Stephanie De Vriese

Essential Fatty Acids and Pregnancy

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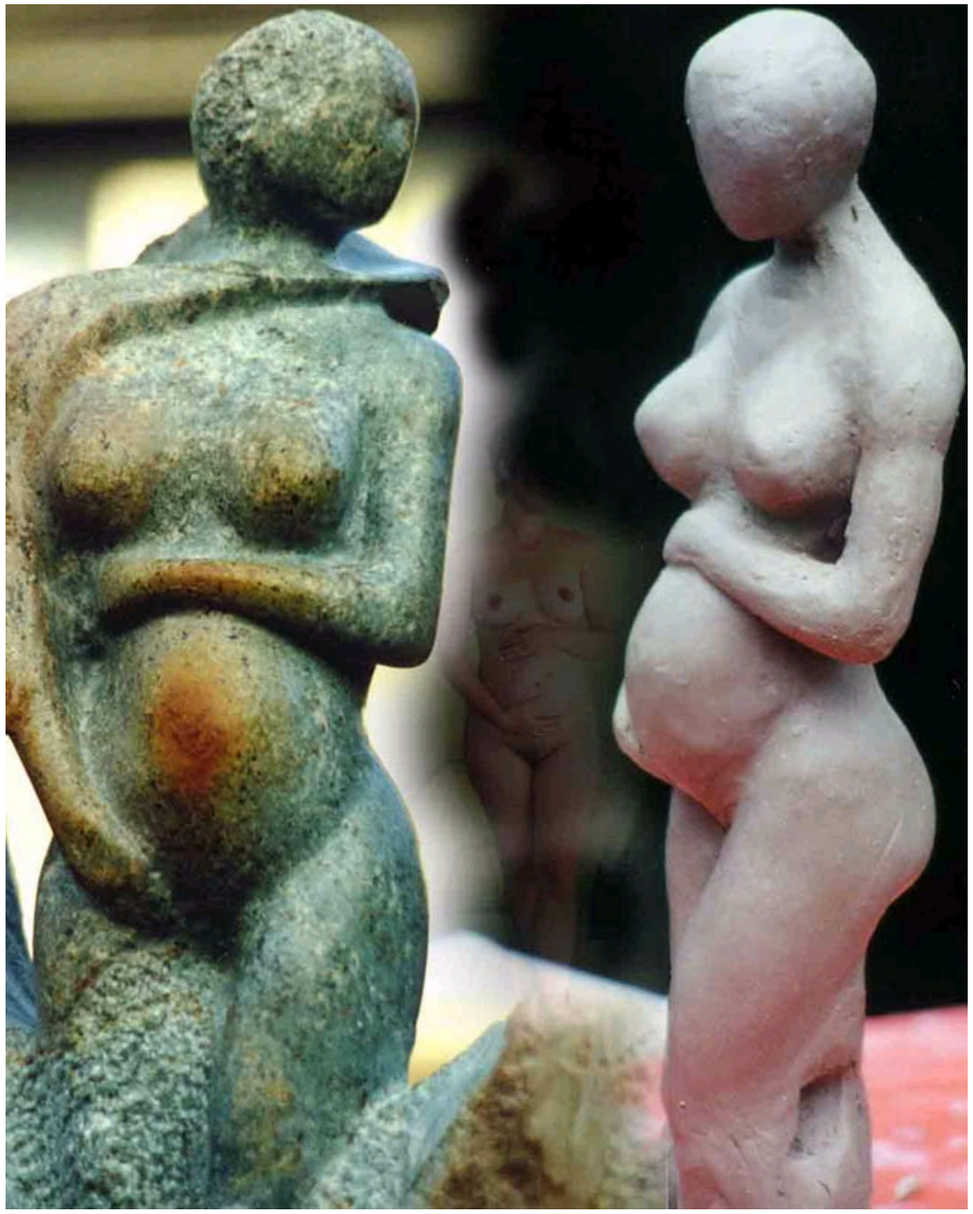

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Essential Fatty Acids and Pregnancy

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Thesis submitted in partial fulfillment of the requirements for the degree of "Doctor in de Medische Wetenschappen"

Promotor: Prof. Dr. A. Christophe

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Aan mijn ouders

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Stephanie De Vriese

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Summary

Essential fatty acids (EFA) are important components of membranes. During pregnancy, neonatal needs for EFA are high because a lot of new tissue is being formed **(chapter 1)**.

In the first experimental part we focused on the nutrient intake during pregnancy. In **chapter 2** we adapted and validated an existing food frequency questionnaire (FFQ), specifically designed to collect data on fat consumption. The relative validity of the FFQ was assessed by comparing the estimated fat intake with that obtained from the seven day estimated record. It was concluded that the FFQ is an appropriate method for classifying individuals to the right part of the distribution of dietary fat intake. In **chapter 3** we evaluated the maternal dietary intake of macronutrients and micronutrients of our study population by comparing them with the dietary recommendations for pregnant women. We advise pregnant women to increase the intake of milk and milk products to obtain an adequate supply of calcium, vitamin B2 and vitamin D. At the same time they should decrease intake of sauces, fats and oils, cookies and pastries in order to diminish their saturated fat intake. In general, the intake of some vitamins and minerals in this study population is below the recommendations. Some women could benefit from a multivitamin/mineral supplement. In **chapter 4** the dietary intake of fatty acids during pregnancy is described. The results are correlated with parameters of the EFA status. Very few women in this study population meet the adequate intake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) whereas the intake of linoleic acid is rather high compared to the current recommendations. Therefore it would be advisable to increase the intake of long-chain n-3 PUFA and decrease the intake of linoleic acid during pregnancy. Moreover the fraction of EPA and of DHA in maternal and umbilical plasma phospholipids (PL) is positively associated with the dietary intake of these fatty acids.

In the second experimental part the EFA status of mother and neonate was determined based on the fatty acid composition of plasma and umbilical cord vessel wall PL.

First the concept of the calculated mean melting point of fatty acids from plasma PL as surrogate parameter of membrane fluidity is explained. In the mother, the loss of long-chain polyunsaturated fatty acids (PUFA) at delivery compared to mid pregnancy results in a higher mean melting point. Concomitantly long-chain PUFA are preferentially replaced by shorter-chain saturated fatty acids (SFA). In umbilical plasma PL the high content of long-chain PUFA is accompanied with more longer-chain, less fluid SFA. Thus the fatty acid composition of the SFA changes in a way to counteract changes in the mean melting point induced by changed long-chain PUFA composition **(chapter 5)**.

Linoleic acid in cord plasma PL is half of that in maternal plasma PL but arachidonic acid (AA) in cord plasma is twice that observed in the mother. Similarly, the α-linolenic acid

concentration in newborns is half of that in the mother, whereas the DHA concentration is almost double. This situation, in which the relative plasma concentrations of the n-3 and n-6 long-chain PUFA exceed those of their precursors has only been observed in newborns and does not exist in adults. It is obviously an extremely favourable situation for the development of the newborn, especially at a time when large quantities of AA and DHA are required for the development of the brain and retina **(chapters 5 & 6)**.

During early puerperium (from 6 days before until 3 days after delivery) the fatty acid composition of maternal plasma PL and cholesteryl esters (CE) changes significantly. It is unlikely that the observed changes in maternal fatty acid composition in early puerperium are related to changes in the dietary intake of fatty acids. Another possible explanation for the observed differences is the changes in the maternal hormonal status during puerperium, but we were unable to confirm this **(chapter 7)**.

Because umbilical vessel walls do not have vasa vasorum which could deliver fatty acids they can only obtain their fatty acids from the blood passing through the cord vessels. Therefore the fatty acid composition of the umbilical venous vessel wall can be considered a longer-term reflection of the fatty acid supply from mother to foetus whereas the fatty acid composition of the umbilical arterial vessel wall is likely to reflect the longer-term EFA status of the developing foetus. The venous vessel walls have lower concentrations of linoleic and AA and significantly higher concentrations of Mead acid than the afferent blood vessel wall, indicating a marginal EFA status of the newborn **(chapter 8)**.

We found significant differences in the postpartum fatty acid status between women who developed a postpartum depression compared to control mothers. We observed a significant association between the ratio of Σn-6/Σn-3 in PL and the occurrence of postpartum depression. Women who became depressed after delivery had a significantly lower status of DHA and of Σn-3 PUFA in PL and CE compared to women who did not **(chapter 9)**.

The third trimester of pregnancy is associated with the predominance of small and dense LDL-particles which have been shown to be more susceptible to oxidation. We found an increase in the oxidative stability of LDL with progressing gestation. Both the rate of formation and the amount of conjugated dienes formed reached a maximum in the third trimester. This could be due to a change in the composition of LDL near term. Furthermore we observed significant increases in vitamin E levels during pregnancy **(chapter 10)**.

Finally we found in a group of healthy volunteers a seasonal affect in long-chain PUFA, such as AA, EPA and DHA **(chapter 11)**.

Samenvatting

Essentiële vetzuren (EFA) zijn belangrijke bouwstenen voor celmembranen. Tijdens de zwangerschap is de behoefte van de foetus aan EFA hoog omdat veel nieuw weefsel wordt gevormd. Een samenvatting van de literatuur omtrent EFA en hun rol tijdens de zwangerschap wordt gegeven in **hoofdstuk 1**.

In het eerste onderzoeksluik spitsen we ons toe op de voedingsinname tijdens de zwangerschap. In **hoofdstuk 2** valideren we een Nederlandse voedselfrequentievragenlijst (FFQ), om de vet- en vetzuurinname te bepalen, aangepast aan de Belgische situatie. De relatieve validiteit wordt bepaald door de geschatte vetinname uit de FFQ te vergelijken met deze bekomen uit het 7-dagen dagboek. We concluderen dat de FFQ een degelijke methode is om individuen te klasseren in het correcte gedeelte van de distributie van de vetinname. In **hoofdstuk 3** vergelijken we de dagelijkse inname van macro- en micronutriënten van onze studiepopulatie met de aanbevelingen. Wij adviseren zwangere vrouwen om de inname van melk en zuivelproducten te verhogen om zo een adequate calcium, vitamine B2 en D-inname te bekomen. Maar de consumptie van sauzen, vetten, oliën, snoep en gebak moet verminderd worden om de inname van verzadigd vet te verlagen. Doorgaans is de inname van bepaalde vitamines en mineralen in deze studiepopulatie lager dan de aanbevelingen. Sommige zwangere vrouwen zouden baat kunnen hebben van een multivitamine/mineraal supplement. In **hoofdstuk 4** wordt de inname van vetzuren tijdens de zwangerschap geëvalueerd en vergeleken met parameters van de EFA-status. Zeer weinig vrouwen hebben een adequate eicosapentaeenzuur (EPA) en docosahexaeenzuur (DHA) inname daar waar de inname van linolzuur redelijk hoog is in vergelijking met de aanbevelingen. Bovendien zijn de plasmaconcentraties van EPA en DHA zowel bij de moeder als in de navelstreng positief gecorreleerd met de voedingsinname ervan. Vandaar bevelen wij aan om tijdens de zwangerschap de inname van n-3 PUFA te verhogen en deze van linolzuur te verlagen.

In het tweede onderzoeksluik wordt de EFA-status van moeder en neonaat bepaald adhv de analyse van de vetzuursamenstelling van plasma en navelstrengweefsel. Met het voorschrijden van de zwangerschap is er een daling van lang-keten polyonverzadigde vetzuren (PUFA) in plasma. Hoewel mathematisch significant is de grootte ervan beperkt en kleiner dan beschreven in andere landen. Vermindering van lang-keten PUFA resulteert in een verhoging van de "Mean Melting Point" (MMP) wat een parameter is voor de membraanfluiditeit. De verzadigde vetzuren bleken in samenstelling te variëren op een manier dat de wijziging in MMP ten gevolge van de daling van lang-keten PUFA in de loop van de zwangerschap tegengewerkt wordt. Hetzelfde fenomeen werd bij de neonaat waargenomen (hier is er in vergelijking met de moeder bij bevalling een lagere MMP ten gevolge van verhoogde lang-keten PUFA en variëren de andere vetzuren zodat deze

verlaging ten dele gecompenseerd wordt) **(hoofdstuk 5)**. De linolzuurstatus in navelstrengplasma is slechts de helft van deze in het plasma van de moeder maar arachidonzuur (AA) in navelstrengplasma is dubbel zo hoog als in het plasma van de moeder (idem voor α-linoleenzuur en DHA). Deze situatie, waarbij de status van n-3 en n-6 lang-keten PUFA hoger is dan van de voorlopers, is uniek voor pasgeboren en bestaat niet bij volwassenen. Het is duidelijk een zeer voordelige situatie voor de ontwikkeling van de pasgeborene juist op het moment dat grote hoeveelheden AA en DHA nodig zijn voor de ontwikkeling van hersenen en neuronaal weefsel **(hoofdstukken 5 & 6)**. Tevens vinden wij gedurende het puerperium (6 dagen voor tot 3 dagen na de bevalling) dat de vetzuursamenstelling van plasma fosfolipiden en cholesterolesters (PL en CE) bij de moeder significant verandert: de fractie lang-keten PUFA neemt toe en de verzadigde vetzuren wijzigen in samenstelling. Deze verandering is niet te wijten aan een gewijzigd eetpatroon. Een andere mogelijk verklaring is wijziging in de hormonale status tijdens het puerperium **(hoofdstuk 7)**.

Een van de manieren om na te gaan of aan de foetale behoeften van EFA tijdens de zwangerschap voldaan wordt is te bepalen of vetzuren die enkel bij EFA-deficiëntie gevormd worden (Meadzuur) in het navelstrengweefsel aanwezig zijn **(hoofdstuk 8)**. De wand van de vene (afvoerend bloedvat) heeft een lagere concentratie linolzuur en AA en een hogere concentratie Meadzuur dan de wand van de arterie (aanvoerend bloedvat). Dit kan wijzen op een marginale EFA-status bij de pasgeborene.

Vrouwen met een postpartum depressie hebben op het ogenblik van de bevalling een lagere status van DHA en Σn-3 PUFA in plasma PL en CE in vergelijking met controle vrouwen **(hoofdstuk 9).** Er bestaat een significant verband tussen de verhouding van Σn-6/ Σn-3 in plasma PL en het voorkomen van postpartum depressie.

De weerstand van LDL tegen peroxidatie neemt toe met de duur van de zwangerschap. Ook de hoeveelheid oxideerbaar materiaal per LDL partikel neemt toe (hoewel de EFA afnemen). Een mogelijke verklaring hiervoor is dat de samenstelling van LDL tijdens de zwangerschap verandert. Dit werd nagegaan. Er bleek inderdaad met voortschrijdende zwangerschap een toename te zijn van cholesterol en triglyceriden en een daling van de cholesterol/triglyceriden verhouding per partikel. Deze bevinding suggereert dat de bekende stijging van LDL gedurende de zwangerschap niet geïnterpreteerd mag worden als een indicator voor een gestegen atherogeen risico. De toename van LDL-cholesterol tijdens de zwangerschap is immers te wijten aan de toename van grotere en minder dense LDL-partikels. Deze zijn minder atherogeen dan de kleinere meer dense LDL-partikels **(hoofdstuk 10)**.

Ten slotte vinden we in een groep gezonde vrijwilligers dat er een seizoensgebonden variatie bestaat in de concentratie van de lang-keten PUFA (AA, EPA en DHA) in plasma PL **(hoofdstuk 11)**.

Résumé

Les acides gras essentiels sont absolument nécessaires au bon fonctionnement de l'organisme et plus particulièrement au niveau des membranes. Pendant la grossesse, les acides gras essentiels jouent un rôle capital dans le développement du fœtus parce que de nombreux tissus doivent être formés **(chapitre 1).**

Dans la première partie de cette étude, nous nous sommes intéressés à la consommation alimentaire pendant la grossesse. Nous avons validé un questionnaire de fréquence alimentaire développé pour analyser la consommation des graisses **(chapitre 2).** Ce questionnaire a été validé en comparant les résultats de la consommation des graisses avec ceux obtenus avec un cahier alimentaire de 7 jours. Nous en concluons que ce questionnaire est un outil solide pour classifier les individus dans la partie adéquate de la courbe de distribution de la consommation des graisses. Puis, nous avons comparé la consommation des nutriments dans la population étudiée avec les apports journaliers recommandés pour la population belge **(chapitre 3).** Les résultats obtenus nous amènent à recommander aux femmes enceintes d'augmenter leur consommation de produits laitiers pour avoir des apports de calcium, vitamines B2 et D suffisants. Parallèlement, nous recommandons de diminuer la consommation des graisses animales, mayonnaise, friandises et pâtisseries pour réduire la consommation des acides gras saturés. Pour ce qui est du statut en vitamines et minéraux, souvent dans la population étudiée, les femmes enceintes n'atteignent pas les taux recommandés pour quelques-uns d'entre eux. Par conséquent, elles pourraient bénéficier d'une supplémentation multivitamines/minéraux. Ensuite, la consommation des acides gras pendant la grossesse a été évaluée et comparée avec des indicateurs du statut systémique des acides gras essentiels **(chapitre 4).** Très peu de femmes consomment suffisamment d'acide eicosapentaénoique (EPA) et d'acide docosahexaénoique (DHA) alors que, comparée aux recommandations, leur consommation en acide linoléique est plutôt élevée. Alors nous recommandons d'augmenter la consommation des acides gras polyinsaturés à longue chaîne n-3 et de réduire celle de l'acide linoléique 18:2n-6 pendant la grossesse.

Dans la seconde partie de cette étude, le statut en acides gras essentiels de la mère et du nouveau-né a été défini par l'analyse de la composition des acides gras du plasma et du tissu du cordon ombilical.

Chez la mère, avec l'avancement de la grossesse, la diminution du taux d'acides gras polyinsaturés à longue chaîne est reflétée par un "Mean Melting Point" (MMP, un marqueur de la fluidité membranaire) plus élevé. Parallèlement, les PUFA à longue chaîne sont préférentiellement remplacés par des acides gras saturés à chaîne plus courte. Dans le plasma ombilical, le taux élevé de PUFA à longue chaîne est associé à des acides gras saturés à plus longue chaîne, moins fluides. Ainsi les variations de la composition en acides gras saturés se font de manière à contrecarrer les changements de MMP induits par la

modification des d'acides gras polyinsaturés. Le même phénomène est observé chez le nouveau-né (en comparaison du plasma de la mère après l'accouchement, le nouveau-né a beaucoup plus des acides gras polyinsaturés à longue chaîne et un MMP plus bas mais la composition des acides gras saturés varie de sorte que cette diminution est compensée partiellement) **(chapitre 5).**

La concentration en acides linoléique et α-linolénique dans les phospholipides du plasma du cordon ombilical est la moitié de celle du plasma de la mère tandis que la concentration en acide arachidonique (AA) et DHA du plasma du cordon ombilical est le double de celle du plasma de la mère. Cette situation où la concentration des acides gras polyinsaturés à longue chaîne n-3 et n-6 est plus élevée que celle des précurseurs, est unique pour le nouveau-né et n'existe pas chez les adultes. Il est clair que cette condition est favorable à la croissance du fœtus, à un moment où il a besoin de beaucoup de AA et DHA pour le développement de son cerveau et de son rétine **(chapitres 5 & 6).** Nous avons par ailleurs observé que la composition des acides gras du plasma de la mère change de façon considérable pendant le puerperium (6 jours avant et jusqu'à 3 jours après l'accouchement): en effet la concentration des acides gras polyinsaturés à longue chaîne s'élève et la composition des acides gras saturés varie. Cette modification rapide après l'accouchement ne peut être due à une modification de l'alimentation. Une explication possible peut être un changement de la concentration des hormones pendant le puerperium **(chapitre 7).**

Parce que les vaisseaux sanguins du cordon ombilical sont dépourvus de vasa vasorum, ils ne peuvent obtenir leurs acides gras qu'à partir du sang qui y circule. Par conséquent la composition en acides gras des veines du cordon ombilical reflète l'adduction des acides gras de la mère au fœtus tandis que la composition des acides gras des artères du cordon ombilical reflète celui du fœtus vers la mère **(chapitre 8).** Les veines du cordon ombilical contiennent moins d'acide linoléique et de AA et beaucoup plus de 20:3n-9 en comparaison des artères du cordon ombilical. Cette observation peut indiquer que le nouveau-né a un état marginal en acides gras essentiels.

Des femmes avec une dépression post-partum ont, juste après l'accouchement, moins de DHA et des acides gras polyinsaturés à longue chaîne n-3 dans leurs phospholipides plasmatiques en comparaison des femmes sans dépression post-partum. Il y a une corrélation significative entre Σn-6/Σn-3 dans le plasma et la prévalence de la dépression post-partum **(chapitre 9).**

La résistance des LDL à la peroxidation augmente avec l'avancement de la grossesse. En plus la quantité de matériaux oxydables par particule de LDL augmente. Une explication possible est le changement de la composition de LDL pendant la grossesse. En effet, la quantité de cholestérol et des triglycérides augmente et la proportion de cholestérol aux triglycérides par unité de LDL diminue avec l'avancement de la grossesse. Il en est de même avec la concentration en vitamine E du plasma de la mère **(chapitre 10).**

Finalement nous avons observé dans une population des volontaires sains, qu'il existait une variation liée aux saisons de la concentration des acides gras polyinsaturés à longue chaîne (AA, EPA et DHA) des phospholipides du plasma **(chapitre 11).**

List of Abbreviations

αLnA	α-linolenic acid (18:3n-3)
7 day ER	Seven day estimated record
AA	Arachidonic acid (20:4n-6)
AI	Adequate intake
AR	Average requirement
BMR	Basal metabolic rate
CE	Cholesteryl esters
DBI	Double bond index
DHA	Docosahexaenoic acid (22:6n-3)
EFA	Essential fatty acids
en%	Energy %
EPA	Eicosapentaenoic acid (20:5n-3)
FFQ	Food frequency questionnaire
HDL	High density lipoproteins
HUFA	Highly unsaturated fatty acids
LA	Linoleic acid (18:2n-6)
LCAT	Lecithin-cholesterol acyltransferase
LDL	Low density lipoproteins
MCL	Mean chain length
MMP	Mean melting point
MUFA	Monounsaturated fatty acids
OPI	Oxidative potential index
PAL	Physical activity level
PL	Phospholipids
PRI	Population reference intake
PUFA	Polyunsaturated fatty acids
RDA	Recommended dietary allowance
SD	Standard deviation
SFA	Saturated fatty acids
UL	Tolerable upper intake level

Fatty acid notation: number of carbon atoms:number of double bounds n-x with x is the place of the first double bond proximal to the methyl end of the fatty acid.

Aims and outline of the studies

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This thesis consists of three parts. In the first part literature concerning the current knowledge of essential fatty acids (EFA) and their role in pregnancy and foetal development is reviewed **(chapter 1)**.

In the second part we focus on the maternal diet. The dietary intake of macronutrients and micronutrients is described with emphasis on the fat and fatty acid intake during pregnancy. First we adapted and validated an existing food frequency questionnaire (FFQ) which was developed based on a Dutch FFQ. This FFQ was specifically designed to collect data on the fat consumption of Dutch pregnant women [1]. Minor changes were used to adapt this Dutch FFQ to the Belgian diet, in order to enable us to focus on the fat and fatty acid intake of pregnant Belgian women **(chapter 2)**.

Then we described the dietary intake of energy and of macronutrients and micronutrients during the course of pregnancy. The results are evaluated by comparing them with the dietary recommendations for pregnant women (**chapter 3).**

At last dietary intake of fatty acids during pregnancy was determined and correlated with parameters for essential fatty acid status in mother and newborn **(chapter 4).**

In the third part of this work we focus on the maternal and neonatal EFA status. The EFA status was estimated based on the fatty acid composition of maternal and umbilical plasma phospholipids and cholesteryl esters.

First the concept of the calculated mean melting point (MMP) of fatty acids from plasma phospholipids as surrogate parameter of membrane fluidity is explained. When polyunsaturated fatty acids (PUFA) are replaced by saturated (SFA) or monounsaturated (MUFA) fatty acids, the MMP increases and membrane fluidity

decreases [2-5]. The MMP of fatty acids from maternal plasma phospholipids at different stages during pregnancy was calculated and compared with the MMP of fatty acids from umbilical plasma phospholipids **(chapter 5)**.

Then we described the fatty acid profile of phospholipids and cholesteryl esters in maternal plasma on three different cut-off points during pregnancy and at delivery and in umbilical blood shortly after birth are (**Chapter 6).**

Furthermore we investigated whether the fatty acid profile of maternal plasma phospholipids six days before delivery, one and three days postpartum differs significantly from each other **(chapter 7)**.

We described the fatty acid composition of umbilical cord venous and arterial vessel walls and discuss their possible meaning. Because umbilical vessel walls do not have vasa vasorum that could deliver fatty acids they can only obtain their fatty acids from the blood passing through the cord vessels. Therefore the fatty acid composition of the umbilical venous vessel wall is considered to be a long-term reflection of the fatty acid supply from mother to foetus **(chapter 8)**.

Moreover we examined whether pregnant women who develop postpartum depression have a different fatty acid profile in their plasma phospholipids and cholesteryl esters compared to control pregnant women. Indeed, several studies have shown that major depression is accompanied by alterations in serum fatty acid composition: reduced n-3 fatty acids and increased 20:4n-6/20:5n-3 ratio in serum and red blood cells [6-9]. Moreover, pregnancy is associated with a gradual decrease of the relative maternal concentration of 22:6n-3 in plasma PL from the 18^{the} week of gestation on resulting in a decreased DHA sufficiency index. Six months after delivery the maternal DHA sufficiency index had not yet returned to early pregnancy values [10;11]. Therefore it was hypothesised that relative maternal depletion of 22:6n-3 might increase the risk of postpartum depression **(chapter 9)**.

The third trimester of pregnancy is associated with the predominance of small and dense LDL-particles [12;13]. These small and dense LDL-particles have been shown to be more susceptible to oxidation [14]. Hyperlipidemia and the occurrence of small and dense LDL particles during late pregnancy might increase the oxidative damage and impair the outcome of pregnancy. We determined whether the oxidative stability of LDL changes during the course of pregnancy and if so

whether this change correlates with changes in vitamin E, vitamin A, and β-carotene levels or with changes in the fatty acid composition of plasma phospholipids **(chapter 10)**.

In this dissertation we study the fatty acid composition of plasma phospholipids during the course of pregnancy. When performing this kind of long-term studies it is important to consider the possibility of seasonal variation. In the final experiment of this thesis we determined whether there exits seasonal variation in the fatty acid composition of plasma phospholipids in 23 healthy individuals who donated during one year a monthly blood sample **(chapter 11).**

Finally, the general conclusions emerging from these studies are discussed in **chapter 12**. Implications for further research are given.

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Chapter 1

Literature review

Chapter 1: Literature review

The aim of this chapter is to give a literature review on the metabolism of essential fatty acids (EFA) and their role in pregnancy and foetal development. First the metabolism of EFA is given. Then the biosynthesis of plasma lipids and the biochemical indicators of EFA-status are covered. Furthermore, the role of EFA in pregnancy and foetal development is clarified. Finally, the increased oxidative stress during pregnancy and the effect on lipid metabolism is discussed.

1. What are essential fatty acids ?

More than seventy years ago, the essentiality of certain polyunsaturated fatty acids (PUFA) was discovered by Burr and Burr [1;2]. Rats fed a fat-free diet for several months developed symptoms like growth retardation, dermatitis and reproductive failure. Supplementation with small amounts of n-6 and n-3 polyunsaturated fatty acids (PUFA) induced growth promotion and prevention of dermatitis [1;2].

There are two families of essential fatty acids (EFA), the n-6 and n-3 families. They are essential because they are required and cannot be synthesized de novo by humans. The human (and animal) metabolism is unable to introduce a double bond between carbon atoms 3 and 4 (for n-3 fatty acids) or between carbon atoms 6 and 7 (for n-6 fatty acids) proximal to the methyl end of the fatty acid. Linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) can be desaturated and elongated to form longer-chain, more unsaturated PUFA (Figure 1.1). Therefore 18:2n-6 and 18:3n-3 are referred to as the parent EFA. The parent EFA are present in high concentrations in plants and vegetables. Linoleic acid has a well-defined function in the skin, but it is unclear whether α-linolenic acid itself is essential only as a precursor or also in its own right [3]. The long-chain PUFA play a major role in the development of new life as important structural components of cell membrane

phospholipids. Arachidonic acid (20:4n-6, AA) and docosahexaenoic acid (22:6n-3, DHA) are important structural fatty acids in neural tissue such as the brain and retina. In addition some PUFA (20:4n-6 and 20:5n-3, and to a lesser extent 20:3n-6) are involved in the formation of eicosanoids which are hormone-like substances.

Linoleic acid and α-linolenic acid are converted into PUFA through desaturation and elongation steps. The desaturation processes are catalysed by rate-limiting activities of Δ6- and Δ5-desaturases. All these enzymes are bound to the microsomal lipid bilayer and require zinc as co-factor [4;5]. Therefore low zinc intake or other factors that reduce the serum zinc status can induce a decrease in the long-chain fatty acids. The Δ6-desaturase converts 18:2n-6 to γ-linolenic acid (18:3n-6) and 18:3n-3 to stearidonic acid (18:4n-3). These fatty acids are further elongated to 20:3n-6 and 20:4n-3 respectively. The Δ5-desaturase is responsible for the biosynthesis of AA and eicosapentaenoic acid (20:5n-3, EPA). These fatty acids are then elongated twice to form 24:4n-6 and 24:5n-3 which are converted by Δ6-desaturase in 24:5n-6 and 24:6n-3. Finally, peroxisomal chain shortening by β-oxidation is responsible for the formation of docosapentaenoic acid (22:5n-6) and docosahexaenoic acid (22:6n-3).

The two parent EFA compete for the enzyme Δ6-desaturase in the first step of their respective conversions. Although the Δ6-desaturase enzyme prefers 18:3n-3 over 18:2n-6, α-linolenic acid remains at a competitive disadvantage owing to the excess availability of linoleic acid compared to α-linolenic acid in the present Western diet [6]. Recently, the Δ6- and Δ5-desaturases have been cloned [7;8] and it has been demonstrated that linoleic acid inhibits expression of the gene coding for the Δ6-desaturase enzyme [7] which likely leads to the phenomenon called substrate inhibition. Under certain conditions, particularly in early postnatal development, low tissue levels of longer-chain PUFA occur even if relatively high amounts of the parent EFA are present in the diet. Indeed, the parent EFA may cause substrate inhibition of long-chain PUFA synthesis, linoleic acid inhibits synthesis of arachidonic acid and α-linolenic acid inhibits synthesis of docosahexaenoic acid [9].

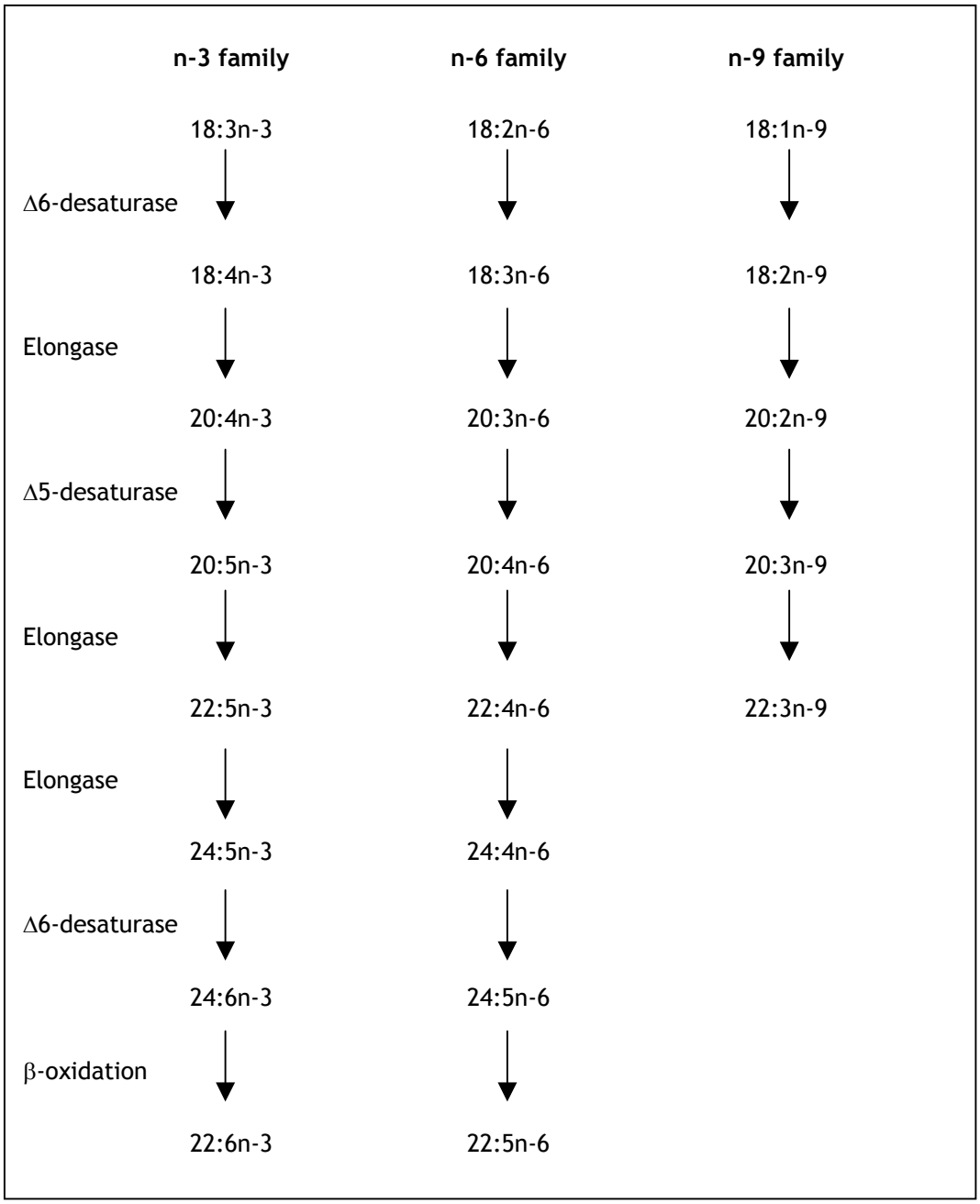

Figure 1.1: Metabolic pathways converting 18-carbon unsaturated fatty acids to more highly unsaturated fatty acids

Fatty acid notation: number of carbon atoms:number of double bounds n-x with x is the place of the first double bond proximal to the methyl end of the fatty acid.

Cunnane introduced the classification of conditionally-indispensable and conditionally-dispensable fatty acids in stead of essential fatty acids [9]. One of the reasons for proposing this change in terminology was that the dietary essentiality of the parent or the longer-chain PUFA always appears to be conditional on developmental age, nutritional circumstances or the presence of diseases that lead to increased fatty acid β-oxidation. Linoleic acid, α-linolenic acid, arachidonic acid and docosahexaenoic acid are conditionally-indispensable fatty acids during infancy, childhood, pregnancy and lactation [9]. α-Linolenic acid is considered the only conditionally-indispensable fatty acid during adulthood [9].

C20 fatty acids of the n-3 and n-6 series are involved in the formation of important bioactive compounds (i.e. eicosanoids) such as the prostacyclins, prostaglandins, thromboxanes, leukotrienes, lipoxines and related substances. These compounds have diverse biological functions in cell growth and development, inflammation, and the cardiovascular system [10-13]. The enzymes involved in the synthesis of the eicosanoids are lipoxygenases and cyclooxygenases. The cyclooxygenase pathway produces prostaglandins and thromboxanes. The lipoxygenase pathway produces leukotrienes and lipoxines. Dihomo-γ-linolenic acid (20:3n-6) is the precursors of prostaglandin series 1 (with one double bond). AA (20:4n-6) is the precursor of the prostanoids of series 2 (prostaglandins and thromboxanes) and of leukotrienes of series 4. EPA (20:5n-3) is the precursor of the prostanoids of series 3 and the leukotrienes of series 5.

EPA competes with AA for cyclooxygenase and lipoxygenase enzymes. The eicosanoids derived from EPA have different biological properties than those derived from AA. The biological response after eicosanoid release is dependent on the net balance of eicosanoids derived from n-6 and n-3 long-chain PUFA [14].

2. EFA deficiency markers

For the assessment of the EFA status of an individual, the total amount of EFA in blood plasma is a useful indicator [15]. In general, phospholipids (PL) have a slower turnover than free fatty acids, triglycerides or cholesteryl esters (CE). Furthermore, phospholipids are the major structural components of membranes and the fatty acid pattern of plasma PL reflects that of tissue PL [16]. Therefore

the EFA profile of plasma or red blood cell PL is considered suitable to document the overall EFA status of a given individual [17].

When insufficient EFA are available (either in the diet or from adipose tissue) to meet the requirements of the body, the human organism starts to synthesise fatty acids, which are hardly present under normal healthy conditions. Under these conditions oleic acid (18:1n-9) will be desaturated and elongated to form Mead acid (20:3n-9) and di-homo-Mead acid (22:3n-9). The presence of Mead acid is an indication of a generalised shortage of the parent EFA and their derived long-chain homologues. The trienoic/tetraenoic ratio (20:3n-9/20:4n-6) was proposed as an index of EFA deficiency [18].

Another suitable indicator is the EFA status index which is the ratio of the sum of all the essential n-3 and n-6 fatty acids to the sum of all the non-essential unsaturated n-7 and n-9 fatty acids. The higher this ratio, the better the essential PUFA status [17].

