43	2.22-4.32	1.34	1.24-1.49	1.98	1.27-2.57
22:6n-3	7.72	6.73-8.28	8.16	6.05-9.46	2.48	2.02-3.06	5.18	2.35-7.92
Σn-3	15.0	12.0-15.2	14.4	10.6-15.4	4.43	3.66-5.60	8.43	4.34-10.8
AA/LA	0.13	0.12-0.15	0.11	0.10-0.13	0.21	0.17-0.27	0.28	0.21-0.34
AA/DHA	0.46	0.41-0.50	0.40	0.33-0.50	1.85	1.70-1.91	1.10	0.67-2.05
DPA/DTA	0.33	0.19-0.39	0.50	0.28-0.54	0.40	0.32-0.56	0.62	0.44-0.92
Σmetn-6/ Σmetn-3	0.53	0.44-0.64	0.49	0.41-0.57	2.06	1.92-2.18	1.41	0.90-2.29
Σn-6/ Σn-3	2.43	2.16-2.66	2.45	1.84-3.24	6.33	5.69-8.01	3.72	2.39-7.18

Table 5.14A. Fatty acids in liver triacylglycerols of the suckling pups (15 days of life). Values are medians with interquartile range (IQR).

	Low-fat control (LFC; n=10)		Low-fat diabetic (LFD; n=9)		High-fat control (HFC; n=16)		High-fat diabetic (HFD; n=6)	
	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR
16:0	25.1	24.2-26.4	24.1	21.6-25.1	21.9	21.2-24.0	22.4	21.8-25.1
18:0	16.8	16.0-17.4	17.4	16.8-18.5	17.1	16.0-17.6	17.8	16.5-18.6
20:0	0.09	0.08-0.09	0.12	0.10-0.14	0.11	0.11-0.12	0.13	0.13-0.15
22:0	0.05	0.03-0.07	0.10	0.07-0.12	0.10	0.07-0.14	0.11	0.08-0.12
24:0	0.04	0.03-0.05	0.04	0.03-0.04	0.04	0.02-0.04	-	-
ΣSFA	41.9	41.5-42.6	41.6	40.3-42.3	38.8	37.5-41.5	41.2	39.8-42.2
16:1	0.83	0.80-0.99	0.68	0.56-0.80	0.95	0.73-1.11	0.82	0.62-0.91
18:1	15.1	14.6-18.3	14.1	13.8-15.4	20.0	18.6-21.4	18.3	17.0-18.9
20:1	0.24	0.23-0.30	0.22	0.19-0.25	0.31	0.25-0.44	0.33	0.26-0.41
22:1	0.05	0.03-0.09	0.10	0.07-0.13	0.10	0.04-0.12	-	-
24:1	0.05	0.04-0.08	0.06	0.04-0.08	0.07	0.05-0.11	0.05	0.03-0.09
Σmono	16.2	15.7-19.6	15.2	14.8-16.7	21.4	19.8-22.7	19.5	18.1-20.2
18:2n-6	4.94	4.36-5.98	5.80	5.13-6.78	6.63	3.85-10.8	5.00	4.30-7.46
18:3n-6	0.11	0.11-0.12	0.13	0.12-0.14	0.08	0.06-0.09	0.08	0.05-0.09
20:2n-6	0.61	0.53-0.65	0.83	0.67-0.86	0.55	0.51-0.60	0.61	0.54-0.67
20:3n-6	0.96	0.88-1.15	1.11	1.03-1.23	0.97	0.83-1.12	0.85	0.56-1.08
20:4n-6	23.8	20.0-24.7	22.6	21.4-24.4	20.4	17.7-23.1	22.6	19.5-24.3
22:4n-6	1.43	1.21-1.53	1.48	1.29-1.68	1.77	1.20-1.92	1.64	1.33-1.80
22:5n-6	0.29	0.26-0.38	0.48	0.43-0.51	1.17	0.95-1.48	0.85	0.76-0.94
Σn-6	32.2	29.4-32.9	32.3	31.4-34.9	32.5	30.6-33.5	32.6	30.1-33.1
18:3n-3	0.07	0.04-0.08	0.07	0.06-0.08	0.04	0.02-0.07	0.03	0.01-0.08
20:5n-3	0.32	0.29-0.36	0.32	0.29-0.35	0.12	0.10-0.15	0.12	0.11-0.14
22:5n-3	1.49	1.21-1.64	1.63	1.34-1.70	0.64	0.52-0.80	0.52	0.45-0.68
22:6n-3	4.96	3.98-5.33	4.98	4.53-5.28	2.80	2.28-3.18	4.15	3.88-4.61
Σn-3	6.68	5.75-7.30	6.88	6.36-7.44	3.71	3.11-4.07	5.02	4.60-5.29
AA/LA	4.87	3.37-5.59	3.81	3.37-4.68	3.22	1.56-5.50	4.33	2.82-5.70
AA/DHA	4.70	4.55-5.23	4.70	4.45-5.12	6.42	5.57-9.68	5.37	4.47-6.22
DPA/DTA	0.26	0.19-0.28	0.31	0.28-0.40	0.81	0.50-1.12	0.55	0.46-0.67
Σmetn-6/ Σmetn-3	3.91	3.80-4.36	3.99	3.74-4.34	6.63	5.96-8.36	5.23	4.62-6.22
Σn-6/ Σn-3	4.55	4.48-5.31	4.80	4.53-5.25	9.03	8.19-9.78	6.31	5.81-7.18

Table 5.15A. Fatty acids in heart choline phosphoglycerides of the newborn pups (1 day of life). Values are medians with interquartile range (IQR).

	Low-fat control (LFC; n=16)		Low-fat diabetic (LFD; n=8)		High-fat control (HFC; n=7)		High-fat diabetic (HFD; n=4)	
	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR
16:0	22.0	20.4-23.5	20.3	19.8-21.7	18.0	16.9-19.6	19.1	18.2-20.6
18:0	22.1	21.3-23.2	23.2	22.7-24.4	27.3	26.0-28.1	26.4	25.2-27.3
20:0	0.07	0.06-0.07	0.09	0.08-0.09	0.09	0.08-0.10	0.08	0.08-0.10
22:0	0.04	0.03-0.07	0.05	0.04-0.08	0.04	0.03-0.08	0.03	0.02-0.07
24:0	0.03	0.02-0.05	0.03	0.02-0.04	-	-	0.03	0.02-0.04
ΣSFA	45.0	43.8-45.3	44.1	43.7-44.5	45.1	44.9-46.4	45.6	44.9-46.7
16:1	0.18	0.14-0.21	0.17	0.12-0.21	0.23	0.20-0.25	0.24	0.18-0.28
18:1	6.07	5.86-6.50	6.03	5.53-6.44	7.85	6.62-8.03	7.27	4.60-8.52
20:1	0.09	0.08-0.14	0.08	0.07-0.10	0.15	0.13-0.19	0.14	0.12-0.16
22:1	-	-	-	-	-	-	-	-
24:1	0.03	0.02-0.04	0.06	0.02-0.08	0.04	0.02-0.07	0.03	0.02-0.07
Σmono	6.48	6.23-6.87	6.30	5.76-6.77	8.30	7.03-8.50	7.64	5.02-9.08
18:2n-6	5.49	5.30-5.91	5.53	5.24-6.89	3.38	3.26-3.71	3.70	2.84-4.20
18:3n-6	0.16	0.15-0.17	0.19	0.16-0.21	0.08	0.07-0.10	0.08	0.05-0.08
20:2n-6	0.47	0.42-0.51	0.47	0.46-0.49	0.38	0.37-0.43	0.33	0.30-0.38
20:3n-6	0.78	0.75-0.82	0.88	0.82-0.92	0.61	0.60-0.72	0.59	0.55-0.70
20:4n-6	23.1	21.5-24.1	22.6	21.7-23.6	27.6	27.3-29.1	28.3	26.4-29.4
22:4n-6	0.89	0.76-1.28	0.64	0.50-0.74	1.42	1.34-1.68	1.38	1.10-1.51
22:5n-6	0.20	0.17-0.28	0.14	0.10-0.18	0.70	0.68-0.74	0.59	0.46-0.65
Σn-6	30.7	29.4-32.8	31.1	29.9-31.5	34.6	34.2-35.9	34.6	33.0-36.1
18:3n-3	0.05	0.03-0.06	0.05	0.04-0.06	0.02	0.02-0.04	-	-
20:5n-3	0.34	0.24-0.40	0.42	0.37-0.58	0.08	0.06-0.08	-	-
22:5n-3	4.04	3.68-4.41	4.18	3.54-4.67	2.70	2.58-2.97	2.89	1.91-3.11
22:6n-3	10.8	9.79-11.5	10.6	10.5-11.5	6.47	5.46-7.03	7.00	5.84-8.45
Σn-3	15.1	14.3-16.1	15.3	14.9-16.2	9.28	8.17-10.1	10.1	8.48-11.5
AA/LA	4.17	3.68-4.38	4.24	3.19-4.42	8.02	7.43-8.68	7.89	6.31-10.2
AA/DHA	2.06	1.94-2.53	2.12	2.06-2.16	4.57	4.03-5.26	4.14	3.44-4.56
DPA/DTA	0.23	0.20-0.24	0.21	0.17-0.27	0.47	0.39-0.52	0.43	0.37-0.49
Σmetn-6/ Σmetn-3	1.65	1.53-1.85	1.62	1.59-1.72	3.40	3.16-3.94	3.16	2.80-3.45
Σn-6/ Σn-3	2.05	1.86-2.19	2.03	1.91-2.12	3.73	3.48-4.43	3.52	3.05-3.94

Table 5.16A. Percent fatty acids in heart choline phosphoglycerides of the suckling pups (15 days of life). Values are medians with interquartile range (IQR).