Furthermore, an isolated deficiency of DHA stimulates the synthesis of the most comparable long-chain PUFA of the n-6 family, Osbond acid or 22:5n-6 (figure 1.1). Therefore, under steady state conditions, the ratio between 22:6n-3 and 22:5n-6 (i.e. the DHA sufficiency index) is considered to be a reliable biochemical indicator of the DHA status [19]. Since the synthesis of 22:5n-6 also depends on the availability of its precursor 22:4n-6, the ratio of 22:5n-6 over 22:4n-6 (i.e. the DHA deficiency index) is also a reliable marker of the DHA status. A deficit in DHA is accompanied by an increased conversion of 22:4n-6 to 22:5n-6, resulting in a higher DHA deficiency index [17;20].

Finally, Holman *et al* [21] introduced the concept of the calculated mean melting point (MMP) and considered this parameter as the best current single measure of PUFA status although they prefer to use the entire set of fatty acid profiles [22].

3. Determination of the biochemical EFA status

The fatty acid profiles of different lipid fractions (PL, CE, triglycerides, and free fatty acids) extracted from plasma, red blood cell membranes or tissue are useful indicators of the biochemical EFA status of a given individual [23]. Because of different rates of turnover of fatty acids in these constituents, differences arise in

the time required for a change in dietary fat type to be totally reflected in the fatty acid pattern [23].

The fatty acid composition of triglycerides, extracted from fasting serum or plasma, reflects the composition of the last few meals before blood sampling, while the fatty acid composition of CE and PL change more gradually during 2–3 weeks with a change in diet and reflect the average dietary composition during a longer time period.

In a group of institutionalised elderly subjects a good relationship was found for 18:2n-6 between the four major serum fraction (PL, CE, triglycerides and free fatty acids). This implies that these four lipid fractions obtain fatty acids from a common precursor pool or that one lipid class provides fatty acids for synthesis of the other [24].

Since PL are structural lipids and are the richest source of PUFA, changes in PUFA profile are most pronounced in PL and therefore the fatty acid profile of PL reflects best the EFA status [16]. The concentration of PUFA in plasma PL has been used as a marker of recent dietary intake of PUFA [25;26], whereas erythrocyte PUFA patterns have been more commonly used to indicate long-term dietary intake [27]. In a fish-oil supplementation study it was shown that the incorporation half lives of 20:5n-3 in humans are about 5 days for serum CE, almost a month for erythrocytes and longer than a year for subcutaneous fat tissue [28]. Thus, determination of the fatty acid composition of CE gives a reflection of nutritional intakes over the past week or two, erythrocyte membranes over the past months or two and adipose tissue over a period of years. Absolute values of fatty acids reflect intake more accurately but relative proportion of fatty acids could be a more precise functional marker [14].

In our studies we analysed the fatty acid composition of PL and CE because the fatty acid profile of plasma PL and CE changes more gradually with a change in diet and reflects the average dietary composition over a longer time period. The postprandial fatty acid profile of plasma triglycerides or free fatty acids is strongly influenced by the fatty acid composition of the last meal.

Recently, Cunnane [9] stated that tissue and plasma fatty acid profiles are a poor indicator of linoleic and α-linolenic adequacy or inadequacy because there is no implicit reason why a plateau in a specific fatty acid would demonstrate that a

required fatty acid intake has been achieved. Rather, a plateau in the proportional fatty acid data reflects only the maximum amount of that fatty acid that can be accommodated in the tissue under those conditions [9].

3.1. Fatty acid composition of phospholipids and triacylglycerols

Most naturally occurring PL and triacylglycerols have an asymmetric distribution of saturated and unsaturated fatty acids with saturated fatty acids preferentially esterified in the sn-1 position of glycerolipids and unsaturated fatty acids in the sn-2 position in PL and in the sn-2 and sn-3 position for triacylglycerols. Therefore, saturated fatty acids at the sn-1 position account for nearly one-half of the fatty acids in PL (ca 40 to 45 w%) and about one-third of the fatty acids in triacylglycerols (ca 25 to 35 w%). In contrast, the 16 and 18 carbon unsaturated fatty acids are esterified at the sn-2 position during de novo synthesis and the 20 and 22 carbon highly unsaturated fatty acids (HUFA) also at the sn-2 position during the retailoring process of PL.

3.2. Cholesteryl esters biosynthesis

The plasma enzyme lecithin-cholesterol acyltransferase (LCAT) catalyses the transfer of the fatty acid of phosphatidylcholine to unesterified cholesterol with the formation of lysophosphatidylcholine and of CE. LCAT has a specificity for the sn-2 position of phosphatidylcholine [29]. Although LCAT utilises the sn-2 acyl group from most of the phosphatidylcholine species it preferentially utilises the sn-1 acyl group from phosphatidylcholine species containing 20:4n-6 and 22:6n-3 at the sn-2 position [30]. Furthermore, the enzyme LCAT is able to convert two molecules of 1-acyl-lyso-phosphatidylcholine (which contain mainly saturated fatty acids) into one molecule of disaturated phosphatidylcholine and one molecule of glycerylphosphorylcholine. These disaturated phosphatidylcholines can be a substrate for LCAT and may explain the unexpectedly high transfer of saturated fatty acids during the cholesterol esterification reaction. Supplementation with n-3 fatty acids in humans increases the content of n-3 PUFA (20:5n-3, 22:5n-3 and 22:6n-3) in plasma PL and increases the content of saturated plasma CE [31]. It is possible that the paradoxical increase in saturated CE is due to the utilisation of sn-1 acyl groups from phosphatidylcholine species containing n-3 HUFA at the sn-2 position.

4. Essential fatty acids during pregnancy and neonatal life

The last decade, there is considerable interest in the importance of EFA and their longer-chain more unsaturated derivatives in relation to birth outcome and neonatal health. PUFA are major structural components of the cell membrane and are particularly important for optimal visual and nerve cell development and function. During pregnancy accretion of maternal, placental and foetal tissue occurs and therefore the EFA and long-chain PUFA requirements of pregnant women and the developing foetus are high. During the last trimester of pregnancy, the foetal need for AA and DHA is especially high because of rapid synthesis of brain tissue.

It has been demonstrated that the proportion of long-chain PUFA increases in PL going from maternal blood to placenta to cord blood, foetal liver and foetal brain [32]. This process has been termed "biomagnification" and indicates that the fatty acid composition of PL in the foetoplacental unit is the result of alterations designed to achieve the high proportion of long-chain PUFA necessary for structural components of the developing brain [32]. Biomagnification is only valid for the relative concentration of PUFA (% of total fatty acids) and not for the absolute concentration (mg/L plasma or kg tissue). Figure 1.2 illustrates the biomagnification process with regard to DHA [33].

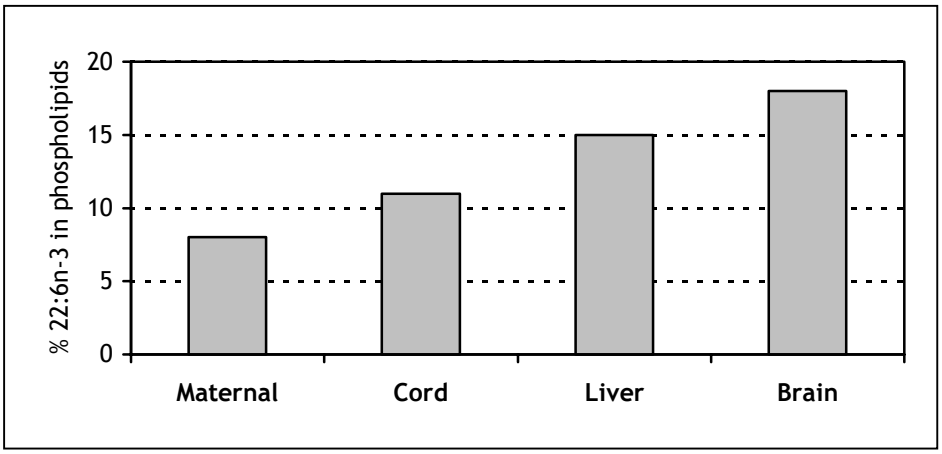

Figure 1.2: The biomagnification process with regard to 22:6n-3. Data were obtained from mid-term abortion material [33].

It is important to understand how the foetus acquires the proper type and amount of long-chain derivatives of the parent EFA. The higher proportion of long-chain PUFA in cord blood could originate from preferential transfer of PUFA from the maternal circulation to the foetal circulation and/or the long-chain PUFA might be formed in either the placenta or the foetal liver. In the following part these different possible sources of long-chain PUFA to the foetus are discussed.

4.1. Desaturation enzyme system of the placenta

Different studies *in vitro* illustrated that the human placenta lacks both Δ6- and Δ5-desaturase activities [15;32;34-36]. Studies in the perfused human placenta obtained from normal or caesarean deliveries have demonstrated that there is no detectable chain elongation and desaturation of the two parent EFA [36]. In the microsomes of human placental tissue obtained at 18 and 22 weeks of gestation, no activity of either the Δ6- or Δ5-desaturase enzymes has been detected [35]. These data suggest that the long-chain derivatives of linoleic and α-linolenic acid in the foetal circulation are synthesised either by the mother or the foetus. The possibility that placental synthesis of long-chain PUFA may occur *in vivo* cannot be ruled out however.

4.2. Desaturation enzyme system of the foetal liver

Chambaz *et al* [35] were the first to detect, *in vitro*, Δ5- and Δ6-desaturase activities in microsomes of human foetal liver after 18 and 22 weeks of gestation. Rodriguez *et al* [37] established *in vitro* significant Δ6- and Δ5-desaturase activities in human foetal liver as early as the 17th week of gestation. The desaturation activities remained stable throughout the third trimester and the Δ6-desaturase activity was higher for n-3 than for n-6 fatty acids, regardless of gestational age. However, their theoretic desaturase capacity calculated from *in vitro* measurements appears to be lower than the foetal PUFA requirements [37;38].

Furthermore, stable isotope tracer studies demonstrated that human preterm infants are capable of synthesising DHA *in vivo* subsequent to enteral administration of [^{13}C]-labelled 18:3n-3 [39;40]. Recently, Su *et al* [41], using [^{13}C]-tracers showed that primate foetuses (baboon) have the capacity to convert α-linolenic acid to DHA *in vivo*. In this study, [U-^{13}C]18:3n-3 or [U-^{13}C]22:6n-3

(extracted from a [U-^{13}C]algal oil) was administered as nonesterified fatty acid to baboon foetuses via intra-venous injection into the jugular artery. This study illustrated that the foetal liver of the baboon is likely to be an important site of 18:3n-3 to 22:6n-3 conversion [41].

Taken together, these data illustrate that PUFA can be synthesised in the human foetus during the second and third trimester. Despite this, desaturation enzyme systems in the human foetal liver seem to be immature and unable to supply enough long-chain PUFA to meet the high demands of rapidly growing tissues and organs [42].

In conclusion, (i) the capacity of the human placenta to synthesise long-chain PUFA from EFA is very limited or absent; and (ii) the desaturation enzyme system in the human foetal liver is immature and probably unable to supply enough long-chain PUFA to meet the high neonatal demand. Therefore it is likely that the long-chain PUFA in the foetal circulation are primarily derived from maternal sources. Consequently, to obtain adequate amounts of parent EFA and their long-chain polyunsaturated derivatives, the developing foetus primarily depends on active transport of these fatty acids from the maternal circulation across the placenta and thus on the EFA status of the mother.

4.3. Fatty acid transport through the placenta

4.3.1 Placenta in general [43]

The placenta, which separates the maternal from the foetal circulation, is composed of large villi of foetal vessels surrounded by intervillous spaces in which maternal blood flows. The placenta allows transmission of gasses, water and a variety of nutrients and waste products. For solutes to move between the maternal and foetal circulation, they must pass through the villous trophoblast which is a syncytium consisting of two membranes (the microvillous or brush-border membrane facing the mother and the basal membrane facing the foetus).

The transfer of any substance depends amongst other factors on the concentration gradient between the maternal and the foetal circulation, the presence of circulating binding proteins, lipid solubility of the substances, etc. Gasses (oxygen, carbon dioxide), sodium and chloride ions, urea and ethanol are passively

transported through the placenta. Free fatty acids and unconjugated steroids have a limited passive transport through the placenta. Glucose is transported by facilitated diffusion according to concentration dependent kinetics. Amino acids are transported through energy dependent processes via selective transporters. Calcium is actively transported across cell membranes of the placenta. Maternal IgG is transported with receptor-mediated endocytosis. The placenta is an effective barrier to the movement of large proteins (except for IgG), thyroid hormones and maternal and foetal erythrocytes. Knowledge about placental transport of lipids is still scant.

4.3.2 Mechanism of placental fatty acid transport

The ability of the placenta to extract long-chain PUFA from the maternal circulation and deliver them to the foetus is extremely important for the neonatal development. Nonesterified fatty acids in the maternal compartment have been proposed as the major source of fatty acids for transport across the placenta, irrespective of the source from which they originate in the maternal circulation [15;32;35]. Kuhn and Crawford [32] showed in the perfused human placenta that neither triglycerides nor PL are taken up from the maternal circulation, only free fatty acids are taken up by the human placenta.

PUFA in maternal plasma in the third trimester and postpartum are mainly esterified and associated with lipoproteins rather than in the form of free fatty acids [44]. Besides, the amount of free fatty acids in plasma of pregnant women is practically negligible compared to the amount of fatty acids associated with circulating lipoproteins. Lipoprotein receptors in the placental trophoblast cells, facing maternal blood, bind maternal triglyceride-rich lipoproteins and mediate their metabolism and subsequent transfer of PUFA to the foetal circulation [44;45].

Thus, PUFA arrive at the microvillous membrane of the placenta (maternal side) as albumin-bound nonesterified fatty acids (minority of the fatty acids) or as triglycerides, PL and CE as components of lipoprotein particles (majority of the fatty acids). Endothelial bound lipases are present that can hydrolyse these lipid classes and generate free fatty acids which can be taken up [46-48]. Intact cholesterol esters can be taken up by placental tissue and be hydrolysed there [49]. The latter mechanism has been suggested to provide the placenta with free

cholesterol but also results in the liberation of free fatty acids. The relative contribution of the different lipid classes to the nonesterified fatty acid pool taken up by the placenta is to our knowledge unclear. Placental uptake of nonesterified fatty acids occurs by facilitated diffusion after hydrolysis by lipase or after dissociation from albumin [10;50;51]. The presence of lipoprotein lipase activity in the human placenta allows the utilization of maternal triglycerides. In guinea pigs, the placental lipoprotein lipase is present only in the microvillous membrane of the placental trophoblast [52]. The placental lipoprotein lipase hydrolyses triglycerides from maternal VLDL but not the triglycerides present in chylomicrons [7;45;46]. The preferential hydrolysis of posthepatic triglycerides (VLDL) by the placental lipoprotein lipase may result in an increased availability of long-chain PUFA for placental uptake and serve as a protection of the foetus from the immediate impact of an unusual fatty acid in a meal [10;53]. The concentration of triglycerides in the maternal circulation increases much more with progressing gestation than the other lipid classes [54]. This suggests that maternal triglycerides are the major source of esterified fatty acids for the placenta. Placental lipoprotein lipase preferentially hydrolyses fatty acids in the sn-2 position of the glycerol backbone and the sn-2 position generally is more unsaturated than the sn-1 or sn-3 positions. This implies selectivity by the placental lipases for the release of long-chain PUFA from triglycerides. Moreover, the preferential incorporation of 22:6n-3 into the triglyceride fraction suggests that triglycerides may play an important role in the placental transport of 22:6n-3 to the foetal circulation [10]. There is a report of the presence of a phospholipase A_2 on the microvillous membrane for the hydrolysis of PL but the hydrolysis of triglycerides by lipoprotein lipase is more pronounced [55]. More recently an endothelial-derived lipase which is expressed amongst other organs in the placenta has been cloned [46]. This endothelial lipase has substantial phospholipase activity and less triglyceride lipase activity. Overexpression of this enzyme in mice reduced plasma concentrations of HDL [46]. As HDL is rich in PL and DHA is highly present in the PL fraction, this enzyme could be important for the hydrolysis of maternal PL and the supply of long-chain PUFA to the foetus.

Once maternal plasma triglycerides are hydrolysed, they are taken up by the placenta, where reesterification and intracellular hydrolysis facilitates diffusion of the released fatty acids to the foetus and their subsequent transport to the foetal liver [44].

The overall picture of the placental transfer of PUFA from maternal to foetal circulation has been schematically summarised in figure 1.3.

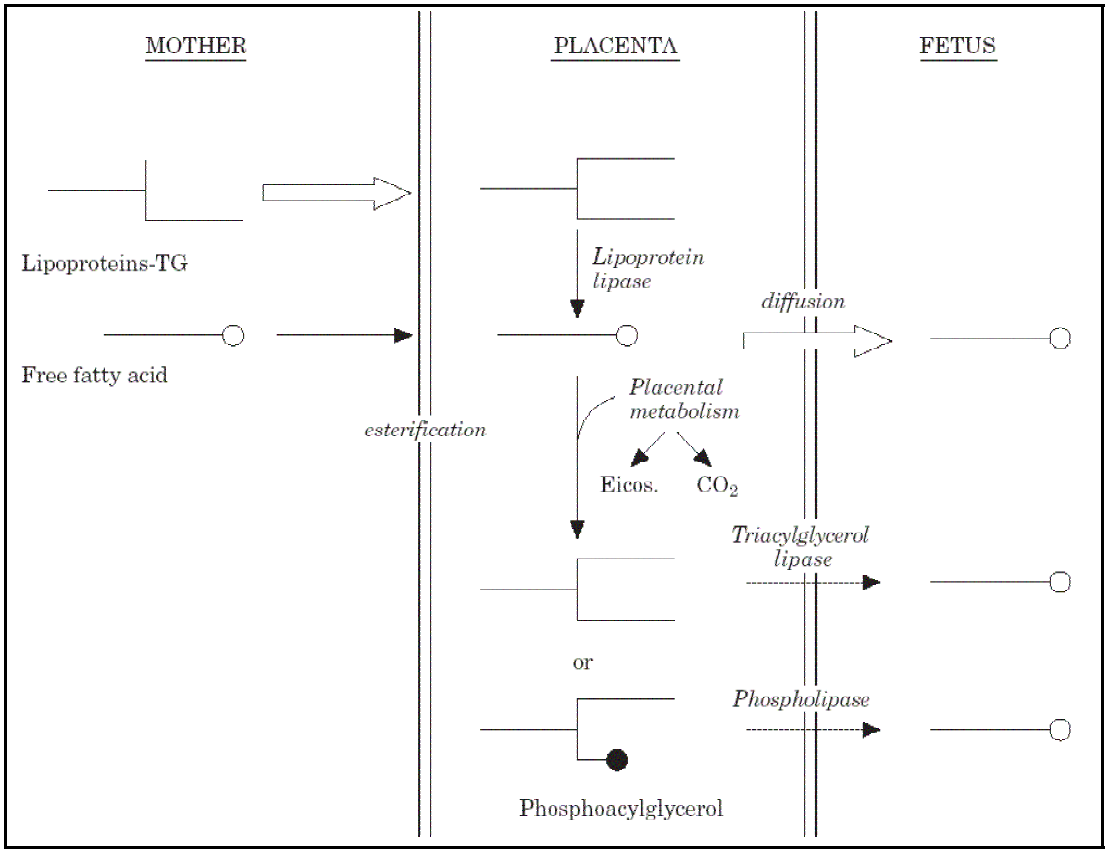

Figure 1.3: Schematic representation of the placental transfer of fatty acids to the foetus [44].

Fatty acids can cross lipid bilayers (as in the syncytiotrophoblast) by simple diffusion. However, fatty acid binding proteins in membranes and cytoplasm are thought to facilitate the transfer across membranes and intracellular channelling of fatty acids. The known placental fatty acid binding proteins are the fatty acid transfer proteins (FAT/CD36 and FATP) both on the microvillous and basal membranes and a placenta specific plasma membrane fatty acid binding protein (p-FABPpm) exclusively on the microvillous membrane [56;57]. Figure 1.4 illustrates the distribution of placental fatty acid binding proteins [57].

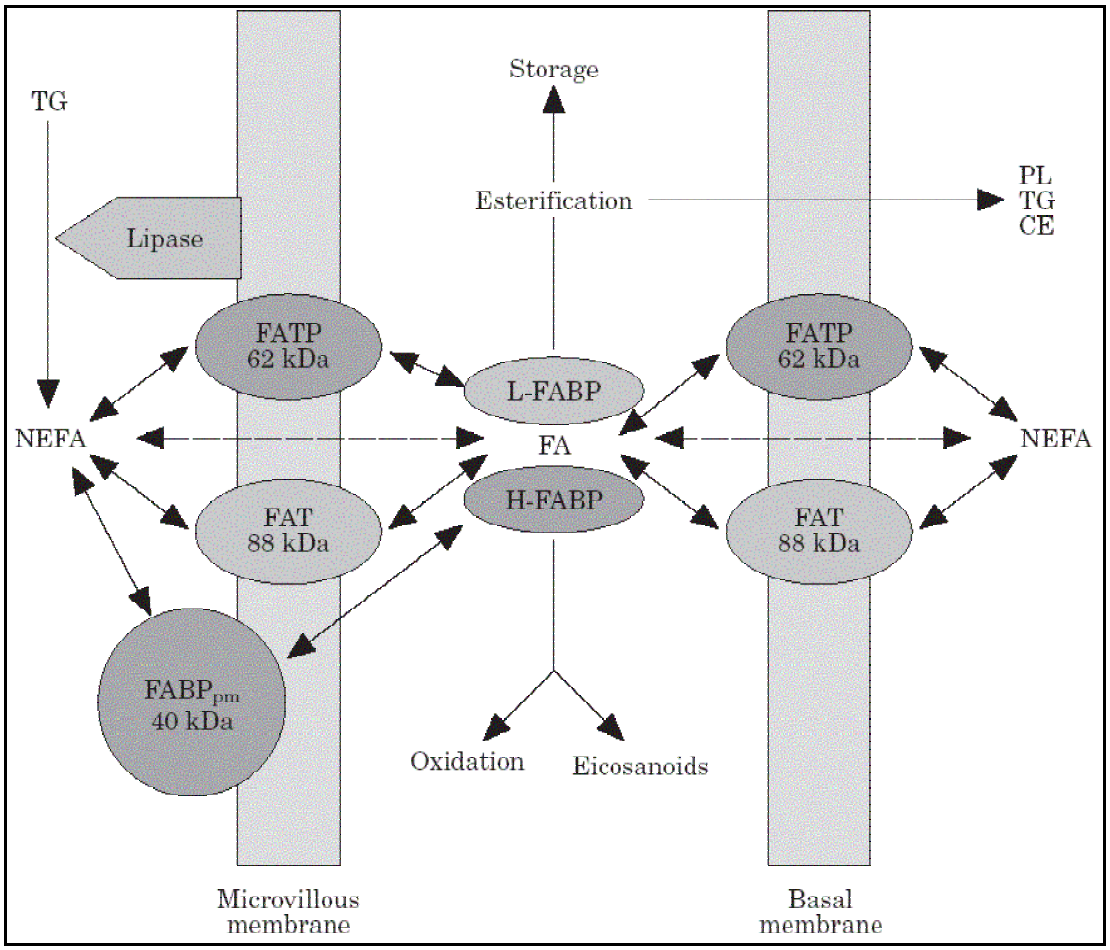

Figure 1.4: Distribution of placental fatty acid binding proteins [57]

Placental perfusion studies demonstrate that linoleic acid is more efficiently transferred from the maternal to the foetal circulation compared to 20:4n-6 [32]. But maternally-derived 18:2n-6 was found mostly in the free fatty acid fraction of foetal circulating lipids whereas 20:4n-6 had been selectively incorporated in PL by the placenta and exported to the foetus in that form [32]. It is known that free fatty acids may cross the placenta in either direction but the movement of PL and triglycerides may be restricted. Thus the selectivity with which the placenta distributes long-chain PUFA into a lipid fraction which does not recross the placental barrier may allow those fatty acids to be retained, relative to parent EFA, in the foetoplacental unit [32]. Furthermore the foetus is provided with preformed structural membrane components.

Campbell *et al* [58] investigated the binding characteristics of human placental membranes using 4 different radiolabeled fatty acids. Binding sites seem to have a strong preference for long-chain PUFA: the order of competition was 20:4n-6 >> 18:2n-6 > 18:3n-3 >> 18:1n-9. When the placenta is perfused with a mixture of fatty acids the order of selectivity for uptake is 20:4n-6 > 22:6n-3 > 18:3n-3 > 18:2n-6 [36]. But the placenta appears to preferentially retain 20:4n-6 in preference to the other fatty acids, resulting in a different order of selectivity for placental transfer to the foetal circulation: 22:6n-3 > 18:3n-3 > 18:2n-6 > 18:1n-9 > 20:4n-6 [36]. Haggarty *et al* [36] concluded that the human placenta has the capacity to selectively transfer individual fatty acids to the foetus with the greatest selectivity being shown for DHA.

In conclusion, these studies illustrate (i) preferential binding of long-chain PUFA by placental membranes [58] and (ii) selective transfer of long-chain PUFA by the human placenta to the foetal circulation [36] in preference to the nonessential fatty acids.

Placental released nonesterified fatty acids at the foetal side are transported in foetal blood bound to a specific foetal protein: the α-fetoprotein [44]. This α-fetoprotein has been shown in a number of studies to bind PUFA more strongly than does albumin [59]. The presence of this protein can account for the relatively high proportion of PUFA found in foetal plasma. The free fatty acids in foetal plasma are rapidly taken up by foetal liver, where they are esterified and released back into the foetal circulation as triglycerides. This may explain the significant linear correlation for certain long-chain PUFA between maternal and cord plasma triglycerides during gestation [60]. A strong correlation between the long-chain PUFA of maternal and umbilical plasma phospholipids has also been observed [17].

To elucidate the role of p-FABPpm in the preferential transfer of long-chain PUFA from the maternal circulation across the placenta, the direct binding of various fatty acids to purified p-FABPpm was determined [61]. It was shown that p-FABPpm preferentially binds with long-chain PUFA despite a high concentration of nonessential fatty acids in the assay mixture [61]. As p-FABPpm is exclusively located on the microvillous membrane (facing the maternal circulation) [56] and p-FABPpm has a preference for long-chain PUFA, a unidirectional flow of maternal long-chain PUFA to the foetus is favoured [10].

The uptake of linoleic acid by brush-border (facing the maternal circulation) preparations from human syncytiotrophoblasts was greater than that for basal membrane (facing the foetal circulation) preparations [62]. It was suggested that this may be due to differences in the concentration of fatty acid binding proteins between the brush-border and basal membrane.

In summary, the observed transplacental gradient of long-chain PUFA (known as biomagnification) suggests the presence of a highly active and specific transplacental long-chain PUFA transport system that may be driven by (i) selectivity of the placental lipoprotein lipases for the release of long-chain PUFA from triglycerides; (ii) specific placental fatty acid binding proteins; and (iii) foetal α-fetoprotein which has a high affinity for long-chain PUFA.

4.4. Maternal and neonatal EFA status

4.4.1 Absolute and relative fatty acid data

Pregnancy is generally associated with a marked hyperlipidemia involving all lipid classes [63]. As a consequence the absolute amounts of all the maternal plasma PL associated fatty acids rise during pregnancy [54;64]. Longitudinal studies indicate that the amounts (mg/L) of all the individual fatty acids in the maternal plasma PL increase from the early onset of pregnancy until delivery [54;64]. Recently, it was shown that plasma total fatty acid content starts to increase within the first ten weeks of pregnancy [65]. It was shown that between early pregnancy and delivery, the plasma amounts of the PL-associated essential PUFA increase by about 40%. For AA and DHA these figures are 23 and 52%, respectively [54]. This probably reflects the high requirement of these fatty acids by the developing brain and retina. On the other hand the saturated and monounsaturated fatty acids increase even more significantly than the essential PUFA (65 versus 40%). Especially the increase in Mead acid is more pronounced (92%), which might indicate a maternal deficit in essential fatty acids [54].

Although the absolute amounts of maternal fatty acids rise with progressing gestation, the relative long-chain PUFA concentrations (w%) decline with progressing gestation. The relative maternal DHA status increases until 18 weeks of gestation. Thereafter DHA gradually decreases near term and even after delivery maternal plasma DHA declines further [54]. The relative concentration of

linoleic acid remains stable, whereas the relative amounts of AA steadily decline from mid gestation till near term [54;64;66]. The ratio of the essential n-3 and n-6 fatty acids to the non-essential n-7 and n-9 fatty acids decreases continuously during pregnancy. This led to the suggestion that pregnancy is associated with reduced maternal EFA status. Overall maternal long-chain PUFA status declines steadily during pregnancy [17].

The amount of all the umbilical plasma PL associated fatty acids is substantially lower than the maternal plasma PL associated fatty acids at delivery because the PL concentration is considerably lower in cord blood than in maternal blood at delivery [54;66]. The relative amounts of AA and DHA on the other hand are significantly higher in umbilical than in maternal plasma PL whereas the parent EFA are much lower in the neonate compared to the mother [54;66].

Several studies [67-69] of the fatty acid composition of foetal and maternal plasma PL showed that at birth, linoleic acid represents ≈ 10% of the fatty acids in cord plasma compared with ≈ 20% in maternal plasma but, surprisingly, the AA concentration of cord plasma is twice (≈ 10%) that observed in the mother (≈ 5%). Similarly, the α-linolenic acid concentration in newborns is half of that in the mother (≈ 0.3% and ≈ 0.6%, respectively), whereas the DHA concentration is double (≈ 6% and ≈ 3%, respectively). This situation, in which the relative plasma concentrations of the n-3 and n-6 long-chain PUFA exceeds those of their precursors has only been observed in newborns and does not exist in adults. It is obviously an extremely favourable situation for the development of the newborn, especially at a time when large quantities of AA and DHA are required for the development of the brain and retina.

In our studies, described in this thesis, we decided to express the fatty acid composition in relative (% of total fatty acids of plasma PL) amounts. Because metabolic reactions of fatty acids occur in a lipid environment, it is the amount of a given fatty acid relative to the other fatty acids present, that determines the rate of this reaction in that environment [70]. Furthermore, the measurement error of the analytical procedure for determining relative concentrations is usually smaller than for determining absolute amounts [70].

4.4.2 Umbilical cord vessel walls

The umbilical vein (afferent or supplying foetal vessel) transports blood and nutrients from the mother to the foetus whereas the blood flows back from the foetus to the mother through the umbilical arteries (efferent or draining foetal vessels). Since umbilical vessel walls do not have a vasa vasorum to obtain nutrients, they can only obtain their nutrients directly from the blood passing through. Therefore the fatty acid composition of the umbilical vessel wall PL can be considered to give a longer term reflection of the neonatal EFA status than that of neonatal plasma or red blood cells [68].

It was shown that the PL of the umbilical arteries contain significantly less 18:2n-6, 20:4n-6, 22:4n-6, 20:5n-3 and 22:5n-3 and significantly more 20:3n-9 and 22:3n-9 compared to the PL of the umbilical vein. The presence of high amounts of Mead acid and 22:3n-9 in the umbilical arterial vessel wall was suggested to be an indication of a marginal EFA status of the newborn [67;68]. Moreover, the DHA deficiency index (22:5n-6/22:4n-6) was significantly higher in the PL of the umbilical arteries compared with the veins. This might indicate that the need for DHA by the foetal tissue is not adequately covered [64;68]. In chapter 8 we will focus on this topic.

4.4.3 Importance of DHA for the neonate

From prepregnancy to 10 weeks of pregnancy and between 10 and 40 weeks of gestation, the absolute amount of DHA associated with maternal plasma PL increased by ≈ 48% [65] and ≈ 52% [54], respectively. Length of gestation is positively correlated with the absolute and the relative maternal DHA values until 15 weeks of pregnancy [71]. This suggests a very important role of DHA for human development.

Daily supplementation of nonpregnant women with either 285 mg DHA as a microalgal oil (DHASCO, Martek Biosciences, Columbia, MD) or with 266 mg DHA as tuna fish oil for a four-week period increased plasma PL DHA by ≈ 34% and ≈ 31% respectively [72]. This shows that the effect of pregnancy on the amount of DHA in plasma PL is considerably stronger than that of increased consumption of ≈ 270 mg DHA/d. The increased DHA content of plasma PL is unlikely of dietary origin alone.

Indeed, many dietary studies show that the dietary habits remain unaltered during pregnancy. Neither the amount and type of fat nor the fatty acid composition of the maternal diet changes during pregnancy [65;73;74]. Because plasma PL mainly originate from hepatic synthesis, the increase in DHA concentrations during pregnancy might reflect a pregnancy-associated adjustment in the hepatic synthesis of DHA (increase in the activity of the desaturation and elongation system), with an enhanced or selective incorporation of this fatty acid into the PL. Other explanations for the improved absolute maternal DHA status could be an increase in the mobilisation of DHA from maternal stores or reduced oxidation or both.

Al *et al* [75] could not distinguish a difference in n-3 fatty acid concentrations in maternal plasma PL from women who delivered preterm (<37 wk), at term (37-42wk) and after prolonged gestation (>42wk). But the DHA status (w%) in umbilical plasma PL significantly increased with progressing gestation. This observation suggests that the efficiency of maternal-foetal transfer of n-3 fatty acids improves with progressing gestation.

After parturition, the elevated concentrations (mg/L) of the n-6 or n-3 fatty acids associated with plasma PL steadily decline both in lactating and nonlactating women. Regarding the relative fatty acid composition (w%), significant changes occur between lactating and nonlactating women for the postpartum changes of the n-3 fatty acids but not for the n-6 fatty acids [76]. The decline in plasma PL 22:6n-3 values was enhanced in lactating women [76]. As human milk contains significant amounts of DHA, the decrease of DHA in plasma PL probably indicates the utilisation of DHA for breast milk. Moreover the reductions in maternal DHA became stronger the longer the duration of breast-feeding [76]. In other words there is an extra drain on DHA stores in women who breast-feed their children and it will probably take them longer to replenish their DHA stores [76].

Some studies report that during pregnancy, the absolute and relative amounts of DHA in maternal plasma PL of women who were previously pregnant (multigravidae) were significantly lower compared to women who were pregnant for the first time (primigravidae) [17;77]. The percentage of DHA in the plasma PL of multigravidae is on average ≈ 10% lower than in primigravidae. In addition, a significant negative relation was observed between the gravida number and the percentage of DHA in the maternal plasma PL [77]. These observations might

indicate that in usual dietary conditions pregnancy is associated with mobilisation of DHA from a store that may not be readily replenished after the first pregnancy [77]. On the other hand in a nonpregnant study population [78], neither a significant difference in relative amount of DHA in plasma PL was found between nonpregnant nulligravidae and nonpregnant multigravidae nor a significant relation between parity and the percentage of DHA in plasma PL. In the latter study the duration between the time of blood sampling and the last partus in the multigravidae was between 1 and 2 years. These observations indicate that the maternal DHA status after pregnancy normalises within 1 year [78]. However the average DHA concentration in erythrocyte PL was significantly lower in the mothers than in the nulligravidae and although there was no significant relationship with parity number, this observation nonetheless suggests that in domains with a slow fatty acid turnover, normalisation of the DHA status after delivery may take longer than 1 year.

4.4.4 Essential fatty acids and birth outcome

AA and DHA are critically important for foetal and infant growth and central nervous system development. Long-chain PUFA are preferentially accumulated in the brain during the last trimester of pregnancy and the first months of life. High levels of DHA are found in the grey matter of the cerebral cortex and in the photoreceptor outer segment membranes of the retina [15]. Post-mortem studies of foetuses, stillbirths and preterm infants have shown foetal accretion of approximately 70 mg n-3 long-chain PUFA per day during the last trimester, most of which is 22:6n-3 [79;80].

There is clinical evidence supporting a relation between long-chain PUFA in plasma and foetal growth. Several authors reported a positive relation between birth weight and 20:4n-6 content in cord plasma, suggesting that intrauterine 20:4n-6 serves as a growth factor. Crawford *et al* [33] documented significantly lower levels of 20:4n-6 and 22:6n-3 in cord plasma phosphatidylcholine and in cord red blood cell phosphatidylethanolamine of low birth weight infants (< 2500 g) compared to infants with birth weights higher than 2500 g. Additionally, 20:4n-6 and 22:6n-3 were significantly lower in cord plasma of the neonates with low head circumference (< 32.5 cm) and with small placentas (< 425 g) compared to neonates with high head circumference (> 35 cm) and with bigger placentas (> 650

g) [33]. Others found a significant and positive correlation between body weight and content of 20:4n-6 and total long-chain n-6 fatty acids in plasma triglycerides in premature infants at four days of age. The authors proposed that during early life AA may have a growth-promoting effect, which could be related to its role as an eicosanoid precursor or to its structural function in membrane lipids [81]. Similar results were found in a group of low birth weight preterm born infants (< 2000 g). The percentages of 22:6n-3, 20:4n-6 and the sum of n-6 long-chain PUFA in cord plasma phosphatidylcholine correlated positively with birth weight and with head circumference. Moreover the percentage of 22:6n-3 showed strongest correlation with length of gestation [82]. In other words, the smallest and least mature infants are born with the lowest circulating levels of long-chain PUFA. After correction for gestational age at birth, relative DHA levels in the umbilical artery wall of preterm infants were significantly related to birth weight and head circumference [83]. These findings might indicate that both 20:4n-6 and 22:6n-3 serve as potential foetal growth factors and are important for foetal and infant development [84].

A recent large cross-sectional study in 627 at term born infants found that both 20:4n-6 and 22:6n-3 in umbilical cord plasma PL were negatively related to weight-for gestational age scores at birth [85]. In other words 20:4n-6, the sum of the n-6 long-chain PUFA, 22:6n-3 and the sum of n-3 long-chain fatty acids were lower in infants born large for gestational age and higher in the smaller infants. But 20:5n-3 and 22:6n-3 were significantly higher in umbilical cord plasma of the neonates born at later gestational age. The DHA-status index (22:6n-3/22:5n-6) was higher in the plasma of infants born at a later gestational age and lower in those with a higher weight for gestational age at birth [85]. The inconsistency between this study and the previous studies [33;81;82] might be explained by the fact that gestational age at birth plays a important role. Indeed, preterm infants have significantly lower EFA and long-chain PUFA statuses than do full-term neonates [86;87].

4.4.5 Singleton versus multiple pregnancy

The concentrations (w%) of the long-chain PUFA, 20:4n-6 and 22:6n-3, in the wall of the umbilical artery and vein are significantly lower in infants born after multiple pregnancy (twins and triplets) compared to infants born after a singleton

pregnancy. The status of Mead acid on the other hand is much higher in twins and triplets [88]. The observed difference in EFA status between infants born after a singleton and after a multiple pregnancy supports the hypothesis that the maternal EFA supply to the developing foetus is limiting. Minor differences occur in the fatty acid composition of maternal plasma PL at delivery between multiplet mothers compared to mothers of singletons. The biochemical EFA status in the cord plasma and vessel walls of twins and triplets is lower than that observed in singletons. It was suggested that a larger total foetal tissue mass is related to an increased EFA accretion and the idea that the supply of EFA is limited [89]. This hypothesis has been confirmed by Rump *et al* [85].

4.4.6 Maternal diet and the effect of supplementation with n-6 or n-3 fatty acids

The developing foetus depends on active transport of the long-chain PUFA from the maternal circulation across the placenta and thus on the EFA and long-chain PUFA status of the mother. Cross country comparisons of cord blood fatty acid composition [64], composition of the cord following supplementation with specific fatty acids [90], and natural variations in diet within a population [91], indicate that the most important determinant of the fatty acid mixture delivered to the foetus is the fatty acid composition of the maternal diet. Therefore, the diet of a pregnant woman should contain sufficient amounts of EFA to cover her own requirement as well as that of her neonate.

It was shown that in a Dutch population dietary habits remained unaltered during pregnancy. Neither the amount and type of fat nor the fatty acid composition of the maternal diet changed from the early onset of pregnancy until delivery [73]. Moreover, Otto *et al* [65] observed no major significant changes in dietary fatty acid intake between the pre-pregnant period and week 10 of pregnancy.

There is epidemiological evidence that populations with a high fish intake (Faroe Islands and the Inuits) have a longer gestation, larger babies and reduced incidence of preeclampsia (pregnancy induced hypertension) compared to populations eating less marine food [92;93].

Dietary marine n-3 fatty acids may reduce the risk of preeclampsia by increasing the physiologically active prostacyclin:thromboxane ratio and by reducing blood pressure. However it was found that pregnant women suffering from pregnancy

induced hypertension have a higher DHA status after delivery compared to women with an uncomplicated pregnancy [94]. The DHA status in pregnancy induced hypertension increased between 32 weeks of gestation and delivery and can thus be considered a late phenomenon not contributing to the pathogenesis of preeclampsia [94].

There is a strong association between gestational age and the DHA concentration (w%) of umbilical plasma PL. In concurrence, DHA in plasma phosphatidylcholine of preterm infants correlates strongly with length of gestation [82]. These observations led to a series of fish oil supplementation studies during pregnancy.

Olsen and his group extensively studied the relationship between the maternal n-3 long-chain PUFA intake and preterm delivery. Initially their results were inconsistent. The first studies from Olsen and co-workers [95;96] showed that maternal dietary intake of n-3 fatty acids by supplementation with fish oil (2.7 g n-3 fatty acids per day) from the 30th week of gestation prolonged gestation with an average of 4 days and increased birth weight by 107 g as an average, as compared with supplementation with olive oil. The suggested explanation for this delayed delivery was that a high intake of n-3 fatty increases the production of n-3 derived eicosanoids (such as prostacyclin PGI_3 which relax the myometrium) at the expense of the n-6 derived eicosanoids (such as prostaglandins $F_{2\alpha}$ and E_2 which initiate labour). However in a later study by Olsen *et al* [97], when the intake of marine n-3 fatty acids during the second trimester was assessed by a semi-quantitative questionnaire no association was observed between gestational length and birth weight on the one hand and dietary intake of n-3 fatty acids on the other hand. Finally, their most recent prospective cohort study among almost 9000 pregnant women clearly demonstrated that length of gestation is positively related to the intake of n-3LCPUFA, and that low fish consumption is a risk factor for preterm delivery [98].

Supplementation with fish oil during the last trimester of pregnancy resulted in a significantly higher concentration of n-3 fatty acids and a decrease in n-6 fatty acids (especially linoleic acid but also AA) in maternal and umbilical plasma PL [90]. In addition the amount of Mead acid was significantly decreased in maternal plasma PL after fish oil supplementation compared to the placebo group. A positive correlation was found between the duration of pregnancy and the DHA status in umbilical plasma [90].

Increased consumption of n-3 fatty acids in the form of sardines and fish oil [99] during the last pregnancy trimester resulted in significantly higher DHA levels in maternal plasma and red blood cell PL compared to control mothers. Similarly DHA levels increased in the blood of the newborn both in plasma as in red blood cells. The length of pregnancy was not affected by this increased consumption of n-3 fatty acids [99].

It is not yet entirely elucidated whether DHA supplementation prolongs gestation. But many studies have shown that neonates born at later gestational age have higher DHA levels in their umbilical plasma. This can be explained by the concept that the maternal-foetal transfer of DHA improves with progressing gestation [75].

Pregnant Spanish women consuming more than 4 fatty fish meals per month, have significantly higher levels of EPA and DHA and significantly lower levels of AA in the PL fraction of their red blood cells at delivery compared to women consuming less than 2 fatty fish meals per month [100]. Similar differences were found in the PL fraction of the red blood cells of their neonates [100].

The effect on the neonatal long-chain PUFA status after administration of pregnant women from the second trimester with three low-dose fish oil supplements (336 mg, 528 and 1008 mg long-chain n-3 fatty acids) was determined by analysing the fatty acid composition of the umbilical arteria and vein [101]. Daily supplements of 528 or 1008 mg long-chain n-3 fatty acids significantly increased foetal long-chain n-3 fatty acid status without impairing the long-chain n-6 fatty acid status of the newborn [101]. The supplement containing 528 mg long-chain n-3 fatty acids was a milk-based supplement which contained also 171 mg 18:3n-3. This supplement resulted in a statistically insignificant higher status of 22:6n-3 compared to the 1008 mg long-chain n-3 fatty acid containing supplement with only 23 mg 18:3n-3 [101].

On the other hand, when pregnant women were supplemented with linoleic acid rich food products from the 20th week of gestation until delivery, the maternal linoleic acid status in plasma PL significantly increased [102]. The neonatal n-6 long-chain PUFA increased whereas the n-3 long-chain PUFA in umbilical plasma became significantly lower compared to that of the neonates of the non supplemented mothers.

Supplementation of pregnant women in their second trimester with 0.57 g DHA and 0.26 g 20:4n-6 for four weeks did not significantly reduce the total n-6 long-chain PUFA levels neither in plasma nor in erythrocyte PL, whereas the plasma levels of 22:6n-3 in the supplemented group had increased already after one week of supplementation and continued to be increased until the end of the intervention [72]. Thus a low dose of 20:4n-6 together with DHA (ratio AA:DHA in the supplement ≈ 1:2.2) increases the levels of DHA without decreasing the levels of total n-6 long-chain PUFA [72].

Recently, a large double-blind randomised study of 341 Norwegian pregnant women showed neither harmful nor beneficial effects of maternal supplementation with long-chain n-3 PUFA regarding pregnancy outcome, cognitive development or growth as compared with supplementation with n-6 fatty acids [103]. In this study, pregnant women were either supplemented with cod liver oil (rich in n-3 long-chain PUFA) or with corn oil (rich in n-6 PUFA). Maternal dietary supplementation with long-chain n-3 fatty acids did not affect gestational length, birth weight, and birth length or head circumference as compared with supplementation with n-6 fatty acids. However, it was observed that neonates with high DHA concentration in umbilical plasma PL had higher gestational age than neonates with low concentration of DHA [103]. There were no differences in cognitive functioning neither at 6 nor at 9 months of age between the two supplementation groups [103]. As expected, the sum of n-6 fatty acids in umbilical plasma PL was increased in the corn oil group whereas the sum of the n-3 fatty acids in umbilical plasma PL was increased in the cod liver oil group [103].

Thus it can be concluded that the n-3 and n-6 long-chain PUFA levels in umbilical plasma PL can be greatly influenced by supplementation during pregnancy [90;99;102;103]. The higher the concentration in the maternal diets the higher the levels of these fatty acids in maternal plasma and in umbilical plasma. Therefore when one wants to increase the neonatal EFA status by maternal dietary supplementation it is advisable to increase both the intake of n-6 and n-3 PUFA.

4.4.7 The effect of long-chain PUFA supplementation in infants born preterm and at term

Two years ago, Simmer reviewed the literature to assess whether supplementation of formula with long-chain PUFA is safe and whether it is of benefit to preterm and at term born infants [104;105].

There is some evidence that long-chain PUFA supplementation of preterm infants increases the rate of visual maturation. However the differences between supplemented and control infants are small and of little clinical significance [104]. It was concluded that data from different randomised clinical trials do not demonstrate a long-term benefit to preterm infants after supplementation with formula with long-chain PUFA [104]. Providing an optimal ratio of 18:2n-6 to 18:3n-3 and sufficient 18:3n-3 for infants to synthesise their own DHA may be adequate. No harm has been demonstrated with respect to growth when formula are supplemented with a balance of n-3 and n-6 long-chain PUFA [104]. The justification for adding long-chain PUFA to formula should be based on the rationale of mimicking the composition of human milk and not on evidence that important clinical benefits have been demonstrated. A supplement with approximately 0.6% 20:4n-6 and 0.4% 22:6n-3 will achieve this [104].

An advantage in visual development was found in at term born infants supplemented with 0.36% DHA compared to infants fed the control formula with only 1.5% α-linolenic acid. This relatively low concentration of 18:3n-3 may have limited the infants' ability to synthesis DHA [105]. When the concentration of α-linolenic acid is increased and the ratio of LA/ALA reduced in the control formula, no difference was found in visual maturation between supplemented and controls infants [105].

There is no evidence that long-chain PUFA supplements impair the growth of term infants because in the current studies a balance of n-3 and n-6 fatty acids is used which is unlikely to result in low arachidonic acid levels and therefore less likely to reduce growth [105].

Both reviews conclude that a large long-term randomised trial is needed to determine whether long-chain PUFA are essential for preterm or at term born infants and whether the fatty acid composition of infant formulas is a determinant of intelligence [104;105].

5. Brain development

The brain is a structural-lipid rich organ that uses highly unsaturated fatty acids, particularly AA and DHA, for structure and function [80;106]. In 2-week-old suckling rats, tests with ^{14}C- and tritium-labelled parent, precursor and product fatty acids showed that the proportions of brain 20:4n-6 derived from 20:4n-6 itself was $\geq$ 10-fold greater than that recovered from the precursor linoleic acid. Similar results were described for a comparison of DHA and its precursor α-linolenic acid [107;108]. Recently these findings were confirmed in foetal primates [41]. In that study, [^{13}C]18:3n-3 or [^{13}C]22:6n-3 was administered to baboon foetuses as nonesterified fatty acid via injection into the jugular artery. DHA in foetal plasma was about 8 times more effective as a substrate for brain DHA accretion compared with its precursor 18:3n-3 [41].

Administration of [^{13}C]α-linolenic acid to rat pups during brain development showed that several times more α-linolenic acid was used for synthesis of brain cholesterol rather than for that of brain DHA. The isotopic data suggest that at least 5 times more α-linolenic acid carbon chain that reaches the brain is used for cholesterol rather than for de novo DHA synthesis [109]. The brain does not import cholesterol but synthesises its own [110]. Cholesterol synthesis is critical for brain development and it seems that α-linolenic acid is more important to the brain as a substrate for cholesterol than for brain DHA [108]. This phenomenon is known as carbon recycling.

PUFA are needed for normal neonatal brain development [15]. Brain lipids contain only trace amounts of linoleic and α-linolenic acid but are extremely rich in long-chain PUFA such as DHA. DHA is the major n-3 fatty acid in the mammalian brain and retina. The majority of DHA is incorporated in the brain during the brain growth spurt, which starts in humans at 26 weeks of gestation until about 2 years of age. An adequate supply of maternal DHA is necessary to support optimal neurobiological development of both foetus and infant [111].

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Chapter 2

Estimation of dietary fat intake of Belgian pregnant women: comparison of two methods

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Estimation of Dietary Fat Intake of Belgian Pregnant Women

Comparison of Two Methods

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Key Words
Dietary fat intake · Food frequency questionnaire · Seven-day estimated food record · Pregnancy · Validity

Abstract
Aim: To evaluate the validity and usefulness of a food frequency questionnaire (FFQ) which was designed to evaluate individual fat consumption for a Dutch population relative to 7-day estimated records (7d ER). The FFQ has been validated previously and was adapted to the Belgian situation. ***Methods:*** Longitudinal study in 26 healthy pregnant women; FFQ and 7d ER were obtained during the 1st and 3rd trimesters. ***Results:*** FFQ was validated with 7d ER. Fat and fatty acid intake estimated by the FFQ did not differ significantly ($p < 0.01$) from data obtained by the 7d ER except for 18:2n–6. Pearson correlation coefficient between the 2 methods ranged from 0.62 to 0.68. On average, 47% of the women were classified in the same quartile with the 2 methods and less than 2% in the opposite quartile. Total fat intake, calculated from FFQ, was on average 87.9 (SD 18.1) g/day. The mean intake of linoleic acid was 13.3 (SD 5.4) g/day and of α-linolenic acid was 1.4 (SD 0.5) g/day. The dietary intake of the saturated, monounsaturated and polyunsaturated fatty acids was respectively 34.7 (SD 10.0) g/day, 29.6 (SD 8.1) g/day and 15.7 (SD 5.9) g/day. ***Conclusion:*** The FFQ gives similar results for fat intake as the 7d ER and is thus considered an appropriate method for classifying individuals to the right part of the distribution of dietary fat intake.

Introduction

In pregnancy studies, it is important to accurately estimate nutrient consumption and relate it to the neonatal outcome. We focus on the importance of essential fatty acids on pregnancy outcome. Therefore, it is important to know exactly the daily intake of the individual fatty acids during the course of pregnancy. The 7-day estimated record (7d ER) is a very time consuming method whereas the food frequency questionnaire (FFQ) method is an easy and cheap method to evaluate dietary intake of a population. An additional advantage of FFQ is that it covers the nutrient intake over a longer time-period compared to 7d ER. In this study we used a FFQ which was specifically designed to collect data on the fat consumption of Dutch pregnant women [1]. Minor changes were used to adapt this Dutch FFQ to the Belgian diet, in order to enable us to focus on the fat and fatty acid intake of pregnant Bel-

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gian women. The aim of the present study was to determine the relative validity and usefulness of a Dutch FFQ [1] adapted to the Belgian diet by comparing dietary fat intake data collected by this FFQ with the 7d ER in a group of pregnant Belgian women during the first and third trimesters of pregnancy. Biological markers to assess the validity were not used in this study. Later, we will compare the results of the FFQ with the fatty acid composition of serum phospholipids [in preparation].

Methods

Study Population

At their first antenatal medical visit, healthy pregnant women attending the Department of Gynaecology of Ghent University Hospital, Belgium were asked to cooperate in this study. All pregnant volunteers signed a written informed consent form, approved by the ethics committee of Ghent University Hospital. Only singleton pregnancies were included. Inclusion criteria were: first pregnancy, diastolic blood pressure below 90 mm Hg, not diabetic, no proteinuria and not suffering from renal or cardiovascular disease. A food frequency questionnaire was filled in twice during the course of pregnancy together with a dietician: between 6 and 22 weeks of gestation (median 15 weeks) and between 32 and 40 weeks (median 35 weeks). Gestational age was calculated from the self-reported first day of the last menstrual period. If the last menstrual period was unknown, gestational age was based on early ultrasound measurements. The pregnant women were asked to complete accurately a food diary for 1 week (7d ER) at these points during their pregnancy. Of the 33 pregnant women willing to participate, 29 women completed twice the 7-day estimated food record and the FFQ.

FFQ

Dietary intake was assessed by a FFQ developed by Al [1], which was specifically designed to collect data on the fat consumption of Dutch pregnant women. The Department of Public Health (Ghent University Hospital) adapted this Dutch FFQ to the Belgian diet and developed a colored photographic booklet as a tool to estimate portion sizes of different foods and courses. The main objective to this FFQ is to estimate the dietary intake of fat and fatty acids in the preceding month. A list of 180 of the most commonly consumed Belgian fat-containing foods are included in the FFQ. The women were visited at home by the same experienced dietician and asked whether they consume those products daily, weekly or monthly and how many units they normally eat of that particular food. At the end of the questionnaire, some additional questions were asked which gave information about the kind of fat used for cooking and the frequency of intake and the amount of fat normally bought per week or month and the number of individuals that generally eat in the same household.

7d ER

For the purpose of assessing overall energy and nutrient intake with high precision on individual level, a 7-day estimated food record method was carried out [2]. For this purpose, a structured food diary was developed, in which each meal (breakfast/morning snack/lunch/afternoon snack/dinner/evening snack) was explicitly mentioned per day. As far as the food groups/food items were concerned, the diary did not contain any predefined entries. For each food item consumed, subjects were asked to give information about time of the day, amount, branch, and location of consumption. For homemade dishes, extra information was asked on ingredients and type of amount of fat. The respondents were given detailed instructions beforehand concerning the information needed for the diary. Special attention was thereby given to portion sizes and to the usual household measures (spoon, cups, glasses, etc.) used by the respondents. The diary was checked for quality and completeness of the information every 2 days by experienced dieticians in the presence of the respondents. The recorded foods were translated into quantities of over 1,400 food items and analysed for nutrient composition. The nutrient estimates from the 7d ER were converted into data for 1 day simply by dividing by 7.

Macronutrients (proteins, carbohydrates and fat) were calculated by self-developed nutritional software based on the Dutch Food Composition Tables [3] and on food consumption data available in Belgium for local food items (e.g. certain margarines).

Nutrient Analysis

Nutrient analysis was performed on the basis of Unilever nutritional software [4]. For the detailed fatty acid analysis (α-linolenic acid, arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid) of the FFQ, a database developed by Staessen et al. [5, 6] was used.

Reliability of the Food Intake Data

The reliability of the food intake data was estimated by comparing the calculated physical activity level (PAL) with published results. The PAL was calculated by dividing the reported energy intake (from 7d ER) by the estimated basal metabolic rate (BMR), which was obtained with the Schofield formula [7]. For calculation of the PAL value during the third trimester, an energy cost for pregnancy of 180 kcal/day [8] was taken into account. Based on measurements with doubly labelled water, PAL values of 1.4–1.7 for seated work and 2.0–2.4 for heavy physical activity have been described [9]. A PAL value lower than 1.35 is likely to indicate an underestimation of habitual dietary intake. Therefore, this value was used to calculate the lower cut-off value [10, 11]. Subjects with PAL values higher than 2.4 were considered overreporters [11]. This should have led to the exclusion of dietary intake data from 11 pregnant women (PAL < 1.35). However, we decided not to be so strict and only exclude those women with a calculated PAL value lower than 1 ($n = 3$). Thus, the final study population consisted of 26 pregnant women.

Statistics

Daily nutrient intakes of all pregnant women were calculated. Normality of distribution was ascertained with the Kolmogorov-Smirnov test. Values are reported as mean and standard deviation of the mean in parentheses (table 1). To assess the relative validity of the FFQ three different statistical tests were performed [12]. Firstly, it was tested whether the two methods did not result in significant differences in dietary fat intake, using the paired Student's t test. $p < 0.01$ was taken as the criterion of significance. Secondly, it was tested how well the results from the two measurements correlated with each other by calculating the Pearson correlation coefficients. Finally, the women were divided into quartiles according to their fat intake assessed by 7d ER and FFQ. When the two measurements classified the subjects in the same or in an adjacent quartile, this was inter-

Table 1. Nutrient intake per day, measured by 7d ER

	1st trimester	3rd trimester	PRI [6, 12]
Energy, kcal	2,157 (362.5)	2,194 (449.5)	
Proteins, en%	16.0 (1.9)	15.4 (2.0)	14
Carbohydrates, en%	46.1 (5.3)	47.0 (4.2)	55–75
Fat, en%	36.5 (5.6)	36.2 (4.7)	15–30
SF, en%	14.8 (2.2)	14.6 (2.5)	<10
MUF, en%	13.6 (2.6)	13.2 (2.0)	–
PUF, en%	5.9 (1.5)	6.1 (1.7)	3–7

Values are reported as mean (SD) (n = 26). Comparison is made with the Belgian Population Reference Intake (PRI) [6, 12].

No significant differences ($p < 0.01$) in nutrient intake or energy distribution between the 1st and 3rd trimesters.

preted as a good classification. When the subjects were classified from one extreme quartile to the other extreme quartile, this was considered as a gross difference in classification. The data were analysed using the MedCalc statistical program, version 6 (MedCalc Software, Mariakerke, Belgium) [13].

Results

Clinical Characteristics of the Study Population

The study population consisted of 26 pregnant Belgian women with a mean age at delivery of 30 years (range 25–37 years). The PAL value (calculated as described previously) ranged from 1.1 to 2.1 (mean 1.56) in the first trimester and from 1.0 to 2.1 (mean 1.45) in the third trimester. The mean Body Mass Index of the women before pregnancy was 22.0 kg/m^2 (range 17.6–29.3 kg/m^2) and mean weight gain at delivery was 15 kg (range 9–20 kg). All pregnant women were nulliparous, all pregnancies were uncomplicated and the infants were born healthy and at term with a mean gestational age of 39.0 weeks (range 38–42 weeks). The mean birth weight of the neonates was 3,270 g (range 2,300–4,020 g) and the mean birth length was 50.7 cm (range 47–54 cm). The Apgar score was measured 1 and 5 min after birth, it is the sum (max. 10 points) of points gained on assessment of the heart rate, respiratory effort, muscle tone, reflex irritability, and color. The median Apgar score 1 min after birth was 8 (range 4–9) and 5 min after birth 9 (range 6–10). The sex ratio of the infants was 16 males and 10 females.

7d ER

From the 7d ER, total energy intake was calculated. Daily energy intake and energy distribution from the first and third trimesters are summarized in table 1 and compared with the Belgian population reference intake (PRI) [8, 14]. The daily caloric intake in our study population is on average 2,175 kcal/day. The average caloric intake only slightly increased (37.3 kcal/day as an average) between the first and third trimesters of pregnancy, significance was not reached. The mean daily intake of carbohydrates (% of energy intake, en%) was below the PRI. Only one woman had a carbohydrate intake higher than 55 en% and 5 women had an intake above 50 en%. 22 of 26 pregnant women (85%) had a protein intake higher than the recommended intake of 14 en% [8]. The mean daily intake of total fat (en%) and saturated fat (SF) was above the PRI. Only two women had a total fat intake lower than 30 en% as recommended and only one woman had a SF intake lower than the recommended maximal level of 10 en%. Mean daily intake of polyunsaturated fat (PUF) met the PRI. No recommendations have been made for monounsaturated fat (MUF). However, with recommendations of maximum 30 en% of total fat, maximum 10 en% SF and 3–7 en% PUF, MUF should represent about 13–17 en%. The mean daily cholesterol intake of this study population is 260 (SD 73.1) mg/day. Eight of 26 pregnant women (31%) had a daily cholesterol intake higher than the maximum recommended daily allowance of 300 mg.

FFQ

Since the FFQ was developed to estimate fat intake, total energy intake could not be calculated. Therefore, intake of total fat, the sum of the saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) and the individual fatty acids (18:2n–6, 18:3n–3, 20:4n–6, 20:5n–3, and 22:6n–3) are expressed as gram per day. Table 2 compares the dietary fat and fatty acid intake calculated with the FFQ between the first and third trimesters of pregnancy. No significant differences ($p < 0.01$) were found either in total fat intake or in intake of individual fatty acids between the first and third trimesters. Additionally, strong and significant correlations between the first and third trimesters for total fat ($r = 0.57$; $p < 0.01$), SFA ($r = 0.47$; $p < 0.01$), MUFA ($r = 0.61$; $p < 0.001$), PUFA ($r = 0.73$; $p < 0.0001$) and sum of n–6 fatty acids ($r = 0.74$; $p < 0.0001$) estimated with the FFQ were found.

Total fat intake was on average 87.9 (SD 18.1) g/day. The most dominant n–6 fatty acid in the diet was the essential fatty acid, linoleic acid, with a mean intake of 13.3 (SD 5.4) g/day and the dominant n–3 fatty acid in the diet was α-linoleic acid with an average intake of 1.4 (SD

Table 2. Total fat and fatty acid intake (g/day) measured by the 7d ER and FFQ during the 1st and 3rd trimesters of pregnancy

	1st trimester		3rd trimester	
	7d ER	FFQ	7d ER	FFQ
Total fat	87.4 (19.6)	85.9 (28.3)	88.5 (22.5)	90.2 (25.0)
SFA	35.3 (7.8)	34.1 (12.1)	35.9 (10.2)	35.3 (11.4)
MUFA	32.5 (8.3)	28.8 (9.6)	32.1 (8.3)	30.5 (8.3)
PUFA	14.2 (4.2)	15.2 (6.2)	15.1 (5.5)	16.1 (6.4)
Sum n–6		13.2 (5.7)		14.0 (5.9)
Sum n–3		1.82 (0.59)		1.98 (0.59)
18:2n–6	10.3 (3.9)	12.9 (5.7)**	11.4 (4.7)	13.7 (5.9)*
18:3n–3		1.3 (0.53)		1.5 (0.55)
20:4n–6		0.13 (0.04)		0.13 (0.04)
20:5n–3		0.17 (0.11)		0.15 (0.09)
22:6n–3		0.30 (0.20)		0.30 (0.19)

Values are reported as mean (SD) (n = 26). No significant differences ($p < 0.01$) in fat intake between the 1st and 3rd trimesters estimated by either FFQ or 7d ER. Significant difference in linoleic acid intake estimated by FFQ compared with 7d ER: ** p = 0.005 and * p = 0.01.

Table 3. Pearson correlation coefficients for total fat and fatty acid intake between the values calculated from 7d ER vs. FFQ (n = 52)

	7d ER vs. FFQ	p
Total fat	0.64	***
SFA	0.63	***
MUFA	0.62	***
PUFA	0.68	***
18:2n–6	0.66	***

Significance of the Pearson correlation coefficients: *** $p < 0.0001$.

0.46) g/day. The dietary intake of the longer chain n–3 fatty acids, 20:5n–3 and 22:6n–3, correlate strongly with each other: $r = 0.94$; $p < 0.0001$.

Validation of FFQ with 7d ER

There are no significant differences ($p < 0.01$) in daily dietary intake (g/d) throughout pregnancy of total fat, SFA, MUFA and PUFA calculated by FFQ versus the values obtained with the 7d ER (table 2). But the intake of linoleic acid was significantly ($p < 0.01$) lower calculated from the 7d ER compared to FFQ. In table 3, the Pearson correlation coefficients for the intake of total fat, SFA, MUFA, PUFA and linoleic acid between FFQ and 7d ER are summarised. All correlations were strong and highly significant ($p < 0.001$).

Table 4 gives the comparison of the classification of fat intake into quartiles according to FFQ with that according to 7d ER. The two methods classified more than 83% of the study population in the same or in an adjacent quartile. In total, less than 2% of the study population had a gross difference in classification (from one extreme quartile to the other).

Discussion

This work illustrates that macronutrient intakes as assessed by the 7d ER do not change significantly in Belgian women during normal pregnancy. In an other publication (in preparation) we will elaborate on the food and nutrient (macro- and micronutrient) intake of Belgian pregnant women. In this study we focused on the dietary fat intake (measured with a Dutch FFQ [1] adapted to the Belgian situation) during normal pregnancy. No significant changes between the first and third trimesters were found for total fat or for the individual fatty acid intake. This finding is in agreement with other studies in preg-

Table 4. Classification into quartiles for the comparison of total fat and fatty acid intake (g/day) measured by the 7d ER vs. FFQ (n = 26)

	Good classification 1st trimester	Grossly misclassification 1st trimester	Good classification 3rd trimester	Grossly misclassification 3rd trimester
Total fat	20	2	23	0
SFA	19	1	23	0
MUFA	21	0	21	0
PUFA	22	0	24	0
18:2n–6	22	0	22	1

Good classification = When classified in the same or in an adjacent quartile; grossly misclassification = when classified from one extreme quartile to the other extreme quartile.

nant women using FFQ [15, 16]. Others found a reduced fat intake in late pregnancy compared to mid-pregnancy as measured by the 7-day weighed dietary record [17]. Brown et al. [18] observed a higher energy and nutrient intake during mid-pregnancy compared to pre-pregnancy. The values for the daily fat intake in our group of pregnant women were in concurrence with other reports [15, 18–20]. Few pregnancy studies evaluated the intake of the individual essential fatty acids and their longer chain homologues. In a group of Dutch pregnant women (n = 176) a daily linoleic acid intake of 15.4 (SD 10.3) g/day in the first trimester and 14.3 (SD 13.6) g/day in the third trimester was reported [15]. These values are comparable to those found in our study population. Lakin et al. [19] found in healthy omnivorous pregnant women a mean intake of n–3 fatty acids of 1.7 (SD 0.29) g/day and a mean intake of eicosapentaenoic acid of 0.14 (SD 0.13) g/day. These findings are consistent with our results. Our study population has higher mean intakes of n–6 fatty acids (13.6 vs. 9.9 g/day), linoleic acid (13.3 vs. 9.5 g/day) and docosahexaenoic acid (300 vs. 173 mg/day) and a lower mean intake of arachidonic acid (129 vs. 198 mg/day) compared to the British omnivorous women [19].

To assess the relative validity of the FFQ, the estimated nutrient intake should be compared with that of a more accurate method. However, there is no gold standard easily applicable for the assessment of dietary intake. Therefore it is important that the errors of the FFQ and the comparing method are as independent as possible to avoid high false estimates of validity [12]. The major sources of error with FFQ are due to restrictions imposed by memory and perception of portion sizes. We used a 7d ER which is likely to have the least correlated errors as this method does not depend on memory (recorded after each meal). Furthermore, for the analysis of the sets of data obtained by the two measurements, different food composition databases were used, which also excludes a correlated source of error. Only one dietician was involved in this study so that observer differences can also be excluded. We found strong and significant correlations (range 0.62–0.68) between the two methods for daily dietary fat intake. In the literature, similar correlation coefficients for total fat intake were described: between FFQ and dietary history, r = 0.78 [21], between FFQ and 7-day weighed record, r = 0.59 [22], between FFQ and 4-day estimated record, r = 0.44 [20], and between FFQ and 14-day weighed record, r = 0.42 [23]. This indicates that the Dutch FFQ which we adapted to the Belgian diet is suitable to assign pregnant women to the appropriate part of the distribution of dietary fat intake. Furthermore, the classification into quartiles was acceptable and much better than would be expected due to chance (25% in the same quartile, 37.5% in an adjacent quartile and 12.5% classified in the opposite quartile). On average, 47% of the subjects were classified in the same quartile and less than 2% were classified in the opposite quartile. These values compare favorably with published values: 34% classified in the same and 8% in the opposite quartile [20], 38% in the same and 3% in the extreme quartile [23], 72% in the same quintile and 3% in the opposite quintile [21], and 45% in the same tertile and 10% in the extreme tertile [22]. No significant differences ($p < 0.01$) in fat intake assessed by FFQ versus 7d ER occurred except for linoleic acid. The daily intake of linoleic acid was consistently lower as assessed by the 7d ER compared to the estimation with the FFQ. This is probably due to the fact that the food composition database used to analyse the 7d ER, lacks a lot of data concerning the linoleic acid content of different foods. It can be concluded that the FFQ in conjunction with the individual fatty acid composition database of Belgian foods [5, 6] is an adequate method to reasonably rank subjects according to their dietary fat intake.

In summary, the Dutch FFQ [1], which we adapted to the Belgian situation, is a valid method which is able to assign subjects to the appropriate part of the distribution of dietary fat intake. As the time required for administrating the FFQ (± 90 min) is much shorter than completing a 7d ER, this method will be used frequently in the future.

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Addendum to chapter 2.

In the publication (Ann. Nutr. Metab. 2001, 45:273-278) described in chapter 2 we validated a food frequency questionnaire (FFQ) with the seven day estimated record (7-day ER).

Different food composition databases were used to analyse the sets of data obtained by the two measurements to exclude a correlated source of error (the errors of the FFQ have to be as independent as possible to avoid high false estimates of validity). The data obtained with the FFQ were analysed with the database developed by Staessen *et al* whereas the data obtained with the 7-day ER were analysed with the database from Unilever (1992).

Only for linoleic acid a significant difference was found between the two measurements. One of the reviewers of this thesis suggested that the discrepancy between these two measurements could be due to the different nutrient databases used during the analysis. Therefore we analysed the two different data sets with the same Unilever nutrient database.

The results are summarised in the following table. When the Unilever nutrient database is used to calculate the linoleic acid intake from the FFQ and from the 7-day ER, no significant differences in fat and fatty acid intake were observed. The correlation coefficient between the two measurements analysed with the same nutrient database for linoleic acid was $r=0.65$, $P<0.0001$ and compares favourably with the correlation coefficient between the two measurements analysed with the two different databases ($r=0.66$, $P<0.0001$).

Table: Total fat and fatty acid intake (g/day) measured by the 7-day ER and FFQ during the first and third trimester of pregnancy.

	1st trimester		3rd trimester	
	7-day ER	FFQ	7-day ER	FFQ
Total fat	87.4 (19.6)	84,8 (27,4)	88.5 (22.5)	88,8 (24,5)
SFA	35.3 (7.8)	33,4 (11,6)	35.9 (10.2)	34,8 (11,3)
MUFA	32.5 (8.3)	31,4 (10,6)	32.1 (8.3)	32,5 (9,1)
PUFA	14.2 (4.2)	14,2 (5,5)	15.1 (5.5)	15,4 (5,7)
18:2n-6	10.3 (3.9)	11,7 (5,0)	11.4 (4.7)	12,8 (5,1)

Both the datasets obtained either with the 7-day ER or with the FFQ were analysed with the same database (Unilever database, 1992).

Chapter 3

Does nutrient intake of healthy Belgian pregnant women differ from recommendations ?

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Chapter 3: Does nutrient intake of healthy Belgian pregnant women differ from recommendations ?

1. Abstract

The objectives: (1) to document the energy intake and the daily dietary intake of macronutrients and micronutrients of a small sample of healthy pregnant women; and (2) to evaluate the nutrient intake by comparing this with the dietary recommendations for pregnant women.

Design and Setting: longitudinal study in 27 healthy pregnant women; seven day estimated record (7 day ER) obtained during the first half of pregnancy and during the third trimester.

Results: Energy and nutrient intake did not differ between the two examination periods during pregnancy. Therefore the average energy and nutrient intake during pregnancy was calculated and is reported in detail. Energy intake during pregnancy was 2148 (SD 386) kcal/d. Protein intake was 1.39 (SD 0.3) g/kg BW/d. Carbohydrate intake (% of energy intake, en%) and total fat intakes were 46.6 (SD 4.1) en% and 36.3 (SD 4.5) en%, respectively. Saturated fatty acids provide 14.7 (SD 1.9) en%, monounsaturated fatty acids 13.3 (SD 2.1) en% and polyunsaturated fatty acids 6.0 (SD 1.4) en%. The dietary intake of vitamins and minerals during pregnancy is described in detail and compared with the Belgian population reference intake.

Conclusion: Pregnant women had an adequate protein intake. Complex carbohydrates should partly replace saturated fat. Some pregnant women could benefit from a balanced multivitamin/mineral supplement.

2. Introduction

The nutrient needs of a pregnant woman are higher than at any other time in her life. The developing foetus depends entirely on the nutrients supplied by the mother's diet, thus good maternal nutrition during pregnancy is important both for the mother as for a healthy foetal development. The pregnant woman needs additional nutrients, including calories, proteins and certain vitamins and minerals. The present study is part of a larger investigation in pregnant women. As described further on we have evaluated the essential fatty acid status of pregnant women and their neonates by analysing the fatty acid composition of maternal and umbilical cord plasma [1;2]. The aim of the present study was (1) to document the energy intake and the daily dietary intake of macronutrients and micronutrients of these healthy pregnant women; and (2) to evaluate their nutrient intake by comparing this with the dietary recommendations for pregnant women.

With a seven day estimated food record (7 day ER) it is possible to calculate the daily dietary intake of macro- and micronutrients. As the purpose of this study was to describe the nutrient intake during the course of pregnancy we asked the pregnant women to fill in a seven day food diary during the first half of pregnancy and during the third trimester. The average of the two estimated nutrient intake values was calculated and compared with dietary recommendations from the Belgian [3;4] or the Dutch Health Council [5;6].