	Low-fat control (LFC; n=10)		Low-fat diabetic (LFD; n=9)		High-fat control (HFC; n=16)		High-fat diabetic (HFD; n=6)	
	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR
16:0	6.97	6.73-7.63	7.03	5.93-7.61	6.13	5.70-6.59	6.74	6.44-7.01
18:0	17.7	17.2-18.2	18.4	17.7-19.1	18.5	17.9-19.2	19.4	19.1-19.8
20:0	0.13	0.12-0.15	0.12	0.10-0.17	0.13	0.11-0.18	0.21	0.19-0.23
22:0	0.10	0.08-0.13	0.04	0.02-0.12	0.12	0.08-0.16	0.16	0.15-0.19
24:0	0.03	0.02-0.04	0.05	0.02-0.06	0.03	0.02-0.05	-	-
ΣSFA	25.1	24.3-26.1	25.4	23.2-26.6	25.0	24.2-26.0	26.4	26.2-27.0
16:1	0.40	0.31-0.52	0.34	0.27-0.40	0.34	0.30-0.43	0.22	0.20-0.25
18:1	5.92	5.51-6.92	6.15	5.11-7.07	7.25	6.68-8.76	6.89	6.34-7.45
20:1	0.08	0.05-0.09	0.06	0.06-0.08	0.12	0.09-0.14	0.09	0.07-0.12
22:1	0.03	0.02-0.07	0.04	0.02-0.13	0.06	0.03-0.11	-	-
24:1	0.05	0.04-0.06	0.07	0.04-0.09	0.07	0.06-0.10	0.08	0.05-0.09
Σmono	6.46	5.96-7.59	6.67	5.52-7.68	7.80	7.30-9.43	7.31	6.86-7.79
18:2n-6	6.68	4.56-8.71	9.40	6.11-9.83	6.90	5.44-9.90	5.23	4.89-5.65
18:3n-6	0.07	0.06-0.07	0.09	0.07-0.11	0.05	0.03-0.06	0.05	0.04-0.06
20:2n-6	0.31	0.26-0.41	0.52	0.41-0.69	0.30	0.26-0.35	0.32	0.30-0.36
20:3n-6	1.24	1.04-1.37	1.37	1.26-1.48	1.13	0.94-1.27	0.90	0.80-1.02
20:4n-6	27.3	25.4-28.2	26.3	25.4-28.1	29.3	28.0-31.2	29.5	28.4-30.1
22:4n-6	3.81	3.39-3.89	3.82	3.44-4.10	4.86	3.83-5.72	4.99	4.42-5.27
22:5n-6	0.72	0.68-0.86	0.93	0.77-1.13	2.60	2.13-2.76	2.14	1.61-2.29
Σn-6	39.4	39.1-40.2	41.1	40.5-43.5	46.0	45.5-47.2	42.7	42.0-43.5
18:3n-3	0.07	0.04-0.07	0.08	0.06-0.11	0.05	0.04-0.06	-	-
20:5n-3	0.51	0.46-0.65	0.50	0.47-0.56	0.16	0.11-0.21	0.14	0.13-0.16
22:5n-3	3.07	2.68-3.40	3.00	2.57-3.30	1.54	1.22-1.92	1.21	1.09-1.41
22:6n-3	11.0	9.82-12.3	9.93	9.35-10.1	5.09	4.47-5.57	8.00	7.16-8.96
Σn-3	14.3	13.6-16.3	13.3	12.6-14.0	6.58	6.38-7.76	9.37	8.85-10.2
AA/LA	4.04	2.96-6.21	2.92	2.55-4.60	4.21	2.94-5.47	5.56	5.16-6.11
AA/DHA	2.52	2.29-2.65	2.64	2.54-2.91	5.88	5.09-6.74	3.75	3.25-4.09
DPA/DTA	0.20	0.18-0.22	0.24	0.21-0.29	0.58	0.42-0.67	0.43	0.34-0.49
Σmetn-6/ Σmetn-3	2.32	2.08-2.47	2.44	2.34-2.65	5.73	5.13-6.47	4.10	3.59-4.24
Σn-6/ Σn-3	2.78	2.36-3.04	3.05	2.89-3.49	6.98	5.94-7.35	4.62	4.12-4.80

Table 5.17A. Fatty acids in heart ethanolamine phosphoglycerides of the newborn pups (1 day of life). Values are medians with interquartile range (IQR).