3. Methods

3.1. Study population and study design

The study population was recruited from the pregnant women attending the Department of Gynaecology of Ghent University Hospital, Belgium in the period from March 1997 to June 1999. At their first antenatal medical visit, the pregnant women were asked to cooperate in this study. Only singleton pregnancies were included. Inclusion criteria were: first pregnancy, diastolic blood pressure below 90 mm Hg, not diabetic, no proteinuria and not suffering from renal or cardiovascular disease. Thirty pregnant women volunteered to cooperate in this part of the study (the food consumption survey). All pregnant volunteers signed a written informed consent form, approved by the ethics committee of Ghent

University Hospital. The pregnant women were asked to complete accurately a food diary for one week (seven day estimated record, 7 day ER) between 6 and 22 weeks of gestation (median 15 wks) and between 32 and 40 weeks (median 35 wks). Length of gestation (in weeks) was calculated from the recorded date of delivery and the self-reported first day of the last menstrual period. If the last menstrual period was unknown gestational age was based on early ultrasound measurements.

3.2. Seven day estimated record

For the purpose of assessing overall energy, macro- and micronutrient intake with high precision on an individual level, a 7 day ER was carried out [7]. For this purpose, a structured food diary was developed, in which each meal (breakfast/morning snack/lunch/afternoon snack/dinner/evening snack) was explicitly mentioned per day. As far as the food groups/food items were concerned, the diary did not contain any predefined entries. For each food item consumed, subjects were asked to give information about time of the day, amount, branch, and location of consumption. For homemade dishes, extra information was asked on ingredients and type and amount of fat. The respondents were given detailed instructions on beforehand concerning the information needed for the diary. Special attention was thereby given to portion sizes and to the usual household measures (spoon, cups, glasses, etc.) used by the respondents. Experienced dieticians, in the presence of the respondents, checked the diary on quality and completeness of the information every 2 days. The recorded foods were translated into quantities of over 644 food items and analysed for nutrient composition. The nutrient estimates from the 7 day ER were converted into data for 1 day by dividing by 7. Nutrient analysis was performed on the basis of Unilever nutritional software [8] based on the Dutch Food Composition Table [9] and on food composition data available in Belgium for local food items (e.g. certain margarines). The Dutch Food Composition Table is more extended than the Belgian Food Composition Table.

3.3. Reliability of the food intake data

The most common reason of the observed bias in self report dietary intake methods is that the procedure is regarded as a burden, which probably promotes underreporting of dietary intake [10]. The reliability of the food intake data was

estimated by comparing the calculated physical activity level (PAL) with published results. The PAL was calculated by dividing the reported energy intake (from 7 day ER) by the estimated basal metabolic rate (BMR), which was obtained with the Schofield formula [11]. For calculation of the PAL value during the third trimester, an energy cost for pregnancy of 180 kcal/d [4] was taken into account. Based on measurements with doubly labelled water, PAL-values of 1.4 – 1.7 for seated work and 2.0 – 2.4 for heavy physical activity have been described [12]. Goldberg, Black and colleagues [13;14] developed a tool to detect the underreporters. They calculated cut-off values based on the ratio of mean reported energy intake and basal metabolic rate, as estimated from weight and height using the Schofield equations [11], with the WHO recommended physical activity level for light activity. Based on doubly-labelled water measurements it is highly unlikely that any normal healthy free living person could habitually exist at a PAL of less than 1.35. Goldberg *et al* [14] suggested that energy intake data lower than 1.35 x BMR as estimates of habitual intake can be rejected (cut-off 1). Furthermore they [14] developed a more specific method (cut-off 2) which takes into account the study period, sample size and use of observed or predicted BMR. For a 7 day measurement in a single individual when the BMR is estimated with Schofield equations, recorded intakes have to be below 1.10 x BMR before they can safely be rejected (cut-off 2). In the population under study 11 women reported an energy intake lower than 1.35 x BMR (cut-off 1) and 3 women reported an energy intake lower than 1.1 x BMR (cut-off 2). We decided to follow the most specific method and excluded those women with a calculated PAL-value lower than 1.1 (n=3). Subjects with a PAL-value higher than 2.4 are considered overreporters [15]. None of the pregnant women had a reported energy intake higher than 2.4 x BMR. Thus the final study population consisted of 27 pregnant women.

3.4. Statistics

Energy and daily macro- and micronutrient intakes of each pregnant woman were calculated from the 7 day ER. The within and between subject variation was calculated by means of coefficient of variation (CV %). Normality of distribution was ascertained with the Kolmogorov-Smirnov test. Values are reported as mean and standard deviation (SD) in parentheses. Comparisons of nutrient intake between the first half of pregnancy and the third trimester were performed with the paired students' t-test. A value of $P<0.01$ was taken as the criterion of

significance. The data were analysed using the MedCalc statistical program, version 6 (MedCalc Software, Mariakerke, Belgium) [16].

4. Results

4.1. Clinical characteristics of the study population

The study population consisted of 27 pregnant Belgian women with a mean age at delivery of 30 years (range 25 - 40 years). The mean Body Mass Index of the women before pregnancy was 22 kg/m^2 (range 18 – 29 kg/m^2) and mean weight gain at delivery was 14 kg (range 8 - 21 kg). All pregnant women were nullipara, all pregnancies were uncomplicated and the infants were born healthy and at term with a mean gestational age of 39.0 weeks (range 38 – 42 weeks). The mean birth weight of the neonates was 3285 g (range 2300 - 4020 g) and the mean birth length was 51.0 cm (range 47 - 54 cm). The sex ratio of the infants was 15 males and 12 females.

4.2. Nutrient intake

Daily energy intake and the energy distribution (macronutrient intake) during the first half of gestation and third trimester were calculated from the 7 day ER. The 7 day ER enabled us also to calculate the vitamin and mineral intake at the two examinations during pregnancy. The students' t-test revealed that there are no significant differences neither in caloric intake, macronutrient nor micronutrient intake between the two examinations during pregnancy. Therefore we calculated the average intake of macro- and micronutrients during the course of pregnancy.

Daily energy intake and the energy distribution during pregnancy are summarised in Table 1 and compared with the Belgian Population Reference Intake (PRI) [3] and with the Dutch dietary reference intake values [5;6].

The between subject variation for macronutrient intake varied from 8.8% to 30.8% with an average CV of 17.6%. The within subject variation for macronutrient intake varied from 5% to 11% with a mean CV of 8.1%.

The energy intake during pregnancy was 2148 (SD 386) kcal/d. This study population had a carbohydrate intake of 250 (SD 52) g/d and a fat intake of 87 (SD 19) g/d. Dietary recommendations for protein intake are best expressed as g/kg body weight/d and the PRI for adults in Belgium is 0.75g/kg BW/d. A net protein

utilisation of 0.7 has to be considered and during pregnancy an extra protein need of 14g/d is recommended. Thus for a pregnant women with a body weight of 65 kg the Belgian Health Council recommends a daily protein intake of (65 x 0.75/0.7) + 14 = 84 g/d [3;5]. The Dutch Health Council has for protein intake a recommended dietary allowance (RDA) of 9 en% or 62 g/d for pregnant women and a tolerable upper intake level (UL) of 25en% [5]. The daily protein intake in our study population was 1.39 (SD 0.3) g/kg BW/d or 84.3 (SD 15.7) g/d.

Table 1: Average macronutrient intake measured by 7 day estimated record at the two examinations during pregnancy. Values are reported as mean (SD) (n = 27).

	Diet of study population	Between subjects CV (%)	Within subject CV (%)	Belgian PRI [3]	Dutch dietary reference intakes [5]
Energy (kcal/d)	2148 (386)	18	8	2250+180=2430 (a)	2727
Energy (MJ/d)	8.98 (1.61)	18	8	10.2	AR: 10.2+1.2=11.4
Proteins (en%)	15.8 (1.8)	11.6	5	± 10	RDA: 9 UL: 25
Proteins (g/kg BW/d)	1.39 (0.3)	23	9	0.75	-
Proteins (g/d)	84.3 (15.7)	18.6	9	70 + 14 = 84 (b)	RDA: 62
Carbohydrates (en%)	46.6 (4.1)	8.8	5	> 55	RDA: > 40
Fat (en%)	36.3 (4.5)	12.5	7	15 – 30	AI: 20 – 40 (c) AI: 20 – 30/35 (d)
SFA (en%)	14.7 (1.9)	13.1	10	< 10	< 10 (UL)
MUFA (en%)	13.3 (2.1)	15.8	7	-	-
PUFA (en%)	6.0 (1.4)	23.9	10	3 – 7	< 12 (UL)
MUFA+PUFA (en%)	19.3 (3.2)	16.7	8		AI: 8 – 38 (c) AI: 8 - 28/33 (d)
18:2n-6 (en%)	4.5 (1.4)	30.8	11	3 - 5	AI: 2.5

-: not given. (a): recommended daily energy intake for women between 18 and 29 years with a body weight of 65 kg and PAL of 1.56 (= light activity level). From the tenth week of pregnancy: extra energy cost of 180 kcal/d or 0.75 MJ/d. (b): (BW x 0.75 g/kg BW/d) / NPU with NPU = Net Protein Utilisation = 0.7 and extra protein cost for pregnancy is 14 g/d. Thus for women with a body weight of 65 kg: PRI = 65 x 0.75 / 0.7 = 70 g/d. (c): for people with an optimal and stable body weight. (d): for people who are overweight or who experience undesirable weight gains. AR: average requirement, RDA: recommended dietary allowance, AI: adequate intake, UL: tolerable upper intake level.

Regarding the composition of dietary fat of the population under study: the mean intake of SFA exceeded the national and international recommendations. Only one woman from this population had a SFA intake lower than 10 en%. For 21 women PUFA intake was within the Belgian PRI range. For 6 women PUFA intake was higher than 7 en% with a maximum intake of 9.6 en% which is still lower than the Dutch UL. Average linoleic acid intake during pregnancy was 4.5 en% and within the recommended Belgian PRI range of 3 – 5 en% and higher than the Dutch adequate intake (AI) of 2.5 en%. In three women linoleic acid intake was lower than 3 en% (PRI) but reached the Dutch AI and in 8 women linoleic acid intake was higher than 5 en% (PRI) with a maximum intake of 8 en%.

Neither the mineral intake nor the vitamin intake did significantly differ between the beginning of pregnancy and near term (students' t-test). Therefore we calculated the average intake of minerals and vitamins during the course of pregnancy. The mineral intake during pregnancy is summarised in Table 2 and the vitamin intake of this population is summarised in Table 3. The data were compared with the Belgian PRI [3] and the Dutch dietary reference intake values [6].

The between subject variation for mineral intake varied from 20.5% to 41.8% with an average CV of 27.2%. The within subject variation for mineral intake varied from 9% to 16% with a mean CV of 12.3%.

Table 2: Daily mineral intake during pregnancy measured by the 7 day ER. Values are reported as mean (SD) (n = 27).

	Diet of study population	Between subjects CV (%)	Within subject CV (%)	Belgian PRI [3]	Dutch dietary reference intakes [5]
Na (g/d)	2.9 (0.63)	21.5	14	0.57 – 3.5	
K (g/d)	3.3 (0.69)	20.5	9	2 – 4	
Ca (mg/d)	967 (337)	34.9	15	1200	AI: 1000 UL: 2500
P (mg/d)	1450 (329)	22.7	10	1000	
Mg (mg/d)	285 (64)	22.6	10	480	
Fe (mg/d)	11.9 (3.6)	30.0	11	10	
Zn (mg/d)	11.4 (4.7)	41.8	14	7	
Cu (mg/d)	1.0 (0.26)	26.0	12	1.1	
Se (µg/d)	38.8 (9.5)	24.5	16	70	

See Table 1 for explanations of abbreviations.

All the pregnant women had a sodium and potassium intake that were in the PRI interval. Two women had a Na intake higher than 3.5 g/d and five women had a K intake higher than 4 g/d. Only 26% (n=7) of the women had a calcium intake higher than 1200 mg/d which is the recommended intake for pregnant women by the Belgian Health Council [3] and 41% (n=11) of the women had a Ca intake higher than 1000mg/d which is the Dutch AI [6]. On the other hand the mean phosphorus intake was rather high (1450 (SD 329) mg/d). 11% of the women had a P intake lower than the PRI. This results in a low Ca/P ratio ranging from 0.4 to 0.9 whereas the recommended ratio is at least 1 [3]. Mg intake was low, none of the pregnant women had an intake as high as the PRI of 480 mg/d [3]. Fe intake was 11.9 (SD 3.6) mg/d and 70% of the women (n=19) had an intake higher than the PRI of 10 mg/d for pregnant women. Similarly the average intake of Zn in this population was good: only 2 women had a Zn intake lower than the PRI. Cu intake was rather low: only 11 women (41%) reached the PRI for Cu. The intake of selenium is very low compared to the Belgian recommendations: all the pregnant women had a Se intake lower than the PRI.

Table 3: Daily vitamin intake during pregnancy measured by the 7 day ER. Values are reported as mean (SD) (n = 27).

	Diet of study population	Between subjects CV (%)	Within subject CV (%)	Belgian PRI [3]	Dutch dietary reference intakes [5]
Retinol (µg/d)	777.9 (353.6)	45.5	22		
β-carotene (mg/d)	1.2 (0.5)	41	33		
Vitamin A (µg/d)	983.5 (388.8)	39.5	22	700 (a)	
Vitamin D (µg/d)	2.1 (0.6)	30.2	18	10	AI: 10 (b) AI: 7.5 (c) UL: 50
Tocopherol (mg/d)	12.2 (5.0)	41	22	10	
Vitamin C (mg/d)	113 (44)	38.7	22	90	
Vitamin B1 (mg/d)	1.3 (0.4)	33.2	14	1.0	RDA: 1.4
Vitamin B2 (mg/d)	1.5 (0.6)	36.8	12	1.6	RDA: 1.4
Vitamin B6 (mg/d)	1.6 (0.5)	33.7	13	1.4	
Niacin (mg NE/d)	15.9 (3.6)	22.7	14	14	AI: 17
Niacin (mg/d)					UL: 35

(a): expressed as retinol equivalents (RE): 1 RE = 1 µg retinol = 6 µg β-carotene. (b): no exposure to sunlight. (c): light-coloured skin, and remain outdoor for at least 15 minutes a day with at least hands and face uncovered. NE = niacin equivalent. For other explanations, see Table 1.

The between subject variation for vitamin intake varied from 22.7% to 45.5% with an average CV of 36.2%. The within subject variation for vitamin intake varied from 12% to 33% with a mean CV of 19.2%.

The intake of the vitamins from the B-complex correlate strongly with each other: B1 versus B2: r= 0.83, P<0.0001 and B1 versus B6: r= 0.91, P<0.0001. Five women had a thiamin intake lower than the PRI of 1 mg/d and 16 women had a riboflavin intake lower than the PRI of 1.6 mg/d. At least 74 % of the study population (n=20) had a vitamin B6 intake higher than the PRI of 1.4 mg/d. At least 70 % (n=19) of the women had a vitamin A (expressed as retinol equivalents), ascorbic acid and tocopherol (expressed as tocopherol equivalents) intake higher than the PRI and 78 % (n=21) of the women had a niacin (expressed as niacin equivalents) intake higher than the PRI. None of the pregnant women reached the PRI or AI for vitamin D intake.

5. Discussion

We did not find any significant differences in macronutrient intake nor in intakes of minerals and vitamins as assessed by the 7 day ER between the first half of pregnancy and the third trimester. However as the inter-subject variability ranges from 8-24% for macronutrients and from 20-42% for micronutrients and the intra-subject variability ranges from 5-10% and from 9-16% for macro- and micronutrients respectively, one could assume that there was no power to detect differences between the two examination periods for nutrient intake. Others [17;18] have shown previously in larger study populations that the fat intake during pregnancy (estimated with food frequency questionnaires) does not change. Average intake values during pregnancy were also obtained by Badart-Smook *et al* [19] who used the cross-check dietary history in 370 pregnant women at approximately 22 weeks of gestation to assess maternal dietary habits during pregnancy. Therefore, we calculated the average intake between the two examinations during pregnancy of each individual nutrient and compared the results with the dietary recommendations. Some women used supplements (vitamin and/or fish oil supplements) during pregnancy. The intake of these supplements was taken into account during the nutrient analysis.

The Belgian dietary recommendations are expressed as Population Reference Intake (PRI). The PRI is defined as the intake that will meet the needs of 97.5% of

all the healthy individuals in a population. The nutritional needs for pregnant women sometimes deviate from the needs of a healthy non pregnant population (such as for calcium, phosphorus, magnesium, iron, and vitamin D). In these cases we compared our results with the PRI for pregnant women. The Belgian Health Council has revised the dietary guidelines in 2000 [3]. In The Netherlands, the Health Council's Committee has reviewed the dietary guidelines for calcium, vitamin D, B1, B2, niacin, pantothenic acid and biotin in 2000 [6] and more recently in 2001 for energy, proteins, fats and digestible carbohydrates [5]. The Dutch Committee expresses its dietary reference intake values either as estimated average requirement (AR), as recommended dietary allowance (RDA), as adequate intake (AI) or as tolerable upper intake level (UL). The RDA is defined as the estimated AR (the level of intake that is adequate for half of the population) plus twice the standard deviation of the requirement. This intake is adequate for virtually all of the individuals in the group in question. When the estimated AR is unknown, the Committee refers to AI. This is the level of intake that is sufficient for the entire population. The UL is the level of intake above which there is a chance that adverse effects will occur.

Most pregnant women in this population had an adequate caloric intake. The daily protein intake in our study population compares favourably with both the Belgian and Dutch dietary guidelines. The average carbohydrate intake of this group of pregnant women was too low with respect to the Belgian guidelines but is considered adequate by the Dutch Health Council. Regarding total fat intake, in Belgium a maximum recommended limit of 30 en% [3] is advised whereas the Health Council of The Netherlands has accepted an upper limit of 40 en% for people with an optimal and stable body weight and an upper limit of 30 or 35 en% for individuals who are overweight or who experience undesirable weight gains [5]. Thus with respect to the Dutch guidelines our study population had an adequate total fat intake, only five women had a total fat intake higher than 40 en% (maximum 45 en%). The Health Council of The Netherlands puts more emphasis on the composition than on the total amount of fat. The average intake of SFA in this study population was far beyond the recommended maximum of 10en%.

Mean intake of calcium was below the recommendations. During pregnancy, calcium needs are increased. It has been shown that a shortage of calcium in the

diet increases the risk of pregnancy induced hypertension [20]. All the pregnant women had a Mg and Se intake lower than the Belgian PRI. These women could become deficient for magnesium or selenium with progressing pregnancy. Magnesium deficiency during pregnancy can cause fatigue, increased risk of premature birth and maternal hypertension (eclampsia). Regarding selenium, a reduction in serum selenium normally occurs in the first trimester of pregnancy but a further highly significant decrease in serum selenium was observed in women who miscarried compared to women who delivered from a healthy neonate [21].

70% or more of this study population had an intake of vitamin C, vitamin A, tocopherol, vitamin B6, thiamine and niacin which was higher than the PRI. Riboflavin intake on the other hand was below recommendations. Maternal intake of riboflavin has been positively associated with birth weight and length [19].

Concerning vitamin D intake, the Belgian PRI for pregnant women is 10 µg/d and the Dutch AI during pregnancy is between 7.5 and 10 µg/d (depending on the exposure to sunlight) [3;6]. The average vitamin D intake in this study population was only 2.1 µg/d. None of the pregnant women reached the PRI or AI. Vitamin D recommendations for non pregnant adult women are 2 µg/d (Belgian PRI) and between 2.5 and 5 µg/d (Dutch AI, depending on the exposure to sunlight) [3;6]. Thus our study population has an adequate vitamin D intake compared to the national and the Dutch recommendations for non pregnant women but during pregnancy vitamin D needs are more than doubled [22]. Besides eating more vitamin D-rich foods (such as fatty fish and vitamin D enriched margarine and milk products), pregnant women should obtain regular sunlight exposure to increase vitamin D production in the skin. Pregnant women could be advised to take vitamin D supplements during the winter period.

We are well aware of the fact that our study population is not representative for the population of Belgian pregnant women and that the sample size is very small. However one cannot ignore the fact that the macronutrient intake in this group is very similar to the macronutrient intake of the Belgian population, calculated from the BIRNH study (Belgian Interuniversity Research on Nutrition and Health) [23-25].

In conclusion, energy and protein intake is adequate in our study population. As saturated fat intake is too high and carbohydrate intake rather low, it could be

advised to partly replace saturated fat in the diet by complex carbohydrates. Only one quarter of this pregnant population had a sufficient Ca intake. We advise pregnant women to increase the intake of milk and milk products (yoghurt and cheese) to obtain an adequate supply of calcium, vitamin B2 and vitamin D. None of the pregnant women reached the PRI for selenium, an important micronutrient present in seafood. Furthermore none of the women in our study population reached the PRI for magnesium. Nuts, seeds and chocolate are good sources of magnesium. Iron supplementation during pregnancy is a controversial issue. First of all, a physiological consequence of normal pregnancy is that the absorption of iron from food is increased. Therefore one opinion is that the increased iron absorption is large enough to meet the increased requirements of pregnancy provided that the dietary intake is adequate and thus that iron supplements during pregnancy are not needed [26]. However others argue that the physiologic adaptations are often insufficient to meet the increased requirements because iron deficiency anemia frequently develops during pregnancy [27]. As a result iron supplementation during pregnancy is often advised. Different strategies for iron supplementation are used. On the one hand, selective iron supplementation after iron status assessment has been recommended because not all women need iron, and compliance is likely to be better when an individual's need is recognized. On the other hand, routine iron supplementation to all women in the second half of pregnancy has been advocated in order to reach all women without the difficulties associated with assessment of iron status [27;28].

In general, the intake of some vitamins and minerals in this study population is below the recommendations. Some women could benefit from a multivitamin/mineral supplement. But one should be aware that balance is important with optimal ratios of iron to zinc (as iron reduces zinc absorption) and optimal ratios of calcium to phosphorus and magnesium.

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Chapter 4

Maternal and umbilical fatty acid status in relation to maternal diet

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Maternal and umbilical fatty acid status in relation to maternal diet

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Summary The aim of this study was to describe the dietary fat intake during pregnancy and to study the relationship between the intake of polyunsaturated fatty acids (PUFAs) and the fatty acid composition of maternal and umbilical plasma phospholipids (PLs) and cholesterol esters (CEs) at delivery. In addition, the contribution of food groups to the intake of total fat and fatty acids in the diet was quantified.

Maternal and umbilical blood samples were collected at delivery from 30 healthy pregnant women. The women completed a food frequency questionnaire during the first and third trimesters. The total fat intake during pregnancy is 85 (SD 24) g/day. The mean intake of saturated fatty acids (SFAs) is 33.4 g/day, of monounsaturated fatty acids (MUFAs) 28.6 g/day and of PUFA 15.2 g/day. Major sources of fat, MUFA and PUFA are fats, oils and sauces. Major sources of SFA are meat and poultry followed by cheese and eggs. Meat and poultry contribute the most to the intake of 20:4n-6 whereas fish is the major source of 20:5n-3 (EPA) and 22:6n-3 (docosahexaenoic acid (DHA)) in the diet. Linoleic acid, EPA and DHA (w%) in PL of maternal plasma are positively related to the intake of these fatty acids during pregnancy. No association is found between the maternal intake of the two parent essential fatty acids (18:2n-6 and 18:3n-3) and their fraction in umbilical PL or CE. EPA and the sum of n-6 fatty acids (w%) in umbilical plasma PL are positively correlated with the dietary intake of these fatty acids. © 2002 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

There are two families of essential fatty acids (EFAs), the n-*6* and n-3 families. They are essential because they are required and cannot be synthesised de novo by humans. Linoleic acid (18:2n-*6*) and α-linolenic acid (18:3n-3) are the parent EFA, they need to be present in the diet. They can be desaturated and elongated by liver enzymes to form long-chain polyunsaturated fatty acids (PUFAs) which play a major role in the development of new life as important structural components of cell membrane phospholipids (PLs).[1,2] Arachidonic acid (20:4n-*6*; AA) and docosahexaenoic acid (22:6n-3; DHA) are important structural fatty acids in neural tissue such as the brain and retina.[3,4] During pregnancy, accretion of maternal, placental and fetal tissue occurs and therefore the EFA requirements of pregnant women and the developing fetus are high. During the last trimester of pregnancy, the fetal need of AA and DHA are especially high because of rapid synthesis of brain tissue. The desaturation enzyme system in the human fetal liver is immature and unable to supply sufficient long-chain PUFA to meet their high neonatal demand. Moreover, the capacity of the placenta to synthesise long-chain PUFA from the parent EFA is very limited.[5] Thus, to obtain an adequate amount of parent EFA and their long-chain polyunsaturated derivatives, the developing fetus depends on active transport of these fatty acids from the mother across the placenta and thus on the EFA status of the mother.[6] Therefore, the diet of a pregnant woman should contain sufficient amounts of EFA to cover her own requirement as well as that of her fetus.

The major objective of the present study was to describe the dietary intake of long-chain PUFA during pregnancy and to study the relationship between the fatty acid intake and their fraction in maternal and

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umbilical plasma PLs and cholesterol esters (CEs) at delivery. In addition, the contribution of food groups to the intake of total fat and fatty acids in the diet was quantified.

PATIENTS AND METHODS

Study population

The study population consisted of 30 healthy pregnant women attending the Department of Gynecology of Ghent University Hospital, Belgium. At their first antenatal medical visit, the pregnant women were asked to cooperate in this study. All pregnant volunteers signed a written informed consent form, approved by the ethics committee of Ghent University Hospital. Only singleton pregnancies were included. Inclusion criteria were: first pregnancy, diastolic blood pressure below 90 mmHg, not diabetic, no proteinuria and not suffering from renal or cardiovascular disease. A food frequency questionnaire (FFQ) was filled in twice during the course of pregnancy together with a dietician: between 6 and 22 weeks of gestation (median 15 weeks) and between 32 and 40 weeks (median 35 weeks). Length of gestation (in weeks) was calculated from the recorded date of delivery and the self-reported first day of the last menstrual period. If the last menstrual period was unknown, gestational age was based on early ultrasound measurements. Shortly after delivery, a maternal and umbilical venous blood sample was collected in EDTA-tubes. Blood samples were temporarily stored at 6°C. Within 24 h of collection, plasma was isolated by centrifugation ($600 \times g$ during 5 min at 4°C) and stored in plastic tubes under nitrogen at −80°C until fatty acid analysis.

Food frequency questionnaire

Dietary intake was assessed by an FFQ developed by Al,[7] which was specifically designed to collect data on the fat consumption of Dutch pregnant women. The Department of Public Health (Ghent University Hospital) adapted this Dutch FFQ to the Belgian diet and developed a coloured photographic booklet as a tool to estimate portion sizes of different foods and dishes. The main objective of this FFQ is to estimate the dietary intake of fat and fatty acids in the preceding month. In a recent publication[8] we have validated this modified Dutch FFQ (adapted to the Belgian diet) with the 7-day estimated record (food diary). The used FFQ was considered to be a valid method that is able to assign the subjects to the appropriate part of the distribution of dietary fat intake.[8] A list of 180 of the most commonly consumed Belgian fat-containing foods are included in the FFQ. Twice the same experienced dietician visited the women at home and asked whether they consume those products daily, weekly or monthly and how many units they normally eat of that particular food. At the end of the questionnaire, some additional questions were asked which gave information about the kind of fat used for cooking and the frequency of intake and the amount of fat normally bought per week or month and the number of individuals that generally eat in the same household.

Fatty acid analysis of FFQ

For the detailed description of fatty acid intake (18:2n-6, 18:3n-3, 20:4n-6, 20:5n-3 and 22:6n-3) of the FFQ, a databank developed by Staessen et al.[9,10] was used. Food items were combined into 12 food groups: meat and poultry; fish and fish products; milk products (except cheese); cheese and eggs; fats, oils and sauces; cookies and pastry; sweets and chips; bread; cereals and pasta; potatoes; vegetables, fruits and nuts; and prepared dishes (such as soups, lasagna, etc.). The relative contribution of each food group to the intake of total fat, saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), PUFA, 18:2n-6, 20:4n-6, 20:5n-3 and 22:6n-3 was determined. These relative contributions were computed on an individual level.[10] For the ith food, the percentage of consumption contributed by that food is calculated as

$$\%\ \text{contribution of nutrient } k \text{ by food } i = \frac{(\text{total of nutrient } k \text{ provided by food } i) \times 100}{(\text{total of nutrient } k \text{ provided by all foods})}.$$

This is given by

$$\sum_{j=1}^{j} (Q_i D_{ik}) \times 100 \Big/ \sum_{j=1}^{j} \sum_{k=1}^{k} (Q_i D_{ik})$$

where Q is the food consumed (g), D the amount of nutrient/g of food, j=1–30 subjects (30 subjects in the study population), k the nutrient factors, and i=1–180 food items.

Fatty acid composition of plasma PLs and CEs

Lipids were extracted from 1 ml serum according to Folch et al.[11] The lipids were separated by thin-layer chromatography on rhodamine-impregnated silica gel plates using petroleum ether (bp 60–80°C; Merck Belgolab, Overijse, Belgium)/acetone 85:15 as mobile phase.[12] The PL and CE fractions were scraped off and the fatty acids converted into methyl esters by transesterification with 2 ml of a mixture of methanol:benzene:HCl (aqueous, 12 N) (80:20:5).[13] After cooling and adding 2 ml of water, fatty acid methyl esters were extracted with petroleum ether (bp 40–60°C), evaporated to dryness under a nitrogen flow at a temperature not exceeding 40°C, and analysed

by temperature-programmed capillary gas chromatography (Varian Model 3500, Walnut Creek, CA, USA) on a 25 m × 250 μm ($L \times$ ID) × 0.2 μm df Silar 10C column.[14] The injection and detection temperatures were set at 285°C. The starting temperature of the column was 150°C, which was increased to 240°C after 3 min at a rate of 2°C/min. The carrier gas was nitrogen with a flow of 25 cm/s. Peak identification was performed by spiking with authentic standards (Sigma–Aldrich, Bornem, Belgium). Peak integration and calculation of the per cent composition was performed electronically with a Varian Model 4290 integrator. The coefficient of variation of intra-assay samples of the entire method of fatty acid analysis is less than 5%.

Statistics

From the FFQ, the daily total fat and fatty acid intake was calculated. Normality of distribution was ascertained with the chi-square test. Dietary intake values obtained from the FFQ and fatty acid fractions of PL and CE that had no normal distribution were log transformed to reach normality of distribution. Values are reported as mean and standard deviation in parentheses. Comparisons of fat and fatty acid intake between the first and third trimesters of pregnancy were performed with the paired Student's *t*-test. A value of $P<0.01$ was taken as the criterion of significance. Pearson correlation coefficients were calculated to test the relationship between dietary fatty acid intake and fatty acid status in maternal and umbilical PL and CE. The data were analysed using the MedCalc statistical program, version 6 (MedCalc Software, Mariakerke, Belgium).[15]

RESULTS

Clinical characteristics of the study population

The study population consists of 30 pregnant Belgian women with a mean age at delivery of 30 years (range 25–37 years). The mean body mass index of the women before pregnancy is 23.0 kg/m^2 (range 17.6–30.0 kg/m^2) and mean weight gain at delivery is 15 kg (range 9–20 kg). All pregnant women are nullipara, all pregnancies are uncomplicated and the infants are born healthy and at term with a mean gestational age of 39.0 weeks (range 38–42 weeks). The mean birth weight of the neonates is 3320 g (range 2300–4200 g) and the mean birth length is 51.0 cm (range 47–55 cm). The sex ratio of the infants is 16 males and 14 females.

Fatty acid intake

Since the FFQ is developed to estimate the fat intake, total energy intake cannot be calculated. Therefore, intake of total fat, SFAs, MUFAs and PUFAs and the different fatty acids are expressed as gram per day. Table 1 summarises the dietary fat and fatty acid intake during pregnancy.

Table 1 Fat and fatty acid intake (g/day) measured by the FFQ[8] during the first and third trimesters of pregnancy. Values are reported as mean (SD) (n=30)

	First trimester	Third trimester	Average during pregnancy[a]
Total fat	83.0 (27.9)	87.0 (27.0)	85.0 (24.5)
SFA	32.6 (12.0)	34.3 (12.2)	33.4 (10.5)
MUFA	27.9 (9.4)	29.3 (8.8)	28.6 (8.2)
PUFA	14.9 (5.9)	15.4 (6.5)	15.2 (5.8)
18:2n-6	12.6 (5.4)	13.1 (5.9)	12.9 (5.3)
18:3n-3	1.26 (0.51)	1.41 (0.58)	1.34 (0.46)
20:4n-6	0.13 (0.04)	0.13 (0.04)	0.13 (0.03)
20:5n-3	0.18 (0.11)	0.15 (0.10)	0.16 (0.08)
22:6n-3	0.31 (0.18)	0.28 (0.19)	0.30 (0.14)
Σ n-3	1.8 (0.6)	1.9 (0.7)	1.8 (0.5)
Σ n-6	12.9 (5.5)	13.4 (6.0)	13.1 (5.3)

No significant differences in fat or fatty acid intake between the first and third trimesters of pregnancy.
[a]Average of values obtained during first and third trimesters of pregnancy.

Fat and fatty acid intake does not change during the course of pregnancy. Therefore, the average fat and fatty acid intake during the course of pregnancy is calculated (Table 1). Total fat intake is on average 85 (SD 24.5) g/day or 765 (SD 220) kcal/day. SFAs provide 301 (SD 94) kcal/day, MUFAs 257 (SD 74) kcal/day and PUFAs 137 (SD 52) kcal/day. The ratio of dietary intake of PUFA/SFA is 0.47 (SD 0.15). The most dominant n-6 fatty acid in the diet is 18:2n-6, with a mean intake of 12.9 (SD 5.3) g/day and the dominant n-3 fatty acid in the diet is 18:3n-3 with an average intake of 1.3 (SD 0.5) g/day. The dietary intake of the two parent EFAs strongly correlate with each other: r=0.89 ($P<0.0001$). The dietary intake of the longer chain n-3 fatty acids is 0.46 (SD 0.21) g/day. The intake of 20:5n-3 and of 22:6n-3 correlate strongly with each other: r=0.93; $P<0.0001$.

Contribution of food groups to the intake of fat

Figure 1 shows the contribution of specific food groups contributing to the total fat intake and to the intake of fatty acids. Fats, oils and sauces provide the major part of total fat in the diet, followed by meat and poultry and by cheese and eggs. The same holds for MUFA in the diet. Meat and poultry are the main source of SFA followed by cheese and eggs and by fats, oils and sauces. Fats, oils and sauces are also the most important source of PUFA and linoleic acid in the diet, followed by meat and poultry and bread. Meat and poultry are the major source of AA in the diet, whereas fish and fish products are the main sources of 20:5n-3 and 22:6n-3.

Relationship between fatty acid composition of maternal and neonatal PL and CE and dietary fatty acid intake

The maternal and umbilical fatty acid composition of PL and CE isolated from plasma is summarised in Table 2. The dominant n-6 fatty acid in maternal plasma PL is linoleic acid (18:2n-6) whereas in umbilical plasma PL it is AA. DHA is the predominant n-3 fatty acid both in maternal and in umbilical plasma PL. 18:2n-6 is the major n-6 fatty acid in the CE fraction of maternal and umbilical plasma. The EPA fraction in umbilical plasma PL correlates significantly with that in maternal plasma PL: $r=0.68$, $P<0.0001$. Neither the fraction of DHA nor of AA in umbilical plasma PL correlates with the fraction of these fatty acids in maternal plasma PL. In CE, no correlations between the fraction of the fatty acids in umbilical and maternal plasma are observed.

In Table 3, the Pearson correlation coefficients (in some cases after log transformation to obtain normal distribution) between the maternal and umbilical fatty acids (w%) of PL and CE with the dietary fatty acid intake estimated by the FFQ (g/kg BW/day) are presented. Linoleic acid intake during pregnancy is positively correlated with the fraction of linoleic acid in maternal plasma PL at delivery. α-Linolenic acid intake is positively correlated with the fraction of α-linolenic acid in maternal plasma CE. No association is found between the maternal intake of the two parent EFAs (18:2n-6 and 18:3n-3) and the fraction

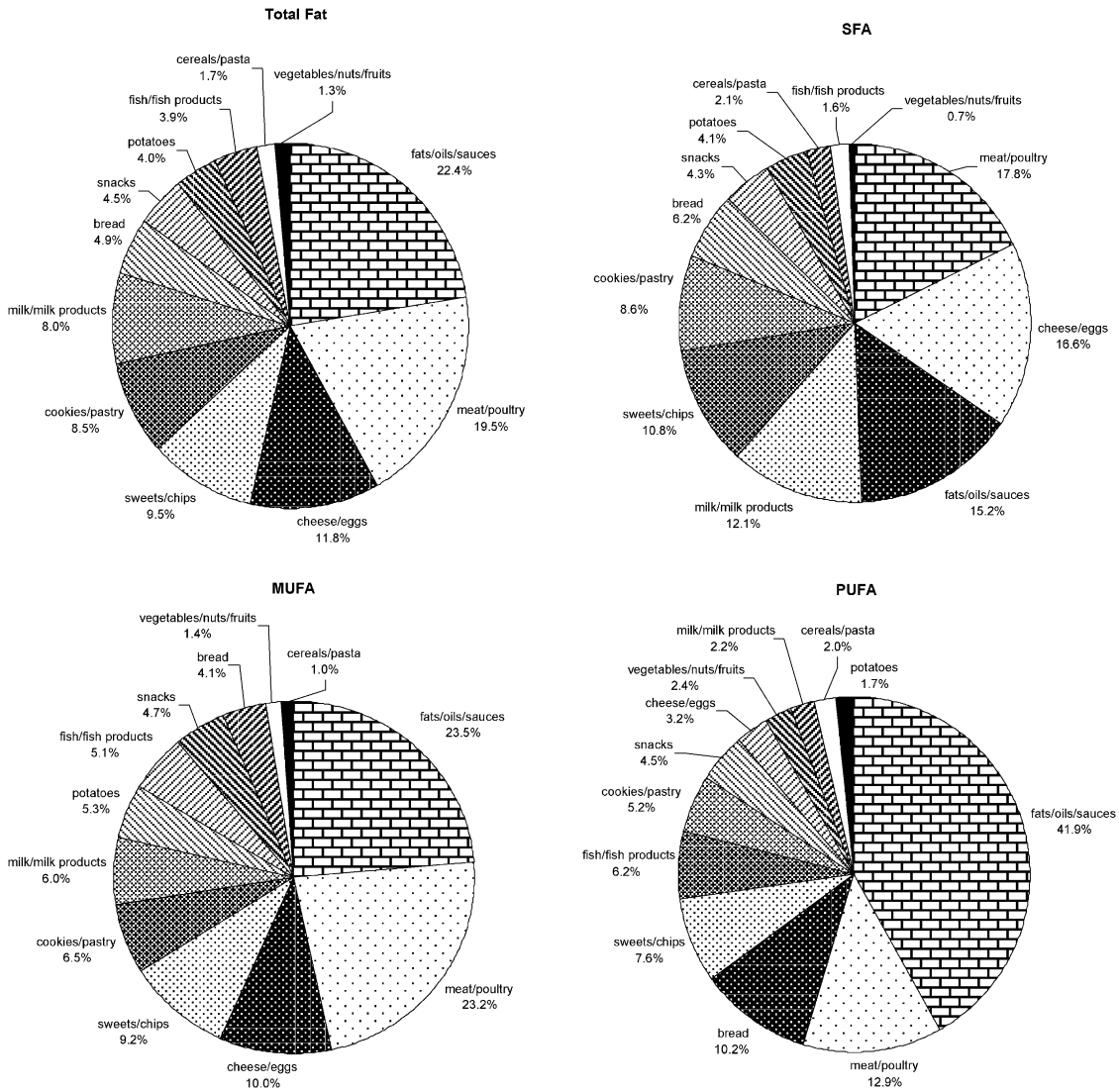

Fig. 1 The relative contribution of specific food groups to the dietary intake of total fat, SFAs, MUFAs, PUFAs, linoleic acid (18:2n-6), AA (20:40n-6), eicosapentaenoic acid (20:5n-3) and DHA (22:6n-3). Food items were combined into 12 food groups: meat and poultry; fish and fish products; milk products (except cheese); cheese and eggs; fats, oils and sauces; cookies and pastry; sweets and chips; bread; cereals and pasta; potatoes; vegetables, fruits and nuts; and prepared dishes (such as soups, lasagna, etc.).

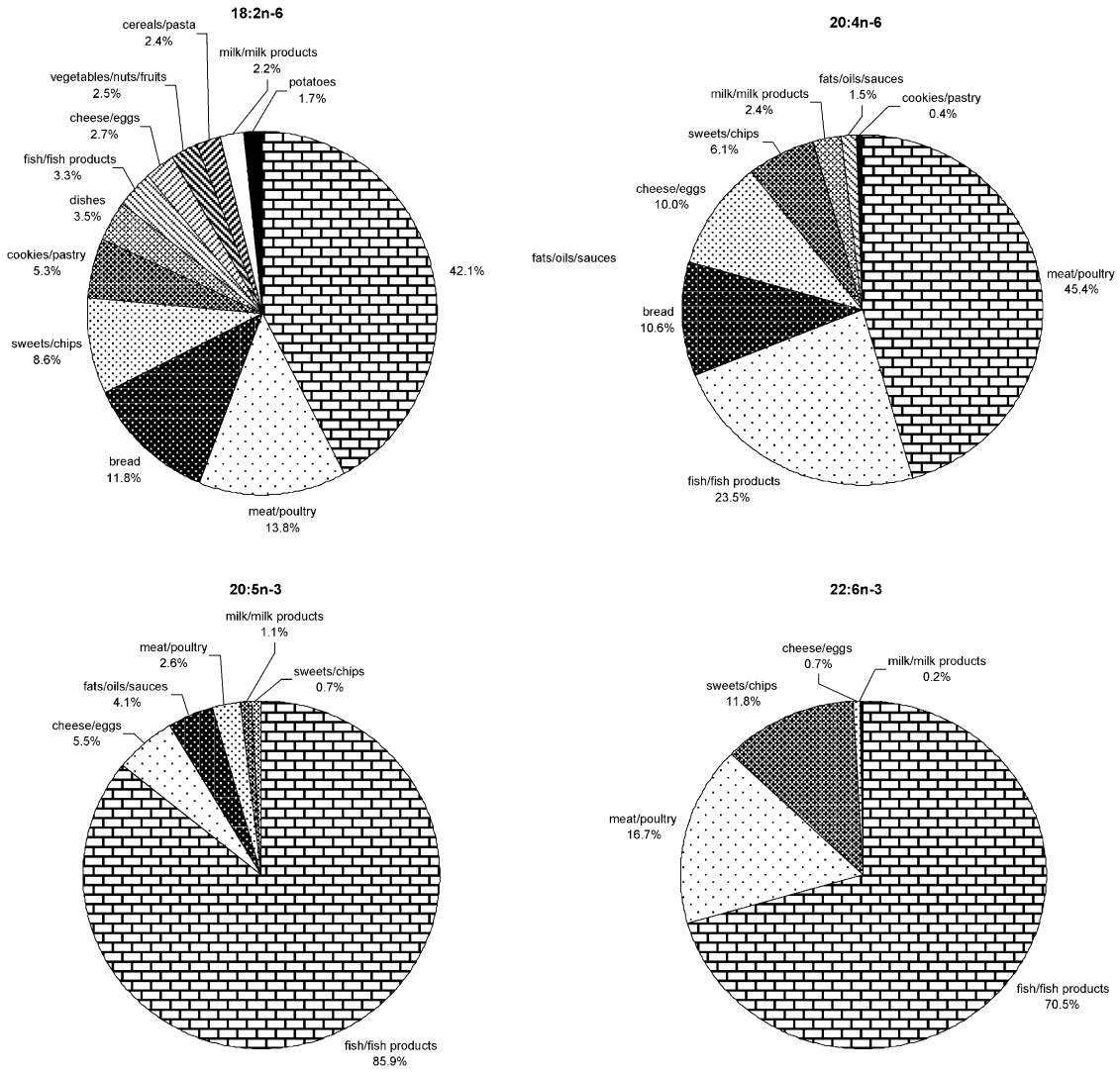

Fig. 1 *(continued)*

Table 2 Fatty acid composition (w%) of PLs and CEs isolated from maternal and umbilical plasma shortly after delivery. Values are reported as mean and (SD) (n=30)

	PLs		CEs	
	Maternal plasma levels	**Umbilical plasma levels**	**Maternal plasma levels**	**Umbilical plasma levels**
16:0	31.9 (SD 2.5)	28.1 (SD 2.5)	11.9 (SD 1.9)	18.1 (SD 3.4)
18:0	9.7 (SD 1.0)	13.8 (SD 2.1)	0.67 (SD 0.44)	3.6 (SD 2.4)
18:2n-6	19.1 (SD 3.2)	8.4 (SD 3.7)	51.4 (6.2)	18.8 (SD 7.3)
18:3n-3	0.22 (SD 0.14)	0.06 (SD 0.08)	0.61 (SD 0.14)	0.19 (SD 0.17)
20:4n-6	8.4 (SD 1.8)	14.9 (SD 3.1)	5.4 (SD 1.6)	10.5 (SD 4.5)
20:5n-3	0.50 (SD 0.31)	0.36 (SD 0.24)	0.38 (SD 0.27)	0.39 (SD 0.32)
22:6n-3	4.8 (SD 1.3)	5.8 (SD 1.7)	0.6 (SD 0.34)	1.2 (SD 1.18)
SFA	46.0 (SD 3.3)	47.3 (SD 2.4)	14.2 (SD 2.9)	25.0 (SD 6.1)
MUFA	12.7 (SD 1.3)	12.6 (SD 1.3)	23.7 (SD 3.2)	32.3 (SD 8.0)
PUFA	38.2 (SD 3.5)	36.4 (SD 2.7)	59.8 (SD 5.9)	33.6 (SD 9.8)

of these fatty acids in umbilical plasma PL or CE. The dietary intake of 20:5n-3 is positively correlated with the fraction of 20:5n-3 in maternal and umbilical plasma PL. The DHA intake during pregnancy is positively correlated with the fraction of DHA in maternal plasma PL and CE. In addition, the intake of long-chain n-3 PUFA

Table 3 Pearson correlation coefficients between maternal fatty acid intake estimated with the FFQ[8] (g/kg BW/day) and fatty acid composition (w%) of PLs or CEs isolated from maternal and umbilical plasma shortly after delivery

Diet 18:2n-6 (g/kg BW/day)		
Maternal plasma PL 18:2n-6	0.53	$P<0.01$
Maternal plasma CE 18:2n-6	0.25	NS
Umbilical plasma PL 18:2n-6	0.02	NS
Umbilical plasma CE 18:2n-6	0.10	NS
Diet 18:3n-3 (g/kg BW/day)		
Maternal plasma PL 18:3n-3	0.06	NS
Maternal plasma CE 18:3n-3	0.53	$P<0.01$
Umbilical plasma PL 18:3n-3	−0.12	NS
Umbilical plasma CE 18:3n-3	0.04	NS
Diet 20:4n-6 (g/kg BW/day)		
Maternal plasma PL 20:4n-6	−0.09	NS
Maternal plasma CE 20:4n-6	0.0	NS
Umbilical plasma PL 20:4n-6	−0.25	NS
Umbilical plasma CE 20:4n-6	−0.27	NS
Diet 20:5n-3 (g/kg BW/day)		
Maternal plasma PL 20:5n-3	0.48	$P<0.01$
Maternal plasma CE 20:5n-3	0.34	NS
Umbilical plasma PL 20:5n-3	0.59	$P<0.01$
Umbilical plasma CE 20:5n-3	−0.05	NS
Diet 22:6n-3 (g/kg BW/day)		
Maternal plasma PL 22:6n-3	0.52	$P<0.01$
Maternal plasma CE 22:6n-3	0.39	$P<0.05$
Umbilical plasma PL 22:6n-3	−0.20	NS
Umbilical plasma CE 22:6n-3	−0.11	NS
Diet EPA+DHA (g/kg BW/day)		
Maternal plasma PL EPA+DHA	0.53	$P<0.01$
Maternal plasma CE EPA+DHA	0.44	$P<0.05$
Umbilical plasma PL EPA+DHA	−0.11	NS
Umbilical plasma CE EPA+DHA	−0.15	NS
Diet Σn-3 (g/kg BW/day)		
Maternal plasma PL Σn-3	0.27	NS
Maternal plasma CE Σn-3	0.34	NS
Umbilical plasma PL Σn-3	−0.15	NS
Umbilical plasma CE Σn-3	−0.07	NS
Diet Σn-6 (g/day)		
Maternal plasma PL Σn-6	0.51	$P<0.01$
Maternal plasma CE Σn-6	0.31	NS
Umbilical plasma PL Σn-6	0.38	$P<0.05$
Umbilical plasma CE Σn-6	0.12	NS

(= EPA+DHA) during pregnancy is positively associated with the fraction of these fatty acids in maternal plasma PL and CE. The dietary intake of the sum of the n-6 fatty acids is positively correlated with the sum of n-6 fatty acids in maternal and umbilical plasma PL.

DISCUSSION

In the present study, we confirmed that neither total fat nor the individual fatty acid intake significantly differs between the first and third trimesters.[8] Therefore, we calculated for each individual the average intake of fat and fatty acids during the course of pregnancy. The mean total fat intake in this study population is 85 g/day (SD 24.5). This is comparable with other pregnancy studies. In a group of Dutch pregnant women, a fat intake of 88.6 (SD 39.0) g/day was measured with an FFQ.[16] One study in the UK calculated a total fat intake by pregnant women of 92 g/day[17] and another study reported in British omnivore pregnant women a total fat intake of 98 g/day.[18] Furthermore, two studies in the USA reported a total fat intake also calculated with FFQ of 69.9 (SD 24.4) g/day[19] and of 74 g/day[20] in pregnant women. Lakin et al.[18] found in British healthy omnivore pregnant women a mean intake of n-3 fatty acids of 1.7 (SD 0.29) g/day and a mean intake of EPA of 0.14 (SD 0.13) g/day. These findings are consistent with our results. Our study population has higher mean intakes of n-6 fatty acids (13.1 vs. 9.9 g/day), linoleic acid (12.9 vs. 9.5 g/day) and DHA (300 vs. 173 mg/day) and a lower mean intake of 20:4n-6 (130 vs. 198 mg/day) compared to the British omnivore women.[18]

The Belgian recommendations concerning the intake of parent EFAs are: the daily intake of linoleic acid should be approximately 3–5% of the total energy intake (en%) and the daily intake of α-linolenic acid should be between 0.5 and 1 en%.[21] The ratio between the intake of 18:2n-6 and 18:3n-3 should be about 5/1. An intake of more than 10 en% of 18:2n-6 and more than 3 en% of 18:3n-3 is not desirable.[21] With the FFQ it is not possible to calculate the daily energy intake. Therefore, we are unable to calculate the contribution of the EFA to the total energy intake. But if we assume that our population has a 2000 kcal diet then the calculated recommended daily intake of 18:2n-6 should be approximately 6.7–11.1 g/day and of 18:3n-3 should be between 1.11 and 2.22 g/day. In this study population, the ratio between the intake of 18:2n-6 and 18:3n-3 averages 9.6 (SD 1.78). Lowest and highest values are 5.1 and 14.0. This indicates that the intake of linoleic acid is too high compared to the intake of α-linolenic acid in the population under study. Indeed, 14 women have an 18:2n-6 intake higher than 11.1 g/day and 10 women have an 18:3n-3 intake lower than 1.11 g/day. In 1999, Simopoulos and co-authors[22] made some recommendations for adequate intake (AI) of fatty acids for adults and pregnant women. The AI is expected to meet or exceed the amount needed to maintain a defined nutritional state of adequacy in essentially all the members of a specific healthy population.[23] Based on a 2000 kcal diet, the AI for 18:2n-6 and for 18:3n-3 should be 4.44 g/day and 2.22 g/day respectively.[22,22] Compared to these recommendations, our study population has a high 18:2n-6 intake and a low intake of 18:3n-3. The AI for the sum of EPA and DHA should be 0.65 g/day of which EPA intake should be at least 0.22 g/day and DHA intake in pregnant women should be at least 0.3 g/day.[22] In only seven women of this study population (23%), the intake of EPA+DHA is higher than the AI. Only eight women (27%) have an EPA intake higher than the AI and 13

women (43%) have a DHA intake higher than the AI for DHA. It is noticeable that the women with a DHA intake higher than the AI have a significantly higher DHA status in plasma PL compared to the women with a DHA intake lower than the AI: 5.4 (SD 1.4) vs. 4.3 (SD 1.1) w%, $P<0.05$. In contrast to the advises of Simopoulos et al.,[22] the AI of the n-3 fatty acids from fish (EPA+DHA) during pregnancy, according to Health Council's Committee of The Netherlands, is only 0.2 g/d.[23] Thus, only two women from this study population have an EPA+DHA intake lower than the Dutch recommendations. In Belgium, there are still no recommendations for the daily intake of the individual long-chain PUFA.

In this study population, the dietary intake of linoleic acid (g/kg BW/day) is positively and significantly correlated with the fraction of 18:2n-*6* in maternal plasma PL. A positive but non-significant ($P<0.1$) association is found between the dietary intake of 18:2n-*6* during pregnancy and the fraction of 18:2n-*6* in maternal plasma CE. In Dutch pregnant women, the linoleic acid intake is positively correlated with the fraction of linoleic acid in maternal (r=0.32) and umbilical (r=0.26) plasma PL, the relationship with the fatty acid fraction in CE is not investigated.[16] In our pregnant study population, the fraction of 20:5n-3 in maternal and umbilical plasma PLs is positively associated with the dietary intake of 20:5n-3. The fraction of DHA in maternal plasma PL and CE is positively associated with the intake of DHA. In the literature, it was found that the dietary intake of long-chain n-3 PUFA during pregnancy shows a slight but significant correlation with maternal (r=0.22) and umbilical plasma levels (r=0.17).[24] Vegetarian pregnant women have a significantly lower intake of 20:4n-*6*, 20:5n-3 and 22:6n-3 compared to omnivore mothers.[18] This was reflected in a reduced ratio of DHA/18:3n-3 in maternal red blood cells.[18]

When pregnant women are supplemented with fish oil (2.7 g n-3 PUFA/day), the maternal plasma PL contain significantly more n-3 fatty acids and less n-*6* fatty acids.[25] Additionally, the concentration of DHA in umbilical plasma PL of neonates born from fish-oil-supplemented mothers was significantly higher compared to the placebo group.[25] Supplementing women in their second trimester of pregnancy with 0.57 g DHA/day and with 0.26 g AA/day resulted in significantly higher DHA levels in plasma PL and in red blood cells compared to controls without a concomitant decline in n-*6* fatty acids.[26]

In a population of 372 pregnant women, the head circumference of the newborn was negatively associated with the maternal intake of 18:2n-*6* and the birth length was positively associated with the sum of n-3 PUFA+AA in the maternal diet.[27] We could not reproduce these findings. The inverse relationship between linoleic acid and n-3 PUFA in maternal diet with fetal growth parameters suggests that the intake of linoleic acid should be decreased in favour of the intake of n-3 PUFA by pregnant women.

Concerning the consumption of food groups, fats/oils/sauces, meat/poultry and cheese/eggs are the most important fat sources. Meat and poultry are the most important source of SFA and of AA whereas fish and fish products are the major sources of EPA and DHA in the diet. Similar results were found in a large Belgian population from the BIRNH study.[10]

In summary, fat and fatty acid intake remain constant throughout pregnancy. Linoleic acid, EPA, DHA and the sum of n-*6* fatty acids (w%) in PL of maternal plasma are positively related to the intake of these fatty acids during pregnancy. EPA and the sum of n-*6* fatty acids (w%) in PL of umbilical plasma are positively associated with the intake of these fatty acids during pregnancy. It our study population, the intake of linoleic acid is rather high whereas the intake of α-linolenic acid is low compared to the current recommendations. The intake of the long-chain n-3 PUFA (EPA+DHA) varies from 0.1 to 0.8 g/day. Taking the Simopoulos et al. recommendations[22] in mind, it would be advisable to increase the dietary intake of long-chain n-3 PUFA in favour of linoleic acid during pregnancy. A high maternal intake of linoleic acid may alter the maternal and neonatal n-3 fatty acid status.

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Chapter 5

The composition of saturated fatty acids in plasma phospholipids changes in a way to counteract changes in the mean melting point during pregnancy

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The Composition of Saturated Fatty Acids in Plasma Phospholipids Changes in a Way to Counteract Changes in the Mean Melting Point During Pregnancy

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ABSTRACT: It has been demonstrated that in pathological conditions with an increase in the calculated mean melting point (MMP) of phospholipid (PL) fatty acids (FA) there are changes in the composition of the saturated FA (SFA), which partially counteract this effect: shorter-chain SFA with lower melting points are increased, while longer-chain less fluid SFA are suppressed. The aim of this study was to determine whether there are differences in MMP during pregnancy and in the newborn and, if so, whether similar adaptive changes occur in the composition of the SFA. The FA composition of plasma PL was determined in healthy women (n = 16) twice during pregnancy (15–24 wk and 29–36 wk) and at delivery and in umbilical venous blood obtained at birth. The MMP of maternal PL was significantly higher at delivery compared to mid-gestation, due to a loss of highly unsaturated FA (HUFA) which were replaced by SFA. In addition, changes in the SFA occurred: 16:0 with lower melting point was higher while 18:0 with higher melting point was lower at delivery. MMP of PL FA in umbilical plasma was lower than in maternal plasma at delivery, which was due to higher HUFA content. In contrast to maternal plasma, 16:0 was lower while 18:0, 20:0, and 24:0 were higher in umbilical plasma resulting in a higher MMP of SFA, tending to raise the overall MMP. It can be concluded that, during pregnancy and in the newborn, the FA composition of SFA changes in a way to counteract changes in MMP induced by reduced and increased HUFA, respectively.

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Holman *et al.* (1–4) introduced the concept of the calculated mean melting point (MMP) and calculated mean chain length (MCL) of fatty acids (FA) from plasma phospholipids (PL) as surrogate parameters of membrane fluidity. PL are the major structural components of membranes, and the FA pattern of plasma PL reflects that of tissue PL. Membrane fluidity depends among others on the amounts of PL, the FA composition of the PL, and the amount of cholesterol. Membrane fluidity plays an important role in the efficiency of ligand binding, the activity of membrane enzymes, membrane transport, and cell deformability (5). Changes in membrane lipid composition and, consequently, changes in membrane fluidity may result in differences in the function of membrane receptors. In preeclamptic women (6), membrane fluidity of platelets was significantly higher compared to that of normotensive women and was accompanied by higher levels of unsaturated FA in the membrane of platelets. When long-chain polyunsaturated FA (PUFA) are replaced by saturated (SFA) or monounsaturated FA (MUFA), the MMP increases and membrane fluidity decreases (1–4). It has been demonstrated that the MMP of FA of plasma PL of pregnant (36 wk of gestation), lactating, and nonlactating women (6 wk postpartum) is higher compared to that from nonpregnant women (3). Furthermore, it has been demonstrated that the MMP of plasma PL is significantly increased in patients with multiple sclerosis. The latter patients have reduced concentrations of PUFA due to impaired chain elongation, and PUFA are replaced with SFA (1). This overall increase in MMP is accompanied by changes in the composition of the SFA, which partially counteract this effect: shorter-chain SFA with lower melting points are increased while longer-chain, less fluid SFA are suppressed.

The objective of the present study was to determine whether similar adaptive changes in MMP occur during the course of pregnancy and in the newborn. Therefore, we determined the FA composition of PL, isolated from maternal venous plasma during the course of pregnancy and from umbilical venous plasma.

MATERIALS AND METHODS

Study population. Healthy pregnant women at the Department of Gynecology of Ghent University Hospital, Belgium, were asked to participate in this study. All pregnant women signed a written informed consent form, approved by the ethics committee of Ghent University Hospital. Only singleton pregnancies were included. Inclusion criteria were: normotensive (DBP <90 mm Hg), not diabetic, no proteinuria, and not suffering from renal or cardiovascular disease. Twenty pregnant women entered the study. Two pregnant women delivered preterm and were excluded from the study. From one pregnancy the umbili-

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Abbreviations: CI, confidence interval; FA, fatty acid; HUFA, highly unsaturated fatty acid; MCL, mean chain length; MMP, mean melting point; MUFA, monounsaturated fatty acid; OPI, oxidative potential index; PL, phospholipid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

cal blood sample was lost and from another pregnancy we only obtained one antenatal sample. These pregnancies were also excluded. The study population thus consisted of 16 healthy pregnant women and their neonates (seven girls and nine boys).

Maternal venous blood was collected in EDTA-tubes twice during the course of pregnancy, between 15 and 24 wk of gestation (median 20 wk) and between 29 and 36 wk (median 32 wk) and at delivery (median 39.7 wk; range 38.0–41.4 wk). After delivery, a sample of umbilical venous blood was collected. Blood samples were temporarily stored at 6°C. Within 24 h of collection, plasma was isolated by centrifugation ($600 \times g$ during 5 min at 4°C) and stored in plastic tubes under nitrogen at –80°C until transportation in dry ice to Maastricht for analysis.

FA analysis. All samples of a given mother-infant pair were analyzed simultaneously. The FA analysis was performed as described previously (7). Previous to the FA analysis an internal standard [dinonadecanoyl lecithin, phosphatidylcholine 19:0] was added to every sample. Total lipid extracts of plasma were prepared using a modified Folch extraction (8). The PL fraction was isolated by solid-phase extraction on an aminopropyl silica column (9). The PL were saponified and the FA converted to the corresponding methyl esters by reaction with BF_3 in methanol (140 g/L) at 100°C during 1 h. The methyl esters were analyzed using a capillary gas–liquid chromatograph equipped with a 50 m BP1 nonpolar column, 0.22 mm i.d. × 0.10 μm film thickness, and a 50 m BP × 70 mm polar column, 0.22 mm i.d. × 0.25 μm film thickness (SGE, Bester BV, Amstelveen, The Netherlands). The injection temperature was set at 250°C and the detector temperature at 300°C. The starting temperature of the columns was 160°C, which after 4 min was increased to 200°C with a rate of 6°C/min. Subsequently, after a stabilization period of 3 min, the temperature was further increased to 270°C at a rate of 7°C/min. The carrier gas was helium, head pressure 370 kPa.

The results are expressed as mole percentage of total FA, and the absolute FA amount in the PL fraction is also reported (mg/L plasma). Thirty-one different FA with chain lengths between 14 and 24 carbon atoms were identified. The sum of all the SFA, the MUFA, the PUFA, the highly unsaturated fatty acids (HUFA: fatty acids with 20 or more carbon atoms and with at least three double bonds), Σn-7, Σn-9, Σn-3, and Σn-6 were calculated and are reported together with the individual FA.

The fluidity of lipids was assessed through the MMP, °C (sum of the mole fraction multiplied by the melting point for each fatty acid) and the MCL (number of carbon atoms, sum of the mole fraction multiplied by the number of carbon atoms in the FA). The oxidative potential index (OPI) of FA in plasma PL was estimated by summing the mole fraction of FA with 1, 2, 3, 4, 5, and 6 double bonds, multiplied by 1, 30, 70, 120, 180, and 240, respectively (10).

Statistical analysis. Normality of distribution was ascertained with the Kolmogorov-Smirnov test. The calculated parameters such as MMP, MCL, and OPI had a normal distribution. FA fractions were arcsin transformed to reach normality of distribution. Values are reported as mean [95% confidence interval of the mean (CI)]. Paired Student *t*-test was performed for FA comparisons between maternal samples of the first and second antenatal visit with maternal samples obtained at delivery and for maternal-umbilical FA comparisons at delivery and birth. In order to avoid type 2 errors, due to multiple comparisons, a value of $P < 0.005$ was taken as the criterion of significance. For maternal plasma, the correlations between the fraction of the PUFA and HUFA in the PL on the one hand and the MMP of SFA and the MMP of MUFA on the other hand, were calculated. For these calculations the results of the two antenatal visits and of delivery were used. The degree of association was calculated using Pearson correlation. The data were analyzed using the MedCalc statistical program, version 6 (MedCalc Software, Mariakerke, Belgium) (11).

RESULTS

Clinical characteristics. The mean age of the mothers ($n = 16$) at delivery was 30 yr (range 20–38 yr). The mean body mass index of the women before pregnancy was 21.2 (range 16.5–24.2). All mothers were nullipara, all pregnancies were uncomplicated, and the infants were born healthy with a mean birth weight of 3169.4 g (range 2570–3860 g) and a mean birth length of 50.3 cm (range 47–53 cm). The median Apgar Score 1 min after birth was 9 (range 4–9) and 5 min after birth 9 (range 9–10).

The FA patterns of plasma PL (mol%) from maternal plasma during the course of pregnancy and at delivery and from umbilical plasma shortly after birth are given in Table 1. Calculated values derived from this FA composition are summarized in Table 2.

Maternal FA composition. In maternal plasma PL, a few significant differences between gestation and delivery occurred. The total amount of maternal plasma PL-associated FA did not differ significantly between gestation and delivery.

Neither PUFA nor MUFA changed significantly between mid-gestation and delivery. HUFA were lower, while SFA were higher at delivery compared to mid-gestation ($P < 0.005$).

Palmitic acid (16:0) and stearic acid (18:0), the two major SFA, changed significantly, but in opposite directions: 16:0 increased while 18:0 dropped during the last 20 wk of pregnancy ($P < 0.001$).

Few of the individual n-6 or n-3 FA differed significantly between mid-pregnancy and delivery; arachidonic acid (20:4n-6) and its elongation product 22:4n-6 declined ($P < 0.005$), but linoleic acid remained stable. In the series of n-3 FA, only 22:5n-3 was lower at delivery ($P < 0.005$).

The sum of the Δ5 desaturation products (20:5n-3, 20:4n-6, and 20:3n-9) was calculated as a parameter for the essential FA status of the mother because in some disorders in which Δ5 desaturation is affected, the conversion of 20:4n-3 to 20:5n-3, of 20:3n-6 to 20:4n-6, and of 20:2n-9 to 20:3n-9 may be equally altered. This would result in an unchanged triene/tetraene ratio. The sum of the Δ5 desaturase products was lower at delivery as compared to mid-pregnancy: 8.2 (7.6 to 8.8%) vs. 9.3 (8.5 to 10.0%); $P < 0.0005$.

Umbilical plasma FA composition. The amount of umbilical

TABLE 1
Amount (mg/L plasma) and Composition (mol% of total fatty acids) of Fatty Acids in Phospholipids Isolated from Maternal Venous Plasma During the Course of Pregnancy and at Delivery and from Umbilical Venous Plasma at Birth: Mean (95% CI of the mean) (n = 16)

Fatty acid	Maternal plasma 1st antenatal visit: 15–24 wk	Maternal plasma 2nd antenatal visit: 29–36 wk	Maternal plasma delivery: 38–41 wk	Umbilical plasma birth: 38–41 wk	Paired t-test[b]
Total (mg/L)	1682.5	1810.1	1845.5	521.4	
	(1532.3 to 1832.7)	(1656.2 to 1964.0)	(1679.1 to 2011.9)	(559.1 to 683.7)	c
14:0	0.5 (0.4 to 0.6)	0.4 (0.4 to 0.5)	0.45 (0.4 to 0.5)	0.4 (0.3 to 0.4)	
15:0	0.2 (0.2 to 0.3)	0.2 (0.2 to 0.25)	0.2 (0.2 to 0.23)	0.1 (0.1 to 0.2)	b,c
16:0	33.5 (32.8 to 34.2)	34.5 (33.8 to 35.3)	35.8 (34.9 to 36.6)	31.9 (30.6 to 33.2)	a,b,c
17:0	0.4 (0.4 to 0.5)	0.4 (0.3 to 0.4)	0.3 (0.3 to 0.4)	0.35 (0.3 to 0.4)	a,b
18:0	11.0 (10.6 to 11.3)	10.5 (10.0 to 11.0)	9.75 (9.4 to 10.1)	14.7 (14.3 to 15.2)	a,b,c
20:0	0.5 (0.5 to 0.5)	0.5 (0.5 to 0.6)	0.5 (0.4 to 0.5)	0.9 (0.8 to 1.0)	c
22:0	1.3 (1.2 to 1.4)	1.3 (1.2 to 1.50)	1.2 (1.1 to 1.4)	1.45 (1.3 to 1.6)	
23:0	0.5 (0.5 to 0.6)	0.5 (0.5 to 0.6)	0.5 (0.45 to 0.6)	0.2 (0.15 to 0.2)	c
24:0	0.9 (0.8 to 1.0)	0.9 (0.8 to 1.0)	0.85 (0.75 to 0.95)	1.4 (1.1 to 1.7)	c
18:3n-3	0.1 (0.1 to 0.2)	0.1 (0.1 to 0.2)	0.2 (0.1 to 0.2)	ND	
20:4n-3	0.1 (0.1 to 0.1)	0.1 (0.1 to 0.2)	0.1 (0.1 to 0.1)	0.1 (0.0 to 0.1)	
20:5n-3	0.5 (0.4 to 0.6)	0.6 (0.4 to 0.7)	0.4 (0.3 to 0.5)	0.3 (0.2 to 0.4)	c
22:5n-3	0.7 (0.6 to 0.8)	0.7 (0.6 to 0.8)	0.6 (0.5 to 0.7)	0.8 (0.5 to 1.1)	b
22:6n-3	4.1 (3.7 to 4.5)	4.2 (3.6 to 4.7)	3.7 (3.2 to 4.3)	6.2 (5.1 to 7.3)	c
18:2n-6	20.4 (19.6 to 22.2)	20.8 (19.2 to 22.4)	20.8 (19.3 to 22.2)	7.7 (6.9 to 8.5)	c
20:2n-6	0.45 (0.4 to 0.5)	0.4 (0.4 to 0.5)	0.4 (0.3 to 0.4)	0.3 (0.3 to 0.3)	
20:3n-6	2.8 (2.5 to 3.0)	2.8 (2.5 to 3.1)	3.0 (2.7 to 3.3)	4.5 (4.1 to 4.95)	c
20:4n-6	8.6 (7.9 to 9.3)	7.9 (7.2 to 8.6)	7.6 (7.0 to 8.2)	15.2 (14.4 to 15.9)	b,c
22:4n-6	0.3 (0.3 to 0.4)	0.3 (0.3 to 0.3)	0.3 (0.3 to 0.3)	0.7 (0.6 to 0.9)	b,c
22:5n-6	0.3 (0.3 to 0.4)	0.3 (0.2 to 0.3)	0.3 (0.3 to 0.4)	0.5 (0.5 to 0.6)	c
24:2n-6	0.2 (0.15 to 0.2)	0.2 (0.2 to 0.3)	0.2 (0.15 to 0.2)	0.6 (0.5 to 0.7)	c
16:1n-7	0.4 (0.3 to 0.5)	0.4 (0.3 to 0.6)	0.7 (0.4 to 0.9)	0.6 (0.5 to 0.7)	
18:1n-7	1.4 (1.2 to 1.5)	1.3 (1.2 to 1.4)	1.3 (1.1 to 1.4)	2.3 (2.1 to 2.5)	c
18:1n-9	8.1 (7.7 to 8.6)	8.3 (7.8 to 8.7)	8.7 (7.9 to 9.6)	6.5 (6.3 to 6.7)	c
20:1n-9	0.1 (0.1 to 0.1)	0.1 (0.1 to 0.1)	0.1 (0.10 to 0.12)	0.1 (0.05 to 0.1)	a,b,c
20:3n-9	0.2 (0.1 to 0.2)	0.1 (0.1 to 0.2)	0.2 (0.14 to 0.2)	0.3 (0.2 to 0.3)	
24:1n-9	1.6 (1.5 to 1.8)	1.7 (1.5 to 1.8)	1.6 (1.4 to 1.7)	1.9 (1.7 to 2.1)	
Σn-3	5.6 (5.0 to 6.2	5.7 (4.8 to 6.5)	5.1 (4.24 to 5.9)	7.4 (6.0 to 8.7)	c
Σn-6	33.5 (32.5 to 34.4)	32.8 (31.6 to 33.9)	32.6 (31.0 to 34.2)	29.6 (28.3 to 30.9)	c
Σn-7	1.7 (1.5 to 2.0)	1.7 (1.5 to 1.9)	1.9 (1.6 to 2.3)	2.9 (2.6 to 3.1)	c
Σn-9	10.05 (9.6 to 10.5)	10.2 (9.7 to 10.7)	10.6 (9.7 to 11.5)	8.7 (8.4 to 9.0)	c
SFA	48.9 (48.2 to 49.5)	49.4 (49.0 to 49.9)	49.6 (48.9 to 50.3)	51.4 (50.4 to 52.5)	b,c
MUFA	11.6 (11.0 to 12.2)	11.76 (11.13 to 12.40)	12.4 (11.2 to 13.5)	11.29 (10.9 to 11.7)	
PUFA	39.2 (38.4 to 40.1)	38.6 (37.9 to 39.2)	37.8 (36.6 to 39.1)	37.2 (36.4 to 38.1)	
HUFA	17.6 (16.7 to 18.5)	17.0 (15.8 to 18.3)	16.3 (15.3 to 17.3)	28.6 (27.4 to 29.8)	b,c

[a]SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; HUFA, highly unsaturated fatty acids; ND, not detectable.
[b]Paired t test performed after arcsin transformation of all the fatty acid fractions. a, significant difference ($P < 0.005$) between maternal values of second antenatal visit and delivery; b, significant difference ($P < 0.005$) between maternal values of first antenatal visit and delivery; c, significant difference ($P < 0.005$) between maternal values at delivery and umbilical venous plasma at birth.

plasma PL-associated FA was only 34% [95% confidence interval (CI) 30.2 to 38.7] of maternal plasma PL-associated FA at delivery. The FA composition of umbilical venous plasma PL was very different from that of maternal plasma PL at delivery.

Both SFA and HUFA were significantly higher in umbilical plasma PL compared to maternal values. No significant differences were observed in MUFA and PUFA between mother and neonate.

The SFA in umbilical plasma PL showed significant differences from the SFA content of maternal plasma PL; 16:0, the major SFA, was lower in umbilical plasma compared to maternal values. In contrast, the 18:0 content was significantly higher in umbilical plasma. The other long-chain SFA were also higher in the neonate compared to the mother, 20:0 ($P < 0.0001$) and 24:0 ($P < 0.001$). The odd-chain FA 15:0 ($P < 0.001$) and 23:0 ($P < 0.0001$) were lower in the neonate.

Umbilical plasma PL were significantly enriched in all the individual n-6 HUFA compared to maternal plasma. In contrast, maternal plasma contained more 18:2n-6. α-Linolenic acid (18:3n-3) was not detected in umbilical plasma. Eicosapentaenoic acid (20:5n-3) was significantly lower in umbilical plasma, while its elongation and desaturation product docosahexaenoic acid (22:6n-3) was significantly higher in umbilical plasma PL compared to maternal plasma PL.

Calculated parameters. (i) Maternal plasma. The MMP of the FA in plasma PL at delivery is significantly elevated compared to mid-pregnancy (increase of 1.13°C, 95% CI, 0.64 to 1.61, $P < 0.001$). The MCL was significantly lower at delivery compared to gestation. These parameters have an effect on

TABLE 2
Calculated Values Derived from the Fatty Acid Composition of Maternal Plasma Phospholipids During the Course of Pregnancy and at Delivery and of Umbilical Venous Plasma at Birth: mean (95% CI of the mean) (*n* = 16)

Calculated values[a]	Maternal plasma 1st antenatal visit: 15–24 wk	Maternal plasma 2nd antenatal visit: 29–36 wk	Maternal plasma delivery: 38–41 wk	Umbilical plasma birth: 38–41 wk	Paired *t*-test[b]
OPI	32.15 (30.87 to 33.44)	31.50 (29.78 to 33.23)	29.94 (28.34 to 31.54)	43.10 (40.54 to 45.66)	a,b,c
MCL (overall)	18.02 (17.98 to 18.06)	18.00 (17.95 to 18.05)	17.92 (17.87 to 17.97)	18.39 (18.30 to 18.47)	a,b,c
MCL SFA	16.86 (16.81 to 16.90)	16.84 (16.79 to 16.90)	16.78 (16.72 to 16.83)	17.04 (16.96 to 17.13)	a,b,c
MCL MUFA	18.80 (18.71 to 18.90)	18.80 (18.72 to 18.89)	18.70 (18.59 to 18.81)	18.90 (18.82 to 18.98)	b,c
MCL PUFA	19.23 (19.15 to 19.30)	19.22 (19.12 to 19.33)	19.18 (19.08 to 19.27)	20.09 (19.99 to 20.19)	c
MCL HUFA	20.62 (20.58 to 20.66)	20.64 (20.59 to 20.68)	20.61 (20.55 to 20.66)	20.57 (20.49 to 20.64)	
MMP (overall)	26.17 (25.49 to 26.84)	26.84 (26.20 to 27.47)	27.30 (26.59 to 28.70)	24.49 (23.48 to 25.50)	b,c
MMP SFA	65.65 (65.52 to 65.79)	65.61 (65.45 to 65.78)	65.41 (65.25 to 65.58)	66.27 (66.02 to 66.53)	a,b,c
MMP MUFA	17.24 (16.78 to 17.69)	17.22 (16.76 to 17.68)	16.64 (16.08 to 17.20)	17.67 (17.27 to 18.07)	b,c
MMP PUFA	–20.49 (–21.58 to –19.40)	–20.04 (–21.40 to –18.67)	–19.27 (–20.42 to –18.13)	–31.12 (–32.38 to –29.86)	c
MMP HUFA	–39.56 (–40.21 to –38.90)	–39.15 (–39.84 to –38.45)	–38.14 (–39.04 to –37.23)	–39.52 (–40.32 to –38.72)	b

[a]OPI, oxidative potential index; MCL, mean chain length, expressed as number of carbon atoms; MMP, mean melting point, expressed as degrees Celsius. For other abbreviations see Table 1.
[b]a, significant difference ($P < 0.005$) between maternal values of second antenatal visit and delivery; b, significant difference ($P < 0.005$) between maternal values of first antenatal visit and delivery; c, significant difference ($P < 0.005$) between maternal values at delivery and umbilical venous plasma at birth.

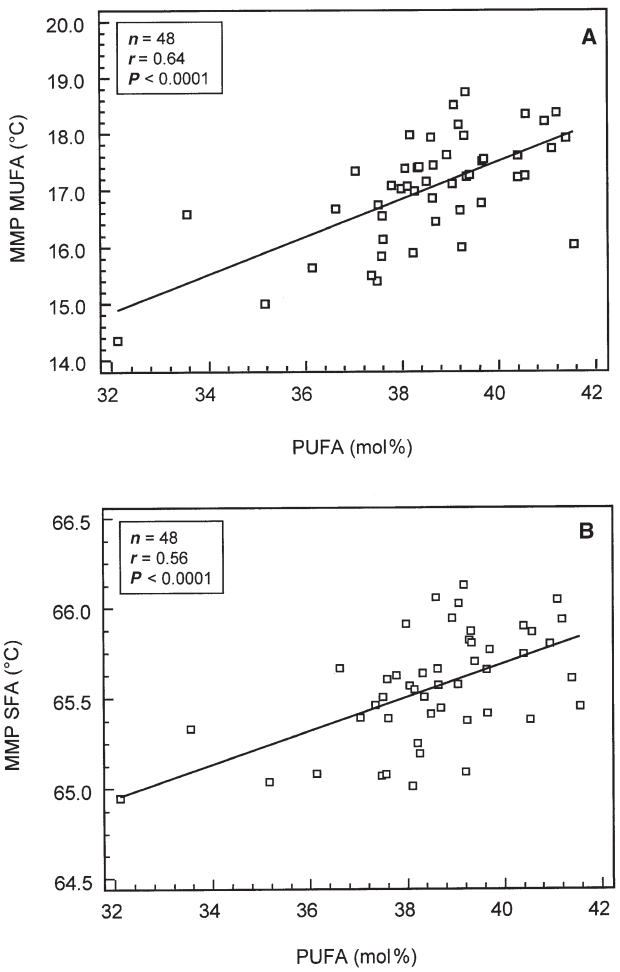

FIG. 1. Correlation between the mean melting point (MMP) of the monounsaturated fatty acids (MUFA) and of the saturated fatty acids (SFA) in plasma phospholipids and the fraction of polyunsaturated fatty acids (PUFA) in maternal plasma phospholipids (mol%).

membrane fluidity. The OPI of plasma PL, an index for susceptibility toward peroxidation, was significantly lower at delivery compared to gestation. The change in composition of the SFA results in a shorter MCL and consequently a lower MMP (decrease of 0.24°C, 95% CI, 0.13 to 0.34, $P < 0.001$) of the SFA. The same phenomenon was observed in MUFA, but it was less pronounced, the MCL of the MUFA is shorter at delivery compared to mid-pregnancy ($P < 0.005$) and their MMP is lower (0.60°C, 95% CI, 0.25 to 0.95, $P < 0.005$).

The mole fraction of the PUFA, but not of HUFA, in the PL correlated positively with both the MMP of SFA ($r = 0.56$, $n = 48$, $P < 0.0001$) and with the MMP of MUFA ($r = 0.64$, $n = 48$, $P < 0.0001$) (Fig. 1). Even without the two values with a low PUFA fraction (<34 mol%), the same association still exists.

(ii) Umbilical plasma. The high concentration of HUFA in umbilical plasma caused a significantly higher OPI, a longer MCL, and a lower MMP compared to maternal values. The lower global MMP in umbilical plasma PL compared to maternal plasma PL is associated with a higher MMP of the SFA (0.86°C, 95% CI, 0.61 to 1.11, $P < 0.001$) and of the MUFA (1.03°C, 95% CI, 0.46 to 1.60, $P = 0.002$).

Higher concentrations of HUFA in the newborn than in the mother are associated with higher concentrations of SFA, and a shift in the composition of SFA toward longer chain lengths. The MCL of the SFA is significantly higher in umbilical plasma compared to maternal plasma at delivery. These changes partially counteract the reduction in MMP induced by higher levels of HUFA.

DISCUSSION

The loss of HUFA (mean 1.30% pts, 95% CI, 0.61–1.99) at delivery compared to mid-pregnancy was about equal to the increase in SFA and MUFA (mean 1.44% pts, 95% CI, 0.48–2.41). The lower content of HUFA in maternal plasma PL resulted in a higher MMP of the FA mixture in plasma PL.

HUFA were preferentially replaced by shorter-chain SFA, especially palmitic acid as was also demonstrated in anorexia nervosa (4). These shorter-chain FA are more fluid than their longer-chain homologs because they have lower melting points. The MMP of SFA was significantly lower at delivery compared to pregnancy. Although the shorter chain length of the SFA compensates only partially for the higher overall MMP, it is possible that FA metabolism in pregnancy attempts to maintain MMP homeostasis as was shown previously in multiple sclerosis (1). An additional indication for this hypothesis is the positive correlation between the fraction of PUFA in PL with the MMP of SFA and of MUFA. This indicates that a loss in PUFA, which would result in an increased MMP, is accompanied by an increase in shorter-chain SFA and MUFA, which have an opposing effect on the MMP. A difference as large as 10.8°C was observed by Holman *et al.* (3) between the MMP of plasma PL FA of nonpregnant healthy controls (15.3°C) and the MMP of plasma PL FA of women at parturition (26.1°C). Taking into account the differences in MMP found in different populations , the latter value compares favorably with ours at delivery (27.3°C). In our study population, the MMP increases only 1.13°C from mid-pregnancy until delivery. The MMP of the FA mixture of PL in umbilical plasma (24.49°C) is comparable with a previously calculated value of 20.07°C of PL in normal cord serum (2), but is much lower than maternal values due to higher HUFA status. A higher HUFA fraction in umbilical plasma PL is accompanied with more longer-chain, less fluid SFA. The observed changes in the FA composition of maternal plasma PL during the course of pregnancy are probably not due to changes in dietary intake. Indeed, analysis of food frequency questionnaires (surveyed at the beginning of pregnancy and in the third trimester) of Belgian pregnant women attended by the same obstetrician revealed no significant differences in the FA composition of the diet nor in fat intake during the course of pregnancy (DeVriese, S.R., Matthys, C., De Henauw, S., Christophe, A.B., and Dhont, M., unpublished results). Others confirm these findings: the maternal dietary fat composition of pregnant Dutch women was consistent during pregnancy (12).

Holman *et al.* (3) found that pregnant women in their 36th wk of gestation have significantly suppressed concentrations of all the products of Δ5 desaturation compared to nonpregnant women. Our data reveal a similar pattern; the concentration of Δ5 products in mid-pregnancy is significantly higher than at delivery.

The essential fatty acid composition of maternal PL slightly changed from mid-pregnancy to delivery; 20:4n-6 and its elongation product 22:4n-6 declined, but 18:2n-6 remained stable. No changes were observed in 18:3n-3, 20:5n-3, or 22:6n-3, while 22:5n-3 decreased. The sum of the Δ5 desaturation products was lower at delivery indicating a lower essential FA status of the mother; however, the differences were small. Similar deviations in maternal essential FA status, but of greater magnitude, were reported by Al *et al.* (10th wk of gestation vs. delivery) (13) and by Otto *et al.* (14th wk of gestation vs. delivery) (14). The FA pattern of umbilical plasma presented here is very different from maternal values as was noted in previous studies (2,11–13,15–17). HUFA, especially 20:4n-6 and 22:6n-3, are higher in umbilical than in maternal plasma and both FA are lower in maternal plasma PL after delivery compared to mid-pregnancy. As FA desaturation and elongation by fetal tissues cannot meet neonatal needs (18–20), this could indicate a preferential placental transfer of these long-chain FA to the fetus (21). The total amount of FA in PL (mg/L plasma), and of each individual FA, is much lower in umbilical plasma compared to maternal values (Table 1). This is in concurrence with literature findings (13).

In summary, small but significant deviations were found in maternal plasma PL essential FA between mid-gestation and delivery. This is in concurrence with previous reports on the essential FA status of the mother during pregnancy.

This study extends the concept that changes in overall MMP are counteracted by changes in the MMP of individual FA classes. We demonstrated that in maternal plasma, the loss of HUFA during gestation is accompanied by a shorter MCL and a decrease in the MMP of the SFA. The high content of HUFA in umbilical plasma is associated with a significantly longer MCL and a higher MMP of the SFA. Thus, the FA composition of the SFA changes in a way to counteract changes in the MMP induced by changed HUFA composition. Similar adaptations in the FA composition of SFA, to maintain homeostasis in the overall MMP, were found in multiple sclerosis (2), cystic fibrosis, and anorexia nervosa (4).

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Chapter 6

Fatty acid composition of cholesteryl esters and phospholipids in maternal plasma during pregnancy and at delivery and in cord plasma at birth

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ARTICLES

FA Composition of Cholesteryl Esters and Phospholipids in Maternal Plasma During Pregnancy and at Delivery and in Cord Plasma at Birth

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ABSTRACT: The purpose of this study was to assess the FA composition of both cholesteryl esters (CE) and phospholipids (PL) in maternal plasma during pregnancy and at delivery and in umbilical plasma at birth. A longitudinal study of 32 normal pregnant women was carried out with three cutoff points during pregnancy (first, second, and third trimester) and at delivery. Few significant differences occurred in the FA profile of maternal CE: 18:1n-9 increased, 18:2n-6 dropped slightly, and 18:3n-3 decreased with progressing gestation. In maternal PL, long-chain highly unsaturated FA concentrations dropped and were replaced by saturated FA as gestation progressed. Additionally, changes in saturated FA in PL occurred: Shorter-chain 16:0 was higher whereas longer-chain 18:0 was lower at delivery compared to early pregnancy. The FA profile of umbilical venous plasma was strikingly different from that of maternal plasma at delivery. Cord plasma CE contained more saturated and monounsaturated FA than maternal CE. The polyunsaturates 18:2n-6 and 18:3n-3 are lower in umbilical CE than in maternal CE whereas 20:4n-6 and 22:6n-3 are twice as high in umbilical CE. Cord plasma PL have a higher content of long-chain highly unsaturated FA than maternal plasma PL at delivery. In contrast to maternal plasma PL, 16:0 was lower and longer-chain saturated FA were higher in cord plasma PL. The FA profile of umbilical plasma at birth shows preferential accumulation of 20:4n-6 and 22:6n-3, with low concentrations of 18:2n-6 and 18:3n-3 in CE and PL, indicating a preferential supply of the fetus with long-chain highly unsaturated FA needed for fetal development.

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The two most important families of long-chain FA for human beings are the n-6 and n-3 families. Linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) can be desaturated and elongated to form long-chain PUFA, which play a major role in the development of new life as important structural components of cell membrane phospholipids (PL) (1,2). During pregnancy, accretion of maternal, placental, and fetal tissue occurs. Therefore, the requirement for PUFA is high for pregnant women and the developing fetus. Arachidonic acid (20:4n-6) and docosahexaenoic acid (22:6n-3) are important structural FA in neural tissue such as the brain and retina (3,4). The FA 18:2n-6, 18:3n-3, 20:4n-6, and 22:6n-3 are conditionally indispensable FA for fetuses, and pregnant and lactating women (5). Cunnane (5) introduced the classification of conditionally indispensable and conditionally dispensable FA instead of EFA (5).

Several authors have analyzed the FA composition of maternal plasma PL throughout pregnancy and of umbilical plasma PL at birth (6–12). Few studies have described the FA composition of cholesteryl esters (CE) in maternal and umbilical plasma at delivery (13–16). The FA composition of maternal plasma CE on three different occasions during the course of pregnancy and at delivery has, to our knowledge, never been described before.

In a previous study we reported a number of changes in the composition and the calculated mean melting point (MMP) of FA in plasma PL throughout pregnancy in a small study population (n = 16) (7). We found that the MMP of maternal PL was significantly higher at delivery compared to mid-gestation due to a loss of highly unsaturated FA, which were replaced by saturated FA (SFA). In addition, changes in SFA occurred: The content of 16:0, with a lower melting point (MP), was higher while 18:0, with a higher MP, was lower at delivery (7). In contrast to maternal plasma, 16:0 was lower in umbilical plasma while the longer chain SFA were higher, tending to raise the overall MMP.

The aim of the present work was to confirm previous findings in a larger study population and to assess whether similar changes occur in the FA profile of plasma CE. Furthermore, the relationship between the FA in the PL and the CE fractions was investigated.

SUBJECTS AND METHODS

Study population. Healthy pregnant women attending the Department of Gynecology of Ghent University Hospital, Belgium, were asked to cooperate in this study. All pregnant volunteers signed a written informed consent form, approved by the ethics committee of Ghent University Hospital. Inclusion criteria were: singleton pregnancy, nullipara, term delivery (38–42 wk), normotensive (diastolic blood pressure below 90 mm Hg). We excluded women (i) who were diagnosed with gestational diabetes mellitus; (ii) who had signs of proteinuria; and (iii) who suffered from renal or cardiovascular disease. We did not exclude women who delivered through a Caesarean section (n = 2) because no important differences in the maternal FA composition of serum PL was found between mothers who had a normal vaginal delivery and mothers who

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Abbreviations: CE, cholesteryl esters; HUFA, highly unsaturated FA; MCL, mean chain length; MMP, mean melting point; MP, melting point; MUFA, monounsaturated FA; PL, phospholipids; SFA, saturated FA.

had a Caesarean section (16). From the 39 pregnant women who entered the study, 34 completed the study. Five women failed to finish the study owing to lack of motivation. Two pregnant women delivered preterm and were excluded from the study. The study population thus consisted of 32 healthy pregnant women and their neonates (13 girls and 19 boys).

Maternal venous blood samples were obtained thrice during the course of pregnancy: (i) between 5 and 14 wk of gestation (median 12 wk), (ii) between 20 and 24 wk (median 22 wk), and (iii) between 29 and 37 wk (median 32 wk) and (iv) at delivery (median 39.5 wk). Umbilical venous blood was collected immediately after the cord had been clamped. Blood was collected in EDTA-containing Vacutainer tubes (Belliver Industrial Estate, Plymouth, United Kingdom) and temporarily stored at 6°C. Within 24 h of collection, plasma was separated from blood cells by centrifugation ($600 \times g$ for 5 min at 4°C) and stored in plastic tubes under nitrogen at –80°C until further analysis.

FA analysis of plasma CE and PL. All samples of a given mother–infant pair were analyzed simultaneously. Lipids were extracted from 1 mL plasma according to a modified Folch *et al.* extraction with methanol/chloroform (1:2) (17). The lipids were separated by TLC on rhodamine-impregnated silica gel plates using petroleum ether (b.p. 60–80°C; Merck Belgolab, Overijse, Belgium)/acetone 85:15 as mobile phase (18). The CE and PL fractions were scraped off and the FA converted into methyl esters by transesterification with 2 mL of a mixture of methanol/benzene/HCl (aqueous, 12 N) (80:20:5) (19). After cooling and adding 2 mL of water, FAME were extracted with petroleum ether (b.p. 40–60°C), evaporated to dryness under a nitrogen flow at a temperature not exceeding 40°C, and analyzed by temperature-programmed capillary GC (Varian Model 3500) on a 25 m × 250 μm (length × i.d.) × 0.2 μm df Silar 10C column (19). The injection and detection temperatures were set at 285°C. The starting temperature of the column was 150°C, which was increased to 240°C after 3 min at a rate of 2°C/min. The carrier gas was nitrogen with a flow of 25 cm/s. Peak identification was performed by spiking with authentic standards (Sigma-Aldrich, Bornem, Belgium). Peak integration and calculation of the percent composition was performed electronically with a Varian Model 4290 integrator. The coefficient of intra-assay variation of the entire method of FA analysis was less then 5%.

The results are expressed as weight percentage (wt%) of total FA. Twenty-six different FA with chain lengths between 14 and 24 carbon atoms were identified. The sum of all the SFA (∑SFA); the monounsaturated FA (∑MUFA); the PUFA (∑PUFA); the long-chain highly unsaturated FA (∑HUFA = FA with 20 or more carbon atoms and with at least three double bonds); ∑n-3; ∑n-6; and ∑*trans* FA were calculated and are reported together with the individual FA. The mean melting point (MMP, °C) (sum of the mole fraction multiplied by the MP for each FA) and the mean chain length (MCL) (sum of the mole fraction multiplied by the number of carbon atoms in the FA) of the plasma lipids were assessed (7).

Statistical analysis. Values are reported as mean and SD in parentheses. The normality of distribution was ascertained with the Kolmogorov–Smirnov test. The FA (wt%) that had a skewed distribution were log-transformed for the statistical analyses of these variables. Group mean differences were assessed by means of ANOVA. Repeated-measures ANOVA was used to test for significant differences in the FA composition of maternal serum PL and CE during the course of pregnancy. The ANOVA model included only a time factor (FA data thrice during the course of pregnancy and shortly after delivery). A paired Student's *t*-test was performed for maternal-umbilical FA comparisons at delivery and birth. In order to avoid type 2 errors, due to multiple comparisons, a value of $P < 0.005$ was taken as the criterion of significance. The degree of association was calculated using the Spearman rank correlation. The data were analyzed using both SPPS (version 10.0 for Windows; SPSS Inc., Chicago, IL) (20) and the MedCalc statistical program (version 6; MedCalc Software, Mariakerke, Belgium) (21). For 4 of our 32 subjects, we were unable to obtain a complete set of matching plasma PL or CE data for maternal and umbilical blood samples. This accounts for the various sample sizes in Tables 1 and 2. However, all repeated-measures ANOVA or paired *t*-tests were made with matching samples.

RESULTS

Clinical characteristics. The mean age of the mothers (n = 32) at delivery was 29 yr (range 21–41 yr). The mean body mass index of the women before pregnancy was 23.6 (range 17.6–35.6). All the infants were born healthy with a mean birth weight of 3155 g (range 2300–4020 g) and a mean crown–heel length of 50.2 cm (range 48–52 cm).

The FA composition of maternal plasma CE and PL (wt%) during the course of pregnancy and at delivery and from umbilical plasma shortly after birth are summarized in Tables 1 and 2.

FA composition of maternal plasma lipid classes. (i) CE. The fraction of the individual SFA remained stable in maternal CE during pregnancy. The major monounsaturate, oleic acid (18:1n-9), and as a consequence ∑MUFA, significantly increased with progressing gestation. Linoleic acid with more than 50 wt% of the FA was the major FA of the CE fraction. The acid 18:2n-6 slightly decreased with progressing gestation. On the other hand, α-linolenic acid increased slightly with progressing gestation and reached a maximum during the third trimester. ∑PUFA significantly decreased in maternal CE during pregnancy. None of the long-chain highly unsaturated acids changed significantly in maternal CE. When we compare maternal values from the first trimester with delivery values, more significant differences were found in CE: 20:4n-6, 20:5n-3, ∑n-6, and ∑HUFA were significantly ($P <$ 0.001) lower at delivery. Neither the MMP nor the MCL of the FA in maternal plasma CE changed significantly with progressing gestation.

(ii) PL. ∑HUFA significantly decreased while ∑SFA significantly increased during gestation. ∑PUFA slightly

TABLE 1
Composition (wt% of total FA) of FA in CE Isolated from Maternal Venous Plasma During the Course of Pregnancy and at Delivery and from Umbilical Venous Plasma at Birth[a]: Mean and SD

	Trimester I (n = 32)		Trimester II (n = 28)		Trimester III (n = 29)		Delivery (n = 30)		RM ANOVA	Umbilical cord (n = 28)		Paired t-test
CE	Mean	SD	Mean	SD	Mean	SD	Mean	SD	(P)	Mean	SD	(P)
14:0	0.84	0.24	0.83	0.24	0.77	0.20	0.77	0.28	0.03	1.20	0.58	0.001‡
15:0	0.24	0.05	0.22	0.08	0.22	0.09	0.29	0.41	0.73	0.43	0.30	0.04
16:0	11.73	1.34	11.83	1.31	11.74	1.32	12.04	1.93	0.18	19.63	3.23	0.0001‡
17:0	0.15	0.12	0.13	0.19	0.13	0.17	0.13	0.13	0.32	0.80	1.32	0.01
18:0	0.97	0.72	0.82	0.50	0.83	0.71	0.70	0.45	0.30	3.92	2.63	0.0001‡
∑SFA	14.28	2.22	14.11	1.91	14.00	2.36	14.29	2.82	0.05	27.05	6.03	0.0001‡
16:1n-9	0.35	0.17	0.33	0.15	0.30	0.16	0.38	0.32	0.59	1.20	1.07	0.0001‡
16:1n-7	2.62	0.90	2.84	1.46	3.00	1.16	3.93	1.66	0.04	5.49	2.55	0.02
18:1n-9	15.96	2.24	16.65	2.37	17.31	2.34	18.58	2.31	0.0001*	24.28	6.29	0.0001‡
18:1n-7	0.70	0.55	0.62	0.46	0.55	0.52	0.58	0.59	0.28	1.95	1.15	0.0001‡
∑MUFA	19.82	2.75	20.56	3.44	21.35	2.92	23.72	3.26	0.0001*	33.89	7.12	0.0001‡
16:2n-6	0.09	0.09	0.08	0.10	0.10	0.10	0.11	0.13	0.62	0.42	0.54	0.007
18:2n-6	55.00	4.74	54.90	5.06	54.68	4.54	52.44	5.97	0.01	20.18	6.66	0.0001‡
18:3n-6	0.37	0.27	0.28	0.25	0.27	0.28	0.41	0.29	0.20	0.41	0.35	0.99
20:3n-6	0.74	0.24	0.79	0.21	0.79	0.17	0.74	0.19	0.45	1.37	0.86	0.001‡
20:4n-6	6.79	1.62	6.42	1.30	5.87	1.16	5.59	1.50	0.05	11.31	4.18	0.0001‡
∑n-6	63.09	4.37	62.53	4.78	61.84	3.92	59.45	5.55	0.01	34.27	7.50	0.0001‡
18:3n-3	0.55	0.25	0.63	0.23	0.69	0.19	0.62	0.14	0.009	0.26	0.25	0.0001‡
18:4n-3	ND		ND		ND		ND		ND	0.25	0.26	0.0001‡
20:5n-3	0.59	0.32	0.63	0.50	0.47	0.24	0.38	0.26	0.02	0.41	0.33	0.80
22:5n-3	0.09	0.13	0.09	0.12	0.11	0.12	0.13	0.19	0.42	0.23	0.23	0.10
22:6n-3	0.76	0.33	0.80	0.27	0.74	0.33	0.61	0.35	0.09	1.24	1.12	0.01
∑n-3	2.01	0.73	2.18	0.75	2.04	0.50	1.75	0.63	0.04	2.40	1.27	0.04
20:3n-9	ND		ND		ND		ND		ND	0.21	0.14	0.0001‡
∑PUFA	65.15	4.10	64.73	4.47	63.91	3.82	61.23	5.45	0.005*	36.88	8.03	0.0001‡
∑HUFA	9.09	1.83	8.79	1.69	8.08	1.35	7.58	1.98	0.03	15.22	5.63	0.0001‡
∑*trans* FA	0.47	0.21	0.43	0.19	0.49	0.26	0.50	0.22	0.67	1.08	1.01	0.007
MMP (°C)	5.36	1.88	5.43	1.61	5.81	1.79	6.42	2.49	0.21	14.84	5.00	0.0001‡
MCL	17.82	0.05	17.80	0.06	17.79	0.04	17.76	0.09	0.04	17.67	0.22	0.04

[a]RM, repeated measurements; ND, not detectable; ∑SFA, sum of the saturated FA; ∑MUFA, sum of the monounsaturated FA; ∑PUFA, sum of PUFA; ∑HUFA, sum of the long-chain highly unsaturated FA (FA with 20 or more carbon atoms and with at least three double bonds); ∑*trans* FA, sum of the *trans* FA;MMP, mean melting point (sum of the mole fraction multiplied by the melting point for each FA); MCL, mean chain length (sum of the mole fraction multiplied by the number of carbon atoms in the FA).
*Significantly different according to repeated measurements ANOVA (maternal values during pregnancy); ‡significantly different according to paired Students' t-test (comparison between maternal values at delivery and umbilical values at birth).

decreased and ∑MUFA slightly increased throughout gestation, but significance was not reached. Palmitic acid (16:0) and stearic acid (18:0), the two major saturates, changed significantly but in opposite directions: 16:0 increased while 18:0 fell during pregnancy. The long-chain 24:0 also dropped significantly during gestation. Few of the individual n-6 or n-3 FA differed significantly with progressing gestation. Arachidonic acid (20:4n-6) declined, but linoleic acid remained stable. In the series of n-3 FA, only 22:5n-3 significantly dropped during pregnancy. When we compare maternal values from the first trimester with delivery values, more significant differences were found in PL: 20:4n-6, 20:5n-3, and 22:5n-3 were significantly ($P < 0.001$) lower at delivery. The MMP of the FA of maternal plasma PL significantly rose with progressing gestation and their MCL significantly decreased.

Maternal-umbilical FA comparisons at delivery and birth. (i) CE. ∑SFA and ∑MUFA were significantly higher in cord plasma CE compared to maternal plasma CE. The two major saturates, 16:0 and 18:0, and oleic acid were much higher in umbilical CE. ∑PUFA were significantly lower (36.9 vs. 61.2 wt%), whereas ∑HUFA were significantly higher in cord plasma CE. The only two FA that were lower in umbilical CE compared to maternal CE were 18:2n-6 and 18:3n-3. Linoleic acid was extremely low in cord plasma (20.2 vs. 52.4 wt%). Arachidonic acid, on the other hand, was twice as high in the neonate as in the mother (11.3 vs. 5.6 wt%). Similarly, 22:6n-3 was also twice as high in cord plasma (1.24 vs. 0.61 wt%). Mead acid (20:3n-9), the marker of combined linoleic and α-linolenic acid deficiency, was significantly higher in the neonate. The MMP of the FA in umbilical plasma CE was significantly higher than the MMP of the maternal plasma CE-associated FA.

(ii) PL. As we found in the CE fraction, ∑SFA and ∑HUFA were significantly higher in umbilical PL compared to maternal values. ∑MUFA and ∑PUFA did not differ between maternal and umbilical plasma PL. The SFA content of umbilical PL was signifcantly different from that of maternal PL. Palmitic acid was lower in cord plasma. In contrast, the 18:0

TABLE 2
Composition (wt% of total FA) of FA in PL Isolated from Maternal Venous Plasma During the Course of Pregnancy and at Delivery and from Umbilical Venous Plasma at Birth[a]: Mean and SD

	Trimester I (n = 32)		Trimester II (n = 28)		Trimester III (n = 30)		Delivery (n = 32)		RM ANOVA	Umbilical cord (n = 32)		Paired t-test
PL	Mean	SD	Mean	SD	Mean	SD	Mean	SD	(P)	Mean	SD	(P)
14:0	0.34	0.09	0.40	0.17	0.38	0.09	0.34	0.20	0.28	0.33	0.12	0.87
15:0	0.25	0.14	0.27	0.13	0.23	0.10	0.24	0.25	0.14	0.18	0.10	0.14
16:0	28.40	1.73	29.77	2.79	30.21	1.03	32.24	2.46	0.0001*	28.69	2.39	0.0001‡
17:0	0.38	0.30	0.47	0.40	0.32	0.13	0.34	0.20	0.17	0.33	0.20	0.85
18:0	11.75	1.01	11.19	1.27	10.58	0.71	9.90	1.04	0.0001*	14.11	2.20	0.0001‡
20:0	0.52	0.10	0.57	0.13	0.56	0.09	0.56	0.14	0.10	0.92	0.20	0.0001‡
22:0	1.54	0.25	1.60	0.28	1.60	0.21	1.48	0.20	0.03	1.73	0.27	0.0001‡
23:0	0.62	0.12	0.66	0.11	0.59	0.17	0.53	0.19	0.02	0.25	0.13	0.0001‡
24:0	1.14	0.19	1.17	0.19	1.08	0.15	0.99	0.16	0.0001*	1.74	0.47	0.0001‡
∑SFA	44.95	1.28	46.10	3.89	45.55	0.99	46.61	3.32	0.001*	48.29	2.50	0.02
16:0 DMA	0.82	0.14	0.73	0.17	0.66	0.11	0.64	0.26	0.0001*	0.98	0.36	0.0001‡
18:0 DMA	0.49	0.28	0.46	0.23	0.36	0.24	0.39	0.16	0.03	0.49	0.28	0.02
∑DMA	1.31	0.34	1.19	0.33	1.02	0.29	1.03	0.31	0.0001*	1.47	0.58	0.0001‡
16:1n-9	0.27	0.11	0.30	0.14	0.27	0.11	0.27	0.15	0.21	0.26	0.11	0.85
16:1n-7	0.41	0.30	0.40	0.19	0.43	0.20	0.54	0.23	0.66	0.60	0.23	0.26
18:1n-9	8.02	1.30	7.83	1.20	8.63	1.17	8.69	1.26	0.54	7.11	1.18	0.0001‡
18:1n-7	1.11	0.35	1.07	0.38	1.10	0.27	1.10	0.37	0.005*	1.92	0.37	0.0001‡
24:1n-9	2.24	0.54	2.20	0.41	2.26	0.39	2.16	0.55	0.72	2.74	0.59	0.0001‡
∑MUFA	12.25	1.52	12.10	1.38	12.92	1.31	12.98	1.23	0.008	12.85	1.22	0.60
18:2n-6	19.90	3.00	19.83	3.04	20.29	2.66	19.56	3.01	0.62	8.85	3.98	0.0001‡
20:2n-6	0.38	0.18	0.36	0.18	0.40	0.13	0.35	0.14	0.08	0.26	0.11	0.0001‡
20:3n-6	3.12	0.66	2.99	0.64	3.12	0.72	3.08	0.58	0.66	4.24	0.84	0.0001‡
20:4n-6	9.76	1.76	8.78	1.49	8.49	1.31	8.70	1.82	0.0001*	14.80	3.33	0.0001‡
22:4n-6	0.28	0.22	0.35	0.17	0.32	0.20	0.30	0.21	0.23	0.67	0.52	0.0003‡
22:5n-6	0.30	0.16	0.33	0.12	0.34	0.15	0.38	0.16	0.006	0.59	0.25	0.0001‡
∑n-6	33.99	2.50	32.84	3.68	33.19	2.43	32.52	3.10	0.12	29.62	2.33	0.0001‡
18:3n-3	0.19	0.08	0.26	0.29	0.26	0.13	0.21	0.14	0.05	0.07	0.08	0.0001‡
20:5n-3	0.70	0.39	0.72	0.52	0.56	0.37	0.49	0.30	0.07	0.36	0.23	0.003‡
22:5n-3	0.85	0.24	0.73	0.21	0.68	0.19	0.62	0.15	0.0001*	0.48	0.17	0.0002‡
22:6n-3	4.93	0.99	5.13	1.16	4.94	1.16	4.73	1.32	0.11	5.95	1.68	0.001‡
∑n-3	6.73	1.47	6.94	1.74	6.52	1.51	6.16	1.66	0.05	7.01	1.81	0.02
20:3n-9	0.07	0.05	0.16	0.26	0.10	0.09	0.11	0.12	0.23	0.28	0.17	0.0001‡
∑PUFA	40.79	2.08	39.93	3.44	39.81	1.76	38.79	3.05	0.007	36.91	2.75	0.01
∑HUFA	20.00	2.55	19.18	2.62	18.55	2.46	18.40	2.94	0.001	27.37	4.73	0.0001‡
∑*trans* FA	0.59	0.28	0.59	0.25	0.60	0.20	0.50	0.22	0.15	0.32	0.17	0.0001‡
MMP (°C)	25.32	1.29	26.35	3.12	26.34	1.16	27.01	2.81	0.0001*	25.90	2.70	0.10
MCL	18.05	0.10	18.00	0.11	17.98	0.08	17.91	0.12	0.0001*	18.25	0.19	0.0001‡

[a]DMA, dimethylacetals; PL, phospholipids; for other abbreviations see Table 1.

content was significantly higher in umbilical plasma. The other long-chain saturates (20:0; 22:0, and 24:0) were also higher in the neonate. The odd-chain 23:0 was lower in the neonate. In contrast to what was found in CE, 18:1n-9 was significantly lower in umbilical plasma PL. Umbilical PL were significantly enriched in all the individual n-6 long-chain highly unsaturated compounds compared to maternal plasma PL. Consistent with their reduced fraction in the CE, 18:2n-6 and 18:3n-3 were significantly lower in umbilical PL. EPA (20:5n-3) and 22:5n-3 were significantly lower in umbilical PL, and 22:6n-3 was significantly higher in cord plasma PL. Mead acid was significantly higher in the neonate compared to the mother. The MMP of the FA in umbilical plasma PL did not differ from that of the maternal plasma PL-associated FA although the MCL of the FA in umbilical plasma PL was significantly higher than that of maternal plasma PL FA.

Relationship between the FA in plasma CE and PL. Strong correlations ($P < 0.0001$) were found between the maternal CE and PL fractions for 18:2n-6 ($r = 0.73$), 20:3n-6 (0.67), 20:4n-6 (0.71), 20:5n-3 (0.79), and 22:6n-3 (0.49). In umbilical plasma the correlations between these FA in the CE and the PL fraction were not so strong: for 18:2n-6 ($r = 0.45$, $P < 0.01$), 20:5n-3 (0.47, $P < 0.01$), and 22:6n-3 (0.36, $P < 0.05$).

DISCUSSION

In this study population very few significant changes in the FA composition of the polyunsaturates with progressing gestation occured: In PL only 20:4n-6, 22:5n-3, and ∑HUFA dropped significantly, and in CE only 18:2n-6 slightly decreased and 18:3n-3 slightly increased. In other longitudinal studies, more significant changes in PUFA composition were

found (6,10). When we compare maternal values from the first trimester with delivery values, more significant differences are found. Our study shows preferential accumulation of 20:4n-6 and 22:6n-3, with low concentrations of 18:2n-6 and 18:3n-3 in fetal plasma CE and PL, indicating a preferential supply of the fetus with HUFA needed for fetal development. These findings are consistent with other reports (6,7,13,16,22). These observations support the hypothesis of placental selectivity for transport of certain FA (23–25). Kuhn and Crawford (24) found that during *in vitro* perfusion of the human placenta, the majority of radiolabeled 20:4n-6 from the maternal circulation was selectively exported to the fetal circulation and incorporated into fetal PL, in contrast to small amounts of 18:2n-6 and 18:3n-3.

It has been established that ∑SFA increases whereas ∑HUFA decreases in maternal plasma PL during pregnancy (7,10). We demonstrated that in maternal plasma PL the loss of HUFA during gestation is accompanied by a shorter MCL of SFA and that the high content of HUFA in umbilical plasma PL is associated with a significantly longer MCL of SFA (7). In this study we confirmed similar changes in the PL fraction. The most remarkable finding in the composition of SFA of maternal plasma PL is the increase in 16:0 together with the decrease in the longer-chain FA 18:0 and 24:0 during gestation. The concentration of HUFA in umbilical plasma PL is much higher than in maternal plasma PL at delivery. Additionally, the composition of SFA in umbilical plasma PL is completely different from maternal plasma PL; 16:0 is lower and the longer-chain FA (18:0, 20:0, 22:0, and 24:0) are much higher. Thus, this study supports our hypothesis that the FA composition of SFA of plasma PL changes in a way to counteract changes in the MCL and consequently in the MMP induced by a changed HUFA composition (7).

The increase in ∑SFA and the decline in ∑HUFA in maternal plasma PL could be related to changes in the dietary intake of FA. However, this is rather unlikely as we found in this study population that the dietary habits remain unaltered during pregnancy (26,27). Neither the amount and type of fat nor the FA composition of the maternal diet changed during pregnancy until 1 mon postpartum, as has been confirmed by others (26–28). We can conclude that in this study population maternal diet cannot be a confounding factor in the plasma FA composition.

Another possible explanation for the observed differences in the maternal plasma FA composition during pregnancy is changes in the maternal hormonal status during gestation. The major pathway for PC synthesis, the Kennedy pathway, preferentially results in the appearance of 16:0 in the *sn*-1 position and 18:2n-6 or 18:1n-9 in the *sn*-2 position. Estrogen enhances an alternative pathway, the Greenberg pathway, resulting in the appearance of more PC with 18:0 in the *sn*-1 position and 20:4n-6 in the *sn*-2 position (29–31). During pregnancy, levels of estrogens and progesterone rise steadily as a result of placental production of these hormones (32). One would expect that the rise in estrogen during pregnancy would result in an increased synthesis of PC along the Greenberg pathway, resulting in an increased ratio of 18:0 over 16:0. On the contrary, an increase in 16:0 together with a decrease in 18:0 and 20:4n-6 with progressing gestation is observed, indicating an enhanced synthesis of PC along the Kennedy pathway in spite of estrogen (7,30,31). Skryten *et al.* (30) suggested subclinical cholestatic changes in the liver during normal pregnancy to explain this discrepancy in PC synthesis. Indeed, intrahepatic cholestasis of pregnancy is well described in humans and is associated with hyperlipidemia during pregnancy (33,34). Cholestasis is characterized by higher levels of 16:0 and lower levels of 18:0 in serum PL (35). Cholestatic conditions enhance the Kennedy pathway (30). Intrahepatic cholestasis of pregnancy generally resolves after delivery (34). The results of our study are in concurrence with the concept of enhanced synthesis along the Kennedy pathway as a result of increase cholestatic influence on liver PC synthesis during pregnancy. Of course, these explanations are rather speculative as we measured neither estrogen levels nor markers for cholestasis. Furthermore, the level of dimethylacetals, which originate from plasmalogens, dropped during pregnancy. Thus, this decrease shows that there is a decrease in plasmalogens relative to diacylphospholipids during gestation. It is not known whether this reflects a change in the relative contribution of both pathways for PL synthesis.

Minor changes were observed in the maternal CE FA composition during pregnancy. The adaptations in the SFA composition to counteract changes in the MCL and MMP found in PL were not confirmed in plasma CE. In maternal CE the loss of linoleic acid during gestation is compensated by an increase in oleic acid. In umbilical plasma, the higher concentration of ∑HUFA is accompanied by considerably higher levels of all the individual SFA (even the shorter-chain 14:0 and 16:0) compared to maternal plasma. In normal, fed, healthy persons, most of the circulating CE are formed in plasma under the action of LCAT (36,37). The esterification takes place in the plasma mainly by transfer of the FA from the *sn*-2 position of PC, the major plasma PL, to the 3-β-OH-group of cholesterol under the influence of LCAT. Human LCAT utilizes the *sn*-2 FA from most PC species (including 16:0–18:1 PC, 16:0–18:2 PC, and 18:1–16:0 PC) (38). In other words, human LCAT preferentially utilizes linoleic acid, which is the predominant FA in CE. However, when the long-chain FA 20:4n-6 and 22:6n-3 are present in *sn*-2 of PC, LCAT prefers the *sn*-1 acyl group. Thus, from 16:0–20:4 PC and 16:0–22:6 PC, the *sn*-1 acyl group is utilized by LCAT, producing 16:0 CE. This mechanism explains why such small amounts of 22:6n-3 are found in CE (38). The substrate preference of LCAT can explain why the FA composition of CE is less influenced by pregnancy than the FA composition of plasma PL.

Striking differences exist between the maternal and umbilical FA profiles of the two plasma lipid classes studied (CE and PL). In agreement with other studies (6,7,13,16,22), we found that the percentage values of 18:2n-6 and 18:3n-3 were markedly lower in cord plasma than in maternal plasma (CE and PL). The long-chain n-3 and n-6 PUFA are markedly higher in cord plasma in the two lipid fractions compared to

maternal plasma. The maternal-umbilical plasma differences are not always consistent for the two lipid fractions (CE and PL). In some cases the differences are even in the opposite direction. Consistent differences were found for 20:3n-6, 20:4n-6, and 22:6n-3 (umbilical values significantly higher than maternal values) and for 18:2n-6 and 18:3n-3 (umbilical values significantly lower than maternal values). Opposite differences were found in the composition of saturated and monounsaturated FA.

In summary, small but significant deviations occurred in PUFA composition of maternal plasma CE and PL during the course of pregnancy. The FA profile of umbilical plasma at birth is very different from maternal values at delivery in the two lipid fractions (CE and PL). This concurs with previous literature findings on the EFA status of the mother during pregnancy and of the neonate at birth.

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Chapter 7

Fatty acid composition of phospholipids and cholesteryl esters in maternal serum in the early puerperium

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Fatty acid composition of phospholipids and cholesteryl esters in maternal serum in the early puerperium

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Abstract

The fatty acid composition of serum phospholipids (PL) and cholesteryl esters (CE) in 26 healthy pregnant women at the end of term and 1 and 3 days after delivery was analysed in order to determine whether the maternal serum fatty acid composition changes in the early puerperium. The composition of the saturated fatty acids significantly changes in the PL fraction: 16:0 decreased and 18:0 increased. Both 20:4n-6 and 20:5n-3 significantly increased after parturition in serum PL while 22:6n-3 remained constant at the three sampling time points. The sum of HUFA was slightly higher 3 days postpartum compared to the prepartum data. The essential fatty acid index significantly increased after delivery. In the CE fraction too differences occurred during puerperium: 18:2n-6 and 20:4n-6 increased and 18:1n-9 decreased after parturition. The sum of the n-3 fatty acids in CE remained unaltered. The EFA index significantly improved both in PL as in CE after delivery.

In conclusion, the previously reported changes in the fatty acid composition of PL and CE during normal pregnancy diminish shortly after delivery. In fact, very soon after delivery the maternal fatty acid composition returns to more normal values.

Keywords: Cholesteryl esters; Phospholipids; Pregnancy; Puerperium; Longitudinal study; Polyunsaturated fatty acids

1. Introduction

Pregnancy is generally associated with a marked hyperlipidemia involving all lipid classes [1]. As a consequence the absolute amounts of all the maternal plasma phospholipid associated fatty acids rise during pregnancy [2,3]. Longitudinal studies indicate that the amounts (mg/l) of all the individual fatty acids in the maternal plasma phospholipids increase from the early onset of pregnancy until delivery [2–4]. During the course of pregnancy, the total serum cholesterol concentration rises by 25−50%, followed by a rapid fall shortly after delivery [5]. Similarly total serum phospholipid (PL) concentrations rapidly fall postpartum.

Although the absolute amounts of fatty acids raise with progressing gestation, the relative long-chain polyunsaturated fatty acid concentrations (wt%) decline with progressing gestation [4,6,7]. The procentual fatty acid composition changes during the course of pregnancy. During the course of pregnancy, 16:0 increases and 18:0 decreases in maternal PL [6]. Recently, we finalised a longitudinal study in pregnant women and found that in plasma cholesterolesters (CE) only the sum of n-6 slightly decreased and 18:1n-9 and 18:3n-3 increased with progressing gestation [26].

The aim of the present study was to examine whether the established rapid fall in total PL and CE concentrations after delivery is associated with changes in the fatty acid composition of these lipid fractions during early puerperium. Therefore, we analysed the fatty acid composition of PL and CE in healthy pregnant women at the end of term and 1 and 3 days after delivery.

2. Subjects and methods

2.1. Study population

Healthy pregnant women attending the Department of Gynecology, ZOL, Genk, Belgium were asked to cooperate in this study without a selection based on previous miscarriages, parity or gravity. We excluded

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women (i) with any medical disorder; (ii) who had signs of an infection before/after delivery; (iii) who went into labour prematurely (<37 weeks); (iv) with ruptured membranes for more than 12 h; and (v) who had a Caesarean section after labour. Not one of the subjects was a regular drinker. All subjects had a normal physical examination, normal values of renal tests (blood urea and creatinine). Only singleton pregnancies were included. The 26 pregnant women, enrolled in the study, had an uncomplicated vaginal delivery. The mean age of the mothers at delivery was 27.7 (SD 3.5) years. Fourteen women were nulliparas, 8 women were primiparas and 4 women had a parity of 2.

The study protocol was approved by the Medical Ethics Committee of the ZOL, Genk, Belgium and written informed consent was obtained from each participant after the study design was fully explained.

Venous blood collections were always performed under standardised conditions to minimise sources of pre-analytical variation [8]. Each women had 3 blood samplings carried out under fasting conditions at 08.00 h (±30 min). The first sample was obtained during the last visit at the antenatal clinic 3–6 days prior to the expected date of delivery; the two postpartum blood samples were collected in the maternity hospital (1 and 3 days after delivery). Serum was stored in plastic tubes under nitrogen at −80°C until thawed for fatty acid analysis.

2.2. Fatty acid analysis of serum phospholipids and cholesteryl esters

All 3 samples of each women were analysed simultaneously by the same technician. Lipids were extracted from 1 ml serum according to a modified Folch extraction with methanol:chloroform (1:2) [9]. The lipids were separated by thin layer chromatography on rhodamine-impregnated silica gel plates using petroleum ether (bp 60–80°C; Merck Belgolab, Overijse, Belgium)/acetone 85:15 as mobile phase [10]. The PL and CE fraction were scraped off and the fatty acids converted into methyl esters by transesterification with 2 ml of a mixture of methanol:benzene:HCl (aqueous, 12 N) (80:20:5) [11]. After cooling and adding 2 ml of water, fatty acid methyl esters were extracted with petroleum ether (bp 40−60°C), evaporated to dryness under a nitrogen flow at a temperature not exceeding 40°C, and analysed by temperature programmed capillary gas chromatography (Varian Model 3900, Walnut Creek, CA, USA) on a 30 m × 250 μm (L × ID) × 0.2 μm df 10% cyanopropylphenyl-90% biscyanopropyl polysiloxane column (Restek Corp, Bellefonte, PA, USA). The injection and detection temperature were set at 285°C. The starting temperature of the column was 150°C, which was increased to 240°C after 3 min at a rate of 2°C/min [11]. The carrier gas was nitrogen with a flow of 25 cm/s. Peak identification was performed by spiking with authentic standards (Sigma-Aldrich, Bornem, Belgium). Retention times of conjugated linoleic acids were determined on basis of a commercial sample (Tonalin®). Peak integration and calculation of the percent composition was performed by computer with Star Chromatography Workstation Version 5.52 software. The coefficient of intra-assay variation of the fatty acid analysis method (5 repeated assays of a single pool) ranges from 1.4% to 4.4% for peaks larger than 5 wt% and from 1.4% to 11.4% for peaks larger than 1 wt%.

The results are expressed as area percent. The sum of all the saturated fatty acids (SFA), the mono-unsaturated fatty acids (MUFA), the polyunsaturated fatty acids (PUFA), the highly unsaturated fatty acid (HUFA: fatty acids with 20 or more carbon atoms and with at least 3 double bonds), ∑n-3, ∑n-6, the sum of the *trans* fatty acids (∑*trans*) and the sum of the different forms of conjugated linoleic acid (CLA) were calculated and are reported together with the individual fatty acids. The sum of the dimethylacetals (DMA) is also reported. The essential fatty acid index (the ratio of essential n-6 and n-3 fatty acids over non-essential n-7 and n-9 fatty acids) was calculated as a parameter for essential fatty acid adequacy.

2.3. Statistical analysis

Values are reported as mean and standard deviation. The normality of distribution was ascertained with the Kolmogorov–Smirnov test. Group mean differences are assessed by means of analysis of variance (ANOVA). Repeated measures analysis of variance was used to test for significant differences in the fatty acid composition of maternal serum PL and CE at the three time-points near delivery. The ANOVA model included only a time factor (fatty acid data shortly before delivery and twice postpartum). In order to avoid type 2 errors, due to multiple comparisons, a value of $P<0.005$ was considered significant. For 4 of our 26 subjects, we were unable to obtain a complete set of matching plasma PL or CE data for maternal pre- and postpartum blood samples. This accounts for the various sample sizes in Tables 1 and 2. However, all repeated measures analysis of variance were made with matching samples. The data were analysed using SPPS (version 10.0 for WINDOWS; SPSS Inc, Chicago) [12].

3. Results

Tables 1 and 2 summarise the fatty acid composition of PL and CE from maternal serum at the end of pregnancy (prepartus) and 1 and 3 days after delivery.

Table 1
Composition (area% of total fatty acids) of fatty acids in PL isolated from maternal serum at the end of pregnancy (prepartus) and 1 (day 1) and 3 (day 3) days after delivery: mean (SD)

	Prepartus ($n = 26$)	Day 1 ($n = 22$)	Day 3 ($n = 23$)	RM ANOVA (P)
14:0	0.39 (0.13)	0.33 (0.07)	0.38 (0.11)	
15:0	0.16 (0.03)	0.17 (0.03)	0.20 (0.04)	0.001
16:0	32.83 (1.11)	32.43 (1.33)	31.27 (1.43)	0.0001
17:0	0.28 (0.05)	0.29 (0.04)	0.33 (0.07)	0.003
18:0	10.41 (1.09)	10.54 (0.90)	11.32 (1.08)	0.001
20:0	0.51 (0.13)	0.51 (0.09)	0.54 (0.10)	
22:0	1.30 (0.26)	1.35 (0.21)	1.50 (0.38)	
23:0	0.62 (0.13)	0.60 (0.10)	0.67 (0.10)	
24:0	0.96 (0.23)	1.00 (0.20)	1.14 (0.41)	
SFA	47.45 (1.09)	47.21 (0.98)	47.36 (1.27)	
16:1n-9	0.10 (0.02)	0.11 (0.02)	0.11 (0.04)	
16:1n-7	0.58 (0.24)	0.58 (0.20)	0.56 (0.18)	
18:1n-9	9.40 (2.53)	8.49 (1.17)	7.74 (1.11)	0.0001
18:1n-7	1.28 (0.26)	1.38 (0.20)	1.46 (0.17)	0.003
20:1	0.16 (0.05)	0.15 (0.02)	0.16 (0.03)	
24:1	2.17 (0.42)	2.39 (0.41)	2.54 (0.42)	0.005
MUFA	13.69 (2.48)	13.11 (1.43)	12.57 (1.56)	
18:2n-6	21.71 (2.48)	21.50 (2.66)	21.38 (2.63)	
20:3n-6	2.88 (0.60)	3.06 (0.52)	2.83 (0.63)	
20:4n-6	7.19 (1.14)	7.83 (0.93)	8.41 (1.02)	0.0001
22:5n-6	0.44 (0.12)	0.44 (0.13)	0.39 (0.11)	0.001
$\sum$n-6	32.22 (2.28)	32.84 (1.90)	33.02 (2.27)	
18:3n-3	0.29 (0.12)	0.22 (0.05)	0.20 (0.06)	
20:5n-3	0.34 (0.13)	0.36 (0.14)	0.43 (0.13)	0.004
22:5n-3	0.40 (0.09)	0.42 (0.09)	0.42 (0.08)	
22:6n-3	2.95 (0.53)	3.06 (0.51)	2.87 (0.42)	
$\sum$n-3	3.98 (0.69)	4.05 (0.65)	3.92 (0.58)	
20:3n-9	0.26 (0.07)	0.26 (0.07)	0.26 (0.08)	
PUFA	37.23 (2.25)	37.97 (1.70)	38.07 (2.30)	
HUFA	14.45 (1.92)	15.42 (1.34)	15.62 (1.46)	
16:0DMA	0.49 (0.07)	0.53 (0.06)	0.63 (0.08)	0.0001
18:0DMA	0.28 (0.11)	0.27 (0.07)	0.34 (0.06)	0.0001
18:1DMA	0.12 (0.04)	0.11 (0.04)	0.15 (0.03)	0.004
$\sum$DMA	0.90 (0.20)	0.92 (0.14)	1.12 (0.16)	0.0001
$\sum$trans	1.31 (0.32)	1.38 (0.26)	1.48 (0.27)	
$\sum$CLA	0.57 (0.15)	0.58 (0.06)	0.61 (0.10)	
EFA index	3.24 (0.67)	3.47 (0.56)	3.73 (0.64)	0.005

SFA: saturated fatty acids; MUFA: mono-unsaturated fatty acids; PUFA: polyunsaturated fatty acids; HUFA: long-chain highly unsaturated fatty acids; DMA: dimethylacetals; CLA: sum of the peaks identified as conjugated linoleic acids; RM: repeated measurements.

Table 2
Composition (area% of total fatty acids) of fatty acids in CE isolated from maternal venous serum at the end of pregnancy (prepartus) and 1 (day 1) and 3 (day 3) days after delivery: mean (SD)

	Prepartus ($n = 26$)	Day 1 ($n = 22$)	Day 3 ($n = 22$)	RM ANOVA (P)
14:0	0.75 (0.22)	0.70 (0.19)	0.67 (0.20)	
15:0	0.15 (0.04)	0.16 (0.04)	0.17 (0.03)	
16:0	11.89 (0.60)	12.07 (1.14)	11.86 (1.03)	
18:0	0.56 (0.10)	0.56 (0.10)	0.65 (0.14)	
20:0	0.47 (0.18)	0.51 (0.22)	0.53 (0.19)	
SFA	13.82 (0.82)	13.99 (1.21)	13.88 (1.17)	
16:1n-9	0.42 (0.12)	0.45 (0.13)	0.45 (0.12)	
16:1n-7	3.76 (1.48)	3.88 (1.67)	3.20 (1.13)	
18:1n-9	18.57 (2.42)	18.67 (2.89)	16.80 (2.46)	0.0001
18:1n-7	0.73 (0.18)	0.84 (0.36)	1.11 (0.96)	
MUFA	23.47 (3.65)	23.83 (4.39)	21.56 (3.50)	0.003
16:2n-6	0.20 (0.06)	0.24 (0.13)	0.17 (0.07)	0.005
18:2n-6	54.91 (4.92)	53.94 (5.85)	56.16 (4.81)	0.005
20:3n-6	0.70 (0.14)	0.79 (0.09)	0.76 (0.14)	
20:4n-6	4.77 (0.82)	5.16 (1.00)	5.51 (0.90)	0.005
$\sum$n-6	60.59 (4.46)	60.05 (5.29)	62.60 (4.58)	0.005
18:3n-3	0.70 (0.21)	0.57 (0.16)	0.52 (0.14)	0.002
20:5n-3	0.24 (0.14)	0.25 (0.09)	0.27 (0.11)	
22:6n-3	0.43 (0.08)	0.47 (0.10)	0.45 (0.10)	
$\sum$n-3	1.37 (0.33)	1.28 (0.24)	1.24 (0.30)	
20:3n-9	0.07 (0.04)	0.07 (0.02)	0.07 (0.02)	
PUFA	62.11 (4.28)	61.56 (5.15)	63.97 (4.53)	
HUFA	6.22 (0.92)	6.74 (1.12)	7.06 (1.02)	
EFA index	2.73 (0.63)	2.69 (0.73)	3.06 (0.69)	0.003

3.1. Fatty acid composition of maternal serum lipid classes

3.1.1. Phospholipids

Palmitic acid (16:0) decreases significantly over time whereas stearic acid (18:0) significantly increases. The ratio of 16:0 over 18:0 drops significantly from 3.2 (0.4) prepartus over 3.1 (0.4) 1 day after delivery to 2.8 (0.4) 3 days postpartum ($P < 0.001$). The odd-chain fatty acids, 15:0 and 17:0, rise significantly after delivery. Oleic acid (18:1n-9) significantly drops. Linoleic acid and α-linolenic acid remain constant after parturition. Both 20:4n-6 and 20:5n-3 significantly increase after parturition. Docosahexaenoic acid (22:6n-3) remains constant at the three sampling time points. The ratio of 18:2n-6 over 20:4n-6 significantly decreases ($P < 0.001$): 3.1 (SD 0.8), 2.8 (SD 0.6) and 2.6 (SD 0.5) respectively before delivery, 1 and 3 days after delivery. The sum of the dimethylacetals is significantly higher postpartum compared to the prepartum data. The essential fatty acid index significantly increases after delivery.

3.1.2. Cholesteryl esters

Only a few significant differences occurred in the fatty acid profile of maternal CE during the puerperium. As in the PL fraction, 18:1n-9 is significantly lower postpartum compared to the status at the end of pregnancy. The sum of the mono-unsaturates also significantly decreases postpartum compared to prepartum values. Linoleic and α-linolenic acid change in opposite directions: 18:2n-6 increases and 18:3n-3 decreases after parturition. Arachidonic acid (20:4n-6) and the sum of the n-6 fatty acids significantly increases after delivery. As in PL, the ratio of 18:2n-6 over 20:4n-6 significantly decreases during puerperium ($P < 0.001$): 12.0 (SD 3.2), 11.0 (SD 3.3) and 10.5 (SD 2.4) respectively before delivery, 1 and 3 days after delivery. The sum of the n-3 fatty acids remains unaltered as do 20:5n-3 and 22:6n-3. Similarly to the PL fraction, the

EFA index significantly increases after delivery in the CE fraction.

4. Discussion

This study shows that even in a short time period, from 6 days before until 3 days after delivery, the fatty acid composition of maternal plasma PL and CE significantly changes. This is in contrast to the previous findings of Al et al. [13] who observed, in a small subset of 5 mothers, no significant differences in the PL fatty acid composition of maternal plasma collected approximately 10 days before delivery with that of maternal plasma collected immediately after delivery. It was concluded that labour has no influence on the fatty acid composition of maternal plasma PL [13]. The observed changes in the maternal plasma fatty acid composition around delivery in our study, do not necessarily mean that labour influences the fatty acid composition of maternal plasma PL or CE. Previously two different research groups examined the effect of labour on maternal serum lipid composition by comparing values obtained from women who delivered by Caesarean section with those who had normal vaginal deliveries [14,15]. Ruyle et al. [14] found no statistical differences between fatty acid mass (mg/l) of maternal serum lipids from women with Caesarean section ($n = 18$) versus women with standard vaginal delivery ($n = 11$). Moreover, Schouw et al. [15] found no important differences in the maternal fatty acid composition of serum PL between mothers after normal vaginal delivery ($n = 15$) as compared with mothers who had a Caesarean section ($n = 5$). Therefore, any significant influence of labour on maternal fatty acid composition of serum lipids is unlikely.

The observed changes in maternal fatty acid composition in early puerperium could be related to changes in the dietary intake of fatty acids. However, this is rather unlikely as we and others, showed that dietary habits remain unaltered during pregnancy [16]. Neither the amount and type of fat nor the fatty acid composition of the maternal diet changed during pregnancy until 1 month postpartum [16,17]. It is possible that the food served in the maternity clinic is different from the dietary habits of the pregnant women at home. But the samples are taken after 1 and 3 days in the maternity clinic and it is known that the fatty acid profile of plasma PL and CE changes more gradually and reflects the average dietary fat composition over a longer time period [18].

Another possible explanation for the observed differences in the maternal serum fatty acid composition during early puerperium is changes in the maternal hormonal status during puerperium. The major pathway, the Kennedy pathway, for phosphatidylcholine synthesis preferentially results in the appearance of 16:0 in the sn-1 position and 18:2n-6 or 18:1n-9 in the sn-2 position. Estrogen enhances an alternative pathway, the Greenberg pathway, resulting in the appearance of more phosphatidylcholine with 18:0 in the sn-1 position and 20:4n-6 in the sn-2 position [19–21]. During pregnancy, levels of estrogens and progesterone rise steadily as a result of placental production of these hormones. With removal of the placenta at delivery, estrogen and progesterone levels drop sharply, reaching prepregnancy levels by the fifth day postpartum [22]. One would expect that the drop in estrogen postpartum would result in an increased synthesis of phosphatidylcholine along the Kennedy pathway, resulting in an increased ratio of 16:0 over 18:0 postpartum. On the contrary the opposite is observed, in this study, the ratio of 16:0 over 18:0 decreases postpartum. Moreover different studies during pregnancy showed an increase in 16:0 together with a decrease in 18:0 and 20:4n-6 with progressing gestation indicating an enhanced synthesis of phosphatidylcholine along the Kennedy pathway in spite of estrogen [7,20,21]. Skryten et al. [20] suggested subclinical cholestatic changes in the liver during normal pregnancy to explain this discrepancy in phosphatidylcholine synthesis. Indeed intrahepatic cholestasis of pregnancy is well-described in humans and is associated with hyperlipidemia during pregnancy [23,24]. Cholestasis is characterised with higher levels of 16:0 and lower levels of 18:0 in serum lipids [25]. Cholestatic conditions enhance the Kennedy pathway [20]. It has been shown that intrahepatic cholestasis of pregnancy generally resolves after delivery [24]. The results of our study are in concurrence with the concept of reduced synthesis along the Kennedy pathway as a result of reduced cholestatic influence on liver phosphatidylcholine synthesis soon after delivery.

Dimethylacetals originate from plasmalogens. Thus this increase shows that there is a relative increase of plasmalogens relative to diacylphospholipids during the puerperium. It is not known whether this reflects a change in the relative contributing of both pathways for PL synthesis.

Of course these explanations are rather speculative as we did not measure estrogen levels nor markers for cholestasis. Fact is that the previously reported gradual changes in the fatty acid composition of PL [7] and CE which occur with the progression of normal pregnancy [26] diminish shortly after delivery. The sum of n-6 slightly decreased and 18:1n-9 and 18:3n-3 was found to increase in CE with progressing gestation whereas in this study 20:4n-6 increased and 18:1n-9 and 18:3n-3 dropped in serum CE postpartum. Similar opposite changes were found in the PL fraction. Moreover the essential fatty acid index improves after parturition whereas it diminishes with progressing gestation. It seems that very soon after delivery the

maternal fatty acid composition returns to more normal values.

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Chapter 8

Fatty acid composition of umbilical vessel walls

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Chapter 8: Fatty acid composition of umbilical vessel walls

1. Abstract

The essential fatty acid (EFA) status of 16 Belgian neonates after at term pregnancy was determined by analysing the fatty acid composition of phospholipids isolated from umbilical veins (supplying vessel) and arteries (draining vessels). The phospholipids of umbilical arteries contained less n-6 fatty acids and considerably more n-9 fatty acids than phospholipids of umbilical veins. Mead acid (20:3n-9) was 7.5 times higher in umbilical arteries compared to umbilical veins and the ratio of 20:3n-9 over 20:4n-6 was ten times higher in arterial than in the venous vessels, indicating that the EFA status of downstream neonatal tissue may be marginal. Furthermore the ratio of 22:5n-6 over 22:4n-6, which is an indicator of the 22:6n-3 status, is twice as high in the phospholipids of umbilical arteries compared to umbilical veins. This might indicate that the need for 22:6n-3 by the foetal tissue is not adequately covered. In conclusion, the findings in this study can suggest that the biochemical EFA status of neonates born at term may not be optimal.

2. Introduction

Since umbilical vessel walls do not have a vasa vasorum to obtain nutrients, they can only obtain their nutrients directly from the blood passing through. The umbilical vein (afferent or supplying foetal vessel) transports blood and nutrients from the mother to the foetus whereas the blood flows back from the foetus to the mother through the umbilical arteries (efferent or draining foetal vessels). Furthermore tissue phospholipids (PL) have slower fatty acid incorporation and turnover rates compared to plasma PL [1;2]. Therefore the fatty acid composition of the umbilical venous vessel wall can be considered a longer-term reflection of the essential fatty acid (EFA) supply from mother to foetus whereas the fatty acid

composition of the umbilical arterial vessel wall is likely to reflect the longer-term EFA status of the developing foetus [3].

The objective of the present study was to assess the longer-term EFA status of healthy at term born infants by determining the EFA levels in the PL fraction of umbilical cord venous and arterial vessel walls.

3. Subjects and Methods

3.1. Study population

Healthy pregnant women attending the Department of Gynaecology of the Ghent University Hospital, Belgium were asked to cooperate in this study. All pregnant women signed a written informed consent form, approved by the ethics committee of Ghent University Hospital. Inclusion criteria were: singleton pregnancy, nullipara, term delivery (38 – 42 weeks), normotensive (diastolic blood pressure below 90 mmHg). We excluded women who were diagnosed with gestational diabetes mellitus, who had signs of proteinuria, and who suffered from renal or cardiovascular disease. From sixteen women in labour the umbilical cord was obtained. Shortly after giving birth, approximately 15 cm of umbilical cord was collected from their infants. The cord vessels were rinsed with saline (NaCl, 0.9% w/v) and the cords packed vacuum into plastic bags and stored at –80°C until transportation in dry ice to Maastricht for analysis.

3.2. Fatty acid analysis

Umbilical vein and arteries were isolated from each umbilical cord and homogenised as described previously [4]. The vessel walls were frozen in liquid nitrogen and pulverized. The pulverized samples were freeze-dried before lipid extraction. Prior to the fatty acid analysis an internal standard (dinonadecanoyl phosphatidylcholine, PC 19:0) was added to every sample for the quantification of absolute fatty acid amounts present in the PL fraction of cord vein and artery walls. Total lipid extracts were prepared using a modified Folch extraction [5]. The PL fractions isolated by solid-phase extraction on an aminopropyl silica column [6] were hydrolysed and the fatty acids methylated with boron-trifluoride in methanol (140 g/L) at 100 °C during one hour. The methyl esters were analysed using capillary gas liquid chromatography with a 50 m BP1 non-polar column, 0.22 ID x 0.10 µm film thickness, and a 50 m BPx70 polar column, 0.22 ID x 0.25 µm

film thickness (SGE, Bester BV, Amstelveen, The Netherlands). The injection temperature was set at 250°C and the detector temperature at 300°C. The starting temperature of the columns was 160°C and after 4 min it was increased to 200°C with a rate of 6°C/min. Subsequently, after a stabilisation period of 3 min, the temperature was further increased to 270°C at a rate of 7°C/min. The carrier gas was helium and head pressure was 370 kPa.

Relative fatty acid levels are expressed as weight percent of total fatty acid methyl esters and the absolute fatty acid amount present in the PL fraction is expressed as mg/g dry weight of cord vessel wall tissue). The sum of all the saturated fatty acids, the mono-unsaturated fatty acids, the polyunsaturated fatty acids, the highly unsaturated fatty acid (fatty acids with 20 or more carbon atoms and with at least 3 double bonds), ∑n-3, and ∑n-6 are calculated and are reported together with the individual fatty acids.

3.3. Statistical analysis

Normality of distribution was ascertained with the Kolmogorov-Smirnov test. Values are reported as mean and standard deviation (SD). Paired Student's t test (two tailed) was performed for fatty acid comparisons between PL of the umbilical venous and arterial vessel walls. In order to avoid type 2 errors, due to multiple comparisons, a value of $P<0.001$ was taken as the criterion of significance. Pearson's correlation coefficients were calculated to study the relation between some fatty acids measured in cord vessel walls (18:2n-6, 20:4n-6 and 22:6n-3) and gestational age at birth, birth weight or birth length. Furthermore, the relationship between the fatty acid composition of cord vessel walls and that of the plasma PL running through was determined by calculating the Pearson's correlation coefficients. The data were analysed using the MedCalc statistical program, version 6 (MedCalc Software, Mariakerke, Belgium) [7].

4. Results

4.1. Clinical characteristics

The study group comprised 16 healthy pregnant women and their neonates (7 girls and 9 boys). All infants were delivered at term with a mean gestational age of 39.6 weeks (range 38.0-41.4 wks). The mean age of the mothers at delivery was 30 years (range 20-38 years). The mean Body Mass Index of the women before

pregnancy was 21.2 (range 16.5-24.2). All mothers were nullipara, all pregnancies were uncomplicated and the infants were born healthy with a mean birth weight of 3169 g (range 2570-3860 g) and a mean crown-heel length of 50.3 cm (range 47-53 cm). The median Apgar Score 1 min after birth was 9 (range 4 - 9) and 5 min after birth 9 (range 9 - 10).

4.2. Fatty acid composition of umbilical vein and artery vessel walls

The fatty acid composition (weight%) of PL in cord vein and artery vessel walls at birth are given in Table 1.

Striking differences exist between the fatty acid composition of the umbilical vein and arteries. The total amount of umbilical vein PL associated fatty acids was 16.00 (SD 1.27) mg/g dry weight and was significantly higher (P=0.001) than the total amount of umbilical artery PL associated fatty acids of 14.58 (SD 1.38) mg/g dry weight. The contents of n-6 fatty acids is significantly higher in cord vein PL compared to cord artery PL. Especially 18:2n-6, 20:3n-6, 20:4n-6 and 22:4n-6 are much higher in umbilical vein. There is one exception: 22:5n-6 is lower in umbilical vein but significance was not reached. Umbilical artery PL contain much more mono-unsaturated fatty acids and poly-unsaturated fatty acids of the n-9 family. Particularly 20:3n-9 and 22:3n-9 occur significantly more in arterial than in venous PL. The trienoic/tetraenoic ratio (20:3n-9/20:4n-6) is ten times higher in the umbilical arteries compared to the umbilical vein. Similarly the docosahexaenoic acid deficiency index (22:5n-6/22:4n-6) is twice as high in umbilical arteries compared to the supplying umbilical vein. No significant differences were observed for the sum of the n-3 fatty acids nor for 22:6n-3. Hardly any 20:5n-3 was detected in the umbilical PL.

4.3. Relationship between fatty acids in cord vessel walls and gestational age or foetal growth

There was a significant positive relation between gestational age at birth and 18:2n-6 ($r=0.63$, $P<0.01$) or 20:4n-6 ($r=0.66$, $P<0.01$) in arterial wall PL but not in vein PL. In addition, the relationship with 22:6n-3 did not reach significance. There was no significant relation between 18:2n-6, 20:4n-6 or 22:6n-3 in cord vessel walls and birth weight or birth length.

Table 1: Fatty acid composition (weight% of total fatty acids) of phospholipids isolated from vessel walls of umbilical vein and artery (n=16) shortly after birth: mean (SD).

Fatty acid	Umbilical vein (afferent or supplying vessel)	Umbilical artery (efferent or draining vessel)	P *
14:0	0.70 (0.14)	0.97 (0.18)	<0.0001
15:0	0.67 (0.23)	0.70 (0.18)	
16:0	24.44 (0.96)	23.22 (0.93)	<0.001
17:0	1.09 (0.14)	0.99 (0.13)	
18:0	13.15 (0.61)	13.02 (0.45)	
20:0	0.47 (0.04)	0.54 (0.04)	<0.0001
22:0	1.31 (0.14)	1.67 (0.12)	<0.0001
23:0	0.40 (0.05)	0.33 (0.04)	<0.0001
24:0	2.52 (0.30)	3.14 (0.27)	<0.0001
SFA	44.75 (0.87)	44.59 (1.04)	
16:1n-7	0.35 (0.15)	0.31 (0.08)	
18:1n-7	2.20 (0.24)	2.62 (0.33)	<0.0001
18:1n-9	7.66 (0.67)	10.93 (1.80)	<0.0001
20:1n-9	0.28 (0.06)	0.56 (0.16)	<0.0001
24:1n-9	4.22 (0.57)	5.07 (0.62)	<0.0001
MUFA	14.71 (1.18)	19.49 (2.70)	<0.0001
20:3n-9	0.40 (0.25)	2.99 (0.90)	<0.0001
22:3n-9	0.34 (0.17)	1.46 (0.39)	<0.0001
18:2n-6	1.89 (0.53)	1.11 (0.36)	<0.0001
20:2n-6	0.38 (0.09)	0.17 (0.03)	<0.0001
20:3n-6	1.98 (0.32)	1.21 (0.21)	<0.0001
20:4n-6	17.45 (1.19)	12.64 (1.65)	<0.0001
22:4n-6	4.77 (0.71)	2.66 (0.45)	<0.0001
22:5n-6	2.46 (0.72)	2.65 (0.57)	
24:2n-6	0.73 (0.18)	0.23 (0.07)	<0.0001
22:5n-3	0.35 (0.15)	0.24 (0.10)	<0.0001
22:6n-3	5.91 (1.03)	5.96 (1.43)	
PUFA	36.70 (1.89)	31.46 (2.18)	<0.0001
HUFA	33.71 (1.41)	29.94 (1.83)	<0.0001
∑n-3	6.31 (1.17)	6.33 (1.52)	
∑n-6	29.65 (2.15)	20.67 (2.16)	<0.0001
MA/AA	0.024 (0.016)	0.25 (0.09)	<0.0001
DHADI	0.53 (0.22)	1.04 (0.33)	<0.0001
DHASI	2.77 (1.53)	2.44 (1.11)	

* Paired Student's 2-sample t-test. SFA: saturated fatty acids, MUFA: mono-unsaturated fatty acids, PUFA: polyunsaturated fatty acids, HUFA: highly unsaturated fatty acids. MA/AA: 20:3n-9/20:4n-6. DHADI: 22:5n-6/22:4n-6. DHASI: 22:6n-3/22:5n-6.

4.4. Correlation between the fatty acid composition of cord vessel walls and that of the plasma phospholipids running through

Linoleic acid in umbilical vein vessel wall correlated slightly with 18:2n-6 in maternal plasma PL: r = 0,52 and P=0.045 and linoleic acid in arterial vessel wall correlated with 18:2n-6 in umbilical plasma PL: r = 0.45 but significance was not reached (P = 0.1).

No significant correlation was found between 20:4n-6 in arterial or venous vessel walls and 20:4n-6 in plasma PL running through.

There was a significant relation between the 22:6n-3 in vein vessel walls and 22:6n-3 in maternal plasma PL: r = 0.68, P=0.005. Similarly the DHA content in arteria vessel walls correlated positively with DHA in umbilical plasma PL: r = 0.80, P<0.001. The other long-chain n-3 fatty acid, 20:5n-3 is hardly detected in the umbilical vein vessel walls. Therefore any correlation with its concentration in plasma PL of maternal blood is not meaningful.

5. Discussion

This study shows striking differences between the fatty acid composition of umbilical vein, which is the supplying foetal vessel, and the umbilical arteries, which are the draining foetal vessels. In general, the contents of the n-6 fatty acids are higher in vein PL than in artery PL whereas the contents of the n-9 fatty acids and monounsaturates are much higher in the umbilical arteries. This might reflect preferential use of polyunsaturated fatty acids by the foetus, as was suggested by Felton *et al* [8]. The PL of umbilical arterial vessel walls contain considerably more Mead acid (20:3n-9) and dihomo-Mead acid (22:3n-9). Mead acid, a desaturation and elongation product of oleic acid, only accumulates in tissues if insufficient amounts of the parent EFA (18:2n-6 and 18:3n-3) are available [9]. Mead acid and its direct elongation product 22:3n-9 are considered reliable markers of EFA-deficiency [9].

Previously, Hornstra *et al* [3] also showed that the PL of the umbilical arteries contain significantly less 18:2n-6, 20:4n-6, 22:4n-6, 20:5n-3 and 22:5n-3 and significantly more 20:3n-9 and 22:3n-9 compared to the PL of the umbilical vein. Mead acid was rarely observed and dihomo-Mead acid was not observed at all in adult blood vessels [3]. Therefore the presence of high amounts of 20:3n-9 and

22:3n-9 in the umbilical arterial vessel wall was suggested to indicate a marginal EFA status of the newborn.

Al *et al* [10] postulated that the presence of these EFA deficiency indicators does not necessarily reflect a shortage of EFA. An alternative explanation may be that the high proportions of 20:3n-9 and 22:3n-9 may simply reflect that the foetal desaturase system is more active than the adult desaturase system and that the desaturase activity is higher in arterial vessel walls compared to venous vessel walls, resulting in the formation of 20:3n-9 and 22:3n-9 even when adequate amounts of EFA are available. However the ratio between the sum of the n-6 derivatives of linoleic acid and 18:2n-6, which is the n-6 desaturation index, in the umbilical arteries was not significantly different from the ratio in the umbilical vein: 10.4 (SD 1.04) versus 9.9 (SD 2.13), respectively, whereas Mead acid was five times higher in the arteries. Therefore the higher content of 20:3n-9 in umbilical arteries does not simply result from a higher desaturase activity in umbilical arteries [10]. However, in a later study of Al *et al* [11], the n-6 desaturation index was significantly higher in the umbilical arterial as compared to the umbilical venous vessel wall: 17.0 (SD 2.35) versus 15.3 (SD 2.21), respectively. In our study population, the n-6 desaturation index was also significantly higher in the umbilical arteries 18.7 (SD 4.8) compared to 14.9 (SD 3.2) in umbilical veins, whereas 20:3n-9 was as an average 7.5 times higher in the arteries than in the veins.

An alternative explanation for the high 20:3n-9 and 22:3n-9 levels in the umbilical artery could be that the arterial vessel walls serve as the "dustbin" for the foetus, to get rid of substances it does not need [12].

Furthermore, we and others found that 22:5n-6 was the only n-6 fatty acid that was higher in the artery compared to the vein [3;10]. Since the synthesis of 22:5n-6 is known to be stimulated when the available amount of 22:6n-3 is too low [13], this suggests that the DHA status of the neonates is not optimal. Indeed, the DHA deficiency index (22:5n-6/22:4n-6) was significantly higher in the PL of the umbilical arteries compared with the veins [3;10]. These findings suggest that the need for DHA by the foetal tissue is not adequately covered [3;10;14].

In our study population of term infants, significant positive correlations were observed between gestational age and 18:2n-6 or 20:4n-6 in arterial cord vessel walls. Previously it was shown in a Dutch population of preterm infants that

gestational age at birth was positively correlated with 20:4n-6, sum of n-6 long-chain polyunsaturates and 22:6n-3 in arterial cord vessel walls [4]. After correction for gestational age, 22:6n-3 in arterial cord vessel walls remained significantly related to birth weight and head circumference at birth [4]. We could not confirm these observations, but this is probably due to the fact that our study population consisted of a term born infants.

Furthermore we found for the long-chain n-3 fatty acids (20:5n-3 and 22:6n-3) significant correlations between their concentrations in umbilical cord vessel walls and their concentrations in the plasma PL running through. This correlation was not found for 20:4n-6.

In conclusion, the biochemical EFA status of neonates after a normal at term pregnancy does not seem to be optimal. Relatively high amounts of Mead acid were found in the walls of the umbilical arteries. Moreover the DHA deficiency index was significantly higher in the walls from umbilical arteries as compared to umbilical veins, which may indicate a relative shortage of foetal DHA.

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Chapter 9

Lower serum n-3 polyunsaturated fatty acids correlate with the occurrence of postpartum depression

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Chapter 9: Lower serum n-3 polyunsaturated fatty acids correlate with the occurrence of postpartum depression

The first part of this chapter gives a literature review on major depression and lipid metabolism. In the second part, we will discuss the results of a study in women with postpartum depression.

Part A: Literature review

1. Major depression

It is now widely accepted that serotonin or 5-hydroxytryptamine (5-HT) plays a key role in the pathophysiology of major depression. The presumed serotonergic disturbances in depression are a combination of decreased peripheral and central serotonin activity and alterations in peripheral and central serotonin uptake mechanisms [1]. Cholesterol and membrane fatty acids (associated with phospholipids) may play a role in the metabolism of serotonin. Alterations in phospholipids and cholesterol, which are structural components of all cell membranes in the brain, may induce changes in membrane microviscosity and, consequently, may affect functioning of various neurotransmitter systems, which are thought to be related to the pathophysiology of major depression (e.g. serotinin and (nor)adrenalin) [2-4]. Membrane fatty acids and cholesterol regulate 5-HT release and uptake and modulate the activity of tryptophan hydroxylase, the rate-limiting enzyme in serotonin synthesis [5]. Consequently, changes in brain-

cell membrane cholesterol and in membrane fatty acids may decrease 5-HT turnover in the brain and hence precipitate depression [6;7].

Moreover it is well established that low concentrations of 5-hydroxyindolacetic acid in cerebrospinal fluid, which is a marker of brain serotonin turnover, are strongly associated with depression and suicide. It has been shown in healthy volunteers that low plasma concentrations of 22:6n-3 correlate with low concentrations of 5-hydroxyindolacetic acid in cerebrospinal fluid [8].

2. Do plasma cholesterol and polyunsaturated fatty acids predict depression ?

Significant lower concentrations of total cholesterol and of high-density lipoprotein cholesterol have repeatedly been described in depressed patients [3;9]. Moreover, there is evidence for an inverse relationship between plasma cholesterol levels and severity of depression [4]. Furthermore, a negative relation has been described between LDL cholesterol and depressive mood in men but not in women [10].

Cholesterol-lowering therapies (used in the treatment of cardiovascular diseases) have been associated with reduced mortality from cardiac causes but increased mortality due to increased suicide, homicide and accidents [2;11-13]. It was suggested that plasma cholesterol reductions by dietary interventions may lead to a more negative emotional state which in turn could increase the risk for suicide or other types of violent deaths [12]. Furthermore, Morgan *et al* [14] found that lower serum cholesterol concentrations are accompanied by a 3-time increase in the development of depressive symptoms and that plasma cholesterol levels are inversely related to the severity of depression. However in the study of Weidner *et al* [15] consumption of a diet low in fat and high in complex carbohydrates resulted in decreased serum cholesterol levels and was associated with reductions in depression and aggressive hostility.

Pekkanen *et al* [16] reported that lower serum cholesterol was associated with lower mortality due to accidents and violence in coastal Western Finland but no association was found in inland Eastern Finland. Consequently, it was hypothesised that the consumption of fish may be protective for depression. An experiment in monkeys suggested that a low-fat diet may be associated with increases in

aggressive behaviour [17]. However the fatty acid composition of the two diets changed from a n-6 to n-3 ratio of approximately 6:1 on a high fat diet to approximately 33:1 on a low fat diet.

Moreover cholesterol-lowering drugs may alter PUFA concentrations in tissue. All classes of fibrates induce peroxisomal proliferation and increase the oxidation of PUFA furthermore decreased levels of PUFA in VLDL and LDL have been described [18]. Bile-sequestering agents interfere with fat absorption and are likely to reduce tissue concentrations of PUFA. Hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitors increase PUFA levels in triglycerides of VLDL and reduce scores of anger and hostility.

Abnormalities in serum cholesterol and fatty acid composition have been described in psychiatric patients, including unipolar depressed and manic bipolar patients [19]. The role of cholesterol and fatty acids in the pathophysiology of affective disorders has been related to changes in structural components of cell membranes in the brain.

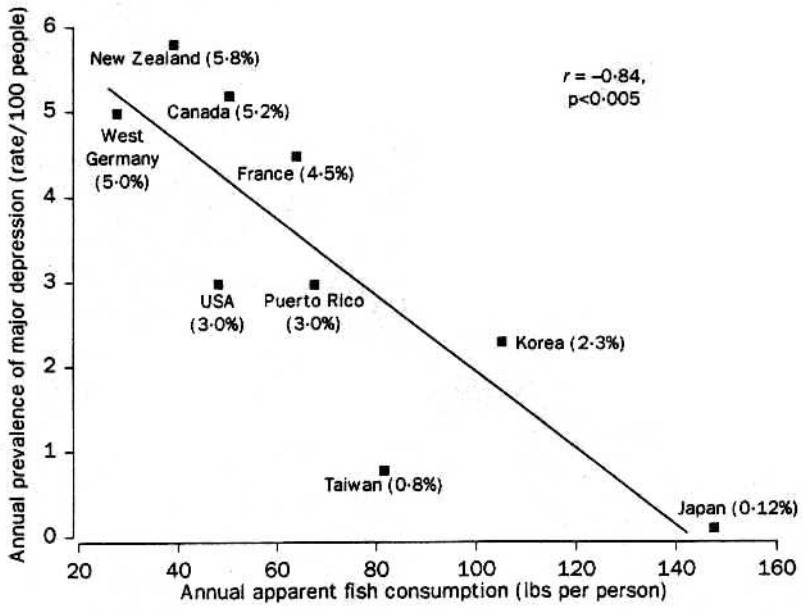

Figure 9.1: Fish consumption and prevalence of major depression. A simple Pearson's product moment correlation was used for regression analysis (r= -0.84, P<0.005) [20;21].

It has been hypothesised by Hibbeln & Salem [19] that a depletion of n-3 fatty acids, particularly an inadequate amount of 22:6n-3 in the nervous system, may increase the vulnerability to depression. This hypothesis is supported by epidemiological observations of a lower prevalence of depression in countries where fish intake is high [8].

A cross-nation study of rates of depression showed that cumulative rates of depression in North American and European populations are 10-fold greater than in a Taiwanese population [22]. Another cross-national comparison with a sample size of 35,000, observed a strong negative correlation between fish consumption and the prevalence of major depression ($r = -0.84$, $P<0.005$) (figure 9.1) [20;21]. Moreover, one recent study reported that a seafood consumption of more than twice a week is associated with a lower risk of both depression (odds ratio = 0.63) and suicidal attempts (odds ratio = 0.57) [23].

Clinical data of plasma fatty acid composition in patients and in supplementation interventions have been consistent with the hypothesis that n-3 fatty acids are protective against depression and hostility, as elaborated below.

In depressed patients reduced n-3 fatty acids and a shift in the balance of fatty acids from n-3 towards n-6 was noticed [4;24-26]. Major depressed patients have significantly lower total n-3 fatty acids in serum cholesteryl esters compared to minor depressed patients or healthy controls [4]. Furthermore the Σn-6/Σn-3 ratio in serum cholesteryl esters is significantly higher in patients with major depression compared to healthy controls [4]. Patients with major depression have an increased ratio of 20:4n-6/20:5n-3 in serum phospholipids and cholesteryl esters [4;26]. Similarly, the severity of illness in major depression was positively correlated with the ratio of 20:4n-6 to 20:5n-3 in serum phospholipids and red blood cell membranes [24]. Depressive patients were found to have reduced levels of n-3 PUFA and particularly DHA in their red blood cell membranes compared to healthy controls [27]. Edwards *et al* [25] confirmed these findings and observed no significant difference between patients and controls for dietary intake of n-3 fatty acids nor for total energy intake. Significant negative correlations were found between the red blood cell membrane n-3 fatty acid levels and the Beck Depression Inventory index on the other hand [25]. Positive correlations were found between dietary intake of n-3 fatty acids and their levels in red blood cell membranes in the patient group. Dietary intake of n-3 fatty acid levels was

negatively correlated with the Beck Depression Inventory score; that is the more n-3 fatty acids consumed by depressive patients in their normal diet the less severe is their level of depression [25]. Finally, Maes *et al* documented a lower status of 18:3n-3, 20:5n-3 and total n-3 PUFA, in cholesteryl esters and an increase in mono-unsaturated fatty acids and n-6 PUFA in phospholipids together with an increased ratio of 20:4n-6/20:5n-3 [26].

All these observations taken together suggest an abnormal metabolism of n-3 PUFA in depression.

In conclusion, affective disorders have repeatedly been associated with abnormalities in cholesterol and fatty acids but it still remains unclear whether these changes are directly related to the pathogenesis of depression. Until now it has not been determined whether the high ratios of 20:4n-6 to 20:5n-3 both in serum and red blood cell phospholipids are the result of depression or whether tissue PUFA changes predate the depressive symptoms. The association between the state of depression and plasma and red blood cell PUFA levels may be a cause, an effect or a reflection of other changes occurring during depression [24]. Furthermore decreased appetite and weight loss in depressed subjects may underlie the relationship between low cholesterol and depressive symptoms.

3. Postpartum depression

Mild depressive symptoms, the so-called postpartum blues, are a common complication of the puerperium and affect 30 – 85% of women in the early postpartum period [28-31]. A cross-national ecological analysis reported that the prevalence of postpartum depression varied from 0.5% in Singapore to 24.5% in South Africa. The mean prevalence rate world-wide was 12.4% (n=22 countries) [32]. The onset of postpartum depression occurs from between 4 weeks and 6 months following delivery.

Pregnancy induces a physiological rise in both serum cholesterol and triglyceride concentrations with peak concentrations at term and a rapid decline within a few days after delivery [33]. The cholesterol concentration normalizes by the 20^{th} week postpartum [34]. Based on these observations it has been suggested that the sudden fall in cholesterol levels after delivery could serve as a 'natural model' to test the association between cholesterol and mood [35].

Ploeckinger *et al* [35] analysed serum concentrations of total cholesterol and triglycerides two weeks before delivery and at the first and third days after delivery in 20 women. These data were correlated with mood scores. A significant correlation was found between the decline in cholesterol and depressive symptoms postpartum [35]. Similarly, a significant association between mood state and serum cholesterol level on the third day postpartum has been found [36]. In contrast, in a longitudinal study of 266 women, rapidity of cholesterol decline had no effect on risk of depression in the weeks after delivery [37]. The decline in serum cholesterol between 32 weeks of pregnancy and week 10 postpartum was similar for women who became depressed postpartum and women who did not [37]. The first studies only lasted until 3 or 4 days postpartum and had a depression score instead of diagnosis of depression as in the latter study. Recently in a study of 47 primiparous women, lower postpartum levels of total cholesterol were associated with symptoms of anxiety, hostility and depression, and lower postpartum levels of HDL cholesterol were associated with symptoms of anxiety [38].

Pregnancy is associated with a gradual relative decrease of 22:6n-3 (w%) in maternal plasma PL from the 18th week of gestation on resulting in a decreased DHA sufficiency index. Six months after delivery the maternal DHA sufficiency index had not yet returned to early pregnancy values [39;40]. After delivery maternal plasma 22:6n-3 steadily declines further both in lactating and nonlactating women [40]. This relative maternal depletion of 22:6n-3 may be one of the complex factors leading to increased risk of depression in the postpartum periods [41].

Hibbeln concluded from a cross-national ecological analysis that both higher concentrations of 22:6n-3 in breast milk and greater seafood consumption were associated with lower prevalence rates of postpartum depression [32]. The concentrations of DHA in mother's milk are a reasonably good parameter of maternal DHA status postpartum [42]. The DHA content of breast milk correlated significantly with the prevalence rate of postpartum depression ($r= -0.84$, $P<0.0001$). Lower national rates of seafood consumption were significantly correlated with higher prevalence rates of major postpartum depressive symptoms ($r= -0.81$, $P<0.0001$) (figure 9.2) [32]. One possible criticism of these cross-national findings is that women from Asian cultures may have been more reluctant

to report depressive symptoms. Even so, despite exclusion of all Asian countries from these analyses, the cross-national relationships remained robust (r= -0.76, P<0.0001).

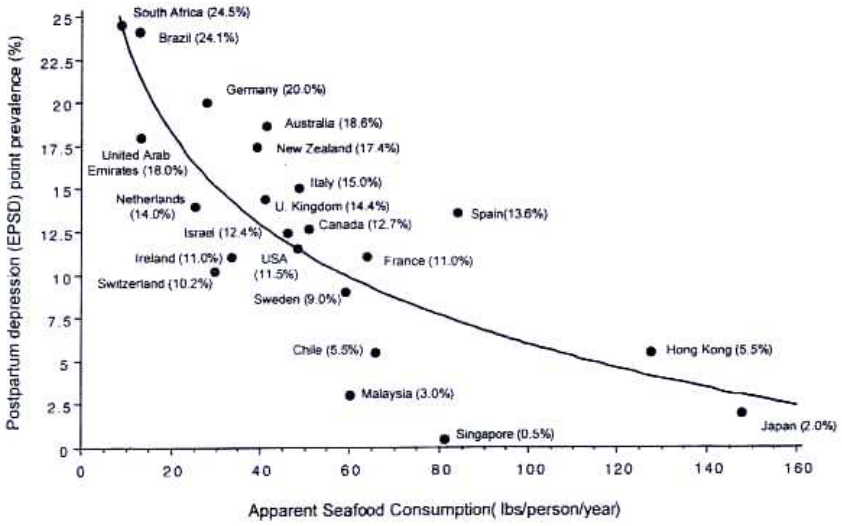

Figure 9.2: Seafood consumption and prevalence rates of postpartum depression. A logarithmic regression was used for analysis (r= -0.81, P<0.0001) [32].

Based on these observations we hypothesised that the relative maternal depletion of 22:6n-3 might increase the risk of postpartum depression. In the next part of this chapter we examine whether the maternal concentration of n-3 fatty acids at delivery differs in women who develop postpartum depression compared to controls.

Part B: Our own study: "Lower serum n-3 polyunsaturated fatty acids predict the occurrence of postpartum depression"

1. Abstract

Several studies have shown that major depression is accompanied by alterations in serum fatty acid composition, i.e. reduced n-3 fatty acids and an increased 20:4n-6/20:5n-3 ratio in serum. Moreover, in several study groups, a gradual relative decrease of maternal serum 22:6n-3 was found from mid gestation till term and a further decline after delivery. Therefore, the aim of the present study was to investigate whether the postpartum fatty acid profile of maternal serum phospholipids (PL) and cholesteryl esters (CE) differs in women who develop postpartum depression compared to controls. We compared the fatty acid composition shortly after delivery of 10 women who developed postpartum depression and 38 women who did not. After delivery, 22:6n-3 and the sum of the n-3 fatty acids in PL and CE was significantly lower in the group of mothers who developed a postpartum depression. The ratio of Σn-6/Σn-3 fatty acids in PL was, postpartum, significantly higher in the depressed group as compared to the controls. The observed abnormalities in fatty acid status in affective disorders were confirmed in postpartum depression.

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2. Introduction

Major depression is associated with alterations in fatty acid composition of serum lipids. In depressed patients reduced n-3 fatty acids and a shift in the balance of fatty acids from n-3 towards n-6 was noticed [4;24-26]. Major depressed patients have significantly lower total n-3 fatty acids in serum cholesteryl esters (CE)

compared to minor depressed patients or healthy controls [4]. Furthermore the Σn-6/Σn-3 ratio in serum CE is significantly higher in patients with major depression compared to healthy controls [4]. The severity of illness in major depression was positively correlated with the ratio of 20:4n-6 to 20:5n-3 in serum phospholipids (PL) and red blood cell membranes [24]. Similarly, patients with major depression have an increased ratio of 20:4n-6/20:5n-3 in serum PL and CE [4;26]. Depressive patients were found to have reduced levels of n-3 fatty acids and particularly 22:6n-3 in their red blood cell membranes compared to healthy controls [27]. Edwards *et al* [25] confirmed these findings and observed no significant difference between patients and controls for dietary intake of n-3 fatty acids nor for total energy intake. These observations suggest an abnormal metabolism of n-3 PUFA in depression [26].

Pregnancy is associated with a gradual relative decrease of 22:6n-3 (w%) in maternal plasma PL from the 18th week of gestation on resulting in a decreased DHA sufficiency index. Six months after delivery the maternal DHA sufficiency index had not yet returned to early pregnancy values [39;40]. Several studies have shown an association between reduced n-3 fatty acids in serum and the occurrence of major depression [4;24-26]. These observations prompted the hypothesis that relative maternal depletion of 22:6n-3 might increase the risk of postpartum depression. Therefore, the aim of the present study was to investigate whether the fatty acid profile of maternal serum PL or CE at delivery differs in women who develop postpartum depression compared to controls.

3. Subjects and Methods

3.1. Study population and blood sampling

Healthy pregnant women attending the Department of Gynaecology, ZOL, Genk, Belgium were asked to cooperate in this study without a selection based on previous miscarriages, parity or gravity. Only singleton pregnancies were included. We excluded women i) with any medical disorder; ii) with a past or present axis-I psychiatric disorder, except depression, as assessed by means of DSM-IV criteria using the Semi-structured Interview for the DSM-III-R (SCID; [43;44]; iii) who ever had used major psychotropic drugs, including antidepressants and antipsychotics; iv) who had signs of an infection before/after delivery; v) who went into labour

prematurely (<37 weeks); vi) with ruptured membranes for more than 12 hours; and vii) who had a Caesarean Section after labour. Finally, 48 pregnant women were enrolled in the study. Not one of the subjects was a regular drinker. All subjects had a normal physical examination, normal values of renal tests (blood urea and creatinine). The study protocol was approved by the Medical Ethics Committee of the ZOL, Genk, Belgium and written informed consent was obtained from each participant after the study design was fully explained.

Shortly after delivery a maternal blood sample was obtained. Serum was obtained after centrifugation and stored in plastic tubes under nitrogen at –80°C until thawed for fatty acid analysis.

3.2. SCID interview

Within six to ten months after delivery a resident in psychiatry trained in the DSM-interview techniques had a telephone interview of the participants in order to make the diagnosis of postpartum depression. Telephone interviews to assess the history of major depression according to DSM-IV criteria using the SCID interview are commonly used in epidemiological studies [43;45]. Although the criteria for the postpartum onset specified in the DSM-IV includes that the onset of the episode occurs within 4 weeks postpartum, we used 3 months as onset specifier, since a review of the literature shows that a considerable number of postpartum depressions may occur after the first month [31].

The study population consisted of 10 pregnant women who developed postpartum depression and 38 pregnant women who did not.

3.3. Fatty acid analysis of serum phospholipids and cholesteryl esters

Lipids were extracted from 1 mL serum according to a modified Folch extraction with methanol:chloroform (1:2) [46]. The lipids were separated by thin layer chromatography on rhodamine-impregnated silica gel plates using petroleum ether (bp 60-80°C; Merck Belgolab, Overijse, Belgium)/acetone 85:15 as mobile phase [47]. The PL and CE fraction were scraped off and the fatty acids converted into methyl esters by transesterification with 2 mL of a mixture of methanol:benzene:HCl (aqueous, 12N) (80:20:5) [48]. After cooling and adding 2 mL of water, fatty acid methyl esters were extracted with petroleum ether (bp

40-60°C), evaporated to dryness under a nitrogen flow at a temperature not exceeding 40°C, and analysed by temperature programmed capillary gas chromatography (Varian Model 3500, Walnut Creek, CA, USA) on a 30m x 250µm (LxID) x 0.2µm df Rtx 2330 column (Restek Corp, Bellefonte, PA, USA). The injection and detection temperature were set at 285°C. The starting temperature of the column was 150°C which was increased to 240°C after 3 min at a rate of 2°C/min. The carrier gas was nitrogen with a flow of 25 cm/s [48]. Peak identification was performed by spiking with authentic standards (Sigma-Aldrich, Bornem, Belgium). Peak integration and calculation of the percent composition was performed by computer with Star Chromatography Workstation Version 5.52 software. The coefficient of intra-assay variation of the fatty acid analysis method (5 repeated assays of a single pool) ranges from 1.4 % to 4.4 % for peaks larger than 5 w% and ranges from 1.4 % to 11.4 % for peaks larger than 1 w%. The results are expressed as weight percent of total fatty acids.

3.4. Statistical analysis

Values are reported as mean and standard deviation. Normality of distribution was ascertained with the Kolmogorov-Smirnov test. Student's t-test was used to examine the difference in the fatty acid composition of maternal serum PL and CE between women who developed a postpartum depression and women who did not.

Since previous research has revealed differences between controls and patients with depression in the Σn-6/Σn-3 and the 20:4n-6/20:5n-3 ratios and total n-3 fatty acids, the significance level concerning the Σn-6/Σn-3 and the 20:4n-6/20:5n-3 ratios, and the 182:n-6, 18:3n-3, 20:4n-6, 20:5n-3, 22:6n-3, Σn-6 and Σn-3 fractions was set at α = 0.05. The data were analysed using the MedCalc statistical program, version 6 (MedCalc Software, Mariakerke, Belgium) [49].

4. RESULTS

The study population consisted of 10 pregnant women who developed postpartum depression and 38 pregnant women who did not.

Table 1 summarises the fatty acid composition of PL and CE from maternal serum immediately after delivery.

After delivery, 22:6n-3 in PL and CE was significantly lower in the groups of mothers who developed a postpartum depression. Similarly the sum of the n-3 fatty acids in PL and CE was significantly lower in the women who became depressed. The ratio of Σn-6/Σn-3 fatty acids in PL postpartum was significantly higher in the depressed group compared to the controls. In CE the ratio of Σn-6/Σn-3 fatty acids was higher in women who became depressed but significance was not reached (P=0.08). Also the ratio of 20:4n-6/20:5n-3 in PL was higher in women with postpartum depression but significance was not reached (P=0.07).

Table 1: Composition (weight% of total fatty acids) of fatty acids in PL and CE isolated from maternal venous serum immediately after delivery: mean (SD).

	Postpartum depression (n=10)	Controls (n=38)	P *
PL 18:2n-6	21.99 (2.93)	20.41 (3.02)	
PL 18:3n-3	0.20 (0.09)	0.21 (0.13)	
PL 20:4n-6	8.21 (1.66)	8.42 (1.47)	
PL 20:5n-3	0.30 (0.08)	0.43 (0.24)	
PL 22:6n-3	3.11 (0.50)	4.22 (1.20)	0.006
PL Σn-6/Σn-3	8.55 (1.48)	6.41 (2.02)	0.003
PL AA/EPA	32.37 (22.34)	23.54 (10.50)	
PL Σn-6	33.60 (1.72)	32.32 (3.08)	
PL Σn-3	4.02 (0.56)	5.43 (1.46)	0.005
CE 18:2n-6	53.99 (6.90)	53.58 (5.99)	
CE 18:3n-3	0.60 (0.20)	0.62 (0.17)	
CE 20:4n-6	4.97 (1.18)	5.67 (1.42)	
CE 20:5n-3	0.21 (0.08)	0.34 (0.21)	
CE 22:6n-3	0.38 (0.14)	0.61 (0.28)	0.02
CE Σn-6/Σn-3	52.87 (15.55)	42.44 (16.39)	
CE AA/EPA	26.66 (12.41)	21.47 (12.83)	
CE Σn-6	59.67 (5.98)	60.02 (5.75)	
CE Σn-3	1.19 (0.26)	1.58 (0.51)	0.03

* unpaired students' t-test; AA: arachidonic acid (20:4n-6); EPA: eicosapentaenoic acid (20:5n-3)

5. DISCUSSION

In this study we found significant differences in the postpartum fatty acid status between women who developed a postpartum depression compared to control

mothers. We observed a significant association between the ratio of Σn-6/Σn-3 in PL and the occurrence of postpartum depression. Moreover, women who became depressed after delivery had a significantly lower concentration of 22:6n-3 and of Σn-3 fatty acids in PL and CE compared to women who did not.

Our observations are in line with several studies in major depression. In depressed patients reduced n-3 fatty acids and a shift in the balance of fatty acids from n-3 towards n-6 was noticed [4;24-26]. Major depressed patients have significantly lower Σn-3 fatty acids in serum CE compared to minor depressed patients or healthy controls [4]. Furthermore the Σn-6/Σn-3 ratio in serum CE is significantly higher in patients with major depression compared to healthy controls [4]. Patients with major depression have an increased ratio of 20:4n-6/20:5n-3 in serum PL and CE or in red blood cell membranes [4;24;26]. Depressive patients were found to have reduced levels of n-3 PUFA and particularly DHA in their red blood cell membranes compared to healthy controls [25;27]. All these observations suggest an abnormal metabolism of n-3 PUFA in depression [26]. Similar deviations were found in our group of women with postpartum depression. From our results, we may conclude that there is an association between 22:6n-3 or Σn-6/Σn-3 and the risk for depression in the postpartum period. Moreover, Hibbeln concluded from a cross-national ecological analysis that both higher concentrations of 22:6n-3 in breast milk and greater seafood consumption (rich in 22:6n-3) are associated with lower prevalence rates of postpartum depression [32]. However, these observations do not prove that higher n-3 fatty acid status causes lower prevalence rates of postpartum depression.

In summary, the observed abnormalities in fatty acid status in affective disorders were confirmed in postpartum depression.

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Chapter 10

Oxidative stability of low density lipoproteins and vitamin E levels increase in maternal blood during normal pregnancy

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Oxidative Stability of Low Density Lipoproteins and Vitamin E Levels Increase in Maternal Blood During Normal Pregnancy

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ABSTRACT: In 24 healthy pregnant women, parameters related to the oxidative stability of low density lipoproteins (LDL) were determined at three times during pregnancy and shortly after delivery. The fatty acid composition of plasma phospholipids (PL) and the plasma concentrations of vitamin E, vitamin A, and β-carotene were assessed in the same samples. Total triglyceride (TG), total cholesterol, LDL-cholesterol, and high density lipoprotein (HDL)-cholesterol concentrations were also determined. The length of the lag phase of isolated LDL challenged with Cu^{2+} ions significantly increased with the progression of pregnancy. The oxidation rate and the amount of conjugated dienes formed increased and reached a maximum at 29–37 wk of pregnancy. Total TG, cholesterol, and LDL-cholesterol reached a maximum in the third trimester of pregnancy. β-Carotene remained stable, vitamin A decreased, and vitamin E significantly increased throughout pregnancy. Vitamin E plasma concentration correlated positively with the length of the lag phase. The increased levels of vitamin E could contribute to the higher resistance of LDL toward oxidation with progressing gestation, measured by the prolonged lag phase. Furthermore, vitamin E plasma levels correlated positively with TG concentration but not with LDL-cholesterol. The level of polyunsaturated fatty acids in PL decreased with the progression of pregnancy. No correlation was found between the fatty acid composition of plasma PL, nor with the cholesterol concentration, and the parameters studied related to the oxidative stability of LDL. The major finding of this study is the increased oxidative resistance of LDL with progressing gestation.

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The third trimester of pregnancy is accompanied by hyperlipidemia. As pregnancy progressess, the maternal plasma triglyceride (TG) concentration, the low density lipoprotein (LDL) content, and total cholesterol concentration increase (1–6). Late pregnancy is also associated with the predominance of small and dense LDL-particles (3,7). These small and dense LDL-particles have been shown to be more susceptible to oxidation (8). Hyperlipidemia and the occurrence of small and dense LDL particles during late pregnancy might increase the oxidative damage and impair the outcome of pregnancy. Many studies have determined either the antioxidative defense systems or peroxidation products during pregnancy (9–12). Uncomplicated pregnancy, but especially preeclampsia and diabetic pregnancy, is associated with high serum levels of lipid peroxides (8–13). During normal pregnancy the higher levels of lipid peroxides are accompanied by higher maternal levels of vitamin E compared to nonpregnant women (9,10,12–14). However, the increase in vitamin E levels is more pronounced, i.e., the vitamin E/lipid peroxide ratio increases with progressing gestation .

The aim of this study was to determine whether the oxidative stability of LDL changes during the course of pregnancy and, if so, whether this change correlates with changes in vitamin E, vitamin A, and β-carotene levels or with changes in the fatty acid composition of plasma phospholipids (PL).

SUBJECTS AND METHODS

Healthy pregnant women attending the Department of Gynecology were asked to cooperate in this study. All pregnant volunteers signed a written informed consent form, approved by the ethics committee of Ghent University Hospital. Only singleton pregnancies were included. Inclusion criteria were: first pregnancy, normotensive (diastolic blood pressure <90 mm Hg), not diabetic, no proteinuria, and not suffering from renal or cardiovascular disease. The study population consisted of 24 healthy pregnant women. None of the women used any medication. Maternal venous blood was collected in EDTA tubes thrice during the course of pregnancy: earlier than 18 wk of gestation (median 12 wk), between 20 and 26 wk (median 23 wk), between 29 and 37 wk (median 32 wk) and shortly after delivery (median 39 wk; range 36–41 wk). Blood samples were temporarily stored on ice, and plasma was isolated by centrifugation (600 × *g* during 5 min at 4°C) within 24 h of collection. Plasma was stored with sucrose (60%, 10 μL/mL) to prevent lipoprotein aggregation during deepfreezing (–80°C).

Preparation and oxidation of LDL. LDL were prepared by sequential ultracentrifugation at 4°C according to Esterbauer *et al.* (15) and isolated from the appropriate density fraction (d = 1.019–1.063 g/mL). EDTA was present throughout all the steps of the isolation in a concentration of 1 mg/mL density solution. Immediately after isolation, the LDL samples

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Abbreviations: HDL, high density lipoprotein; HUFA, highly unsaturated fatty acids (fatty acids with ≥20 carbon atoms and ≥3 double bonds); LDL, low density lipoproteins; PL, phospholipids; PUFA, polyunsaturated fatty acids; TG, triglyceride; *T*lag, length of the lag phase; *T*max, length of time required to obtain maximum levels of conjugated dienes.

were dialyzed at 4°C for 24 h against four changes of buffer (0.01 M Na_2HPO_4, 0.0022 M NaH_2PO_4, 0.16 M NaCl, 10 μM EDTA, and 0.1 μg/mL chloramphenicol; pH 7.4) which was made oxygen-free by vacuum degassing and subsequently was continuously purged with nitrogen. The protein content of the LDL fraction was determined according to Lowry *et al.* (16) with fatty acid-free serum albumin as standard. The concentration