	Low-fat control (LFC; n=16)		Low-fat diabetic (LFD; n=8)		High-fat control (HFC; n=7)		High-fat diabetic (HFD; n=4)	
	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR	Median	25-75% IQR
16:0	8.46	8.12-8.77	8.04	7.53-8.97	6.99	6.97-7.05	7.08	6.37-8.28
18:0	18.1	17.3-18.9	18.4	17.8-18.6	19.1	18.7-20.1	19.6	18.8-20.8
20:0	0.12	0.09-0.13	0.14	0.12-0.15	0.15	0.12-0.16	0.16	0.13-0.17
22:0	0.11	0.10-0.13	0.12	0.09-0.14	0.11	0.10-0.14	-	-
24:0	0.03	0.02-0.05	0.03	0.03-0.05	0.02	0.02-0.04	-	-
∑SFA	27.1	25.6-27.8	26.4	26.1-27.3	26.4	26.0-27.4	27.2	25.4-29.0
16:1	0.10	0.09-0.11	0.08	0.06-0.12	0.12	0.11-0.13	0.13	0.09-0.14
18:1	4.00	3.62-4.48	4.32	3.85-4.43	5.29	4.23-5.43	4.66	4.10-5.89
20:1	0.08	0.06-0.10	0.06	0.04-0.10	0.10	0.07-0.11	0.14	0.10-0.19
22:1	0.04	0.02-0.09	-	-	-	-	-	-
24:1	0.04	0.03-0.06	0.02	0.02-0.03	-	-	-	-
∑mono	4.23	3.83-4.74	4.50	4.02-4.63	5.53	4.42-5.68	5.12	4.38-6.16
18:2n-6	8.29	6.94-9.35	8.52	8.00-8.79	6.21	5.22-6.59	5.13	4.53-5.21
18:3n-6	0.13	0.12-0.14	0.16	0.14-0.17	0.05	0.05-0.06	0.07	0.03-0.08
20:2n-6	0.52	0.48-0.61	0.56	0.54-0.62	0.44	0.40-0.47	0.32	0.23-0.51
20:3n-6	1.46	1.35-1.61	1.52	1.39-1.59	1.24	1.22-1.29	1.02	0.79-1.24
20:4n-6	17.1	16.7-17.9	16.5	16.2-17.8	22.8	22.4-23.1	21.8	21.7-22.4
22:4n-6	1.66	1.53-2.20	1.21	1.05-1.37	3.00	2.92-3.50	2.54	2.25-2.75
22:5n-6	0.55	0.51-0.70	0.41	0.39-0.46	2.38	2.33-2.67	1.69	1.52-1.82
∑n-6	29.0	28.2-33.0	29.5	28.2-30.6	35.9	35.4-36.7	33.0	31.2-33.4
18:3n-3	0.05	0.04-0.06	0.07	0.06-0.09	0.04	0.03-0.05	-	-
20:5n-3	0.34	0.26-0.43	0.48	0.37-0.67	0.10	0.09-0.11	0.09	0.06-0.10
22:5n-3	4.90	4.61-5.22	5.01	4.40-5.39	3.94	3.83-4.31	3.28	2.45-3.95
22:6n-3	22.9	21.2-24.3	22.1	21.7-24.0	15.3	15.1-16.4	16.8	14.7-19.2
∑n-3	28.6	25.7-29.8	27.7	26.9-29.7	19.3	19.2-20.8	20.4	17.2-23.0
AA/LA	2.16	1.87-2.45	2.00	1.87-2.14	3.72	3.52-4.35	4.30	4.23-4.83
AA/DHA	0.76	0.69-0.85	0.77	0.67-0.80	1.43	1.39-1.53	1.34	1.15-1.48
DPA/DTA	0.34	0.32-0.38	0.32	0.31-0.40	0.72	0.65-0.89	0.69	0.58-0.75
∑metn-6/ ∑metn-3	0.75	0.70-0.88	0.75	0.68-0.79	1.51	1.48-1.59	1.38	1.23-1.55
∑n-6/ ∑n-3	1.02	0.95-1.28	1.07	0.95-1.13	1.83	1.72-1.91	1.64	1.45-1.81

Table 5.18A. Fatty acids in heart ethanolamine phosphoglycerides of the suckling pups (15 days of life). Values are medians with interquartile range (IQR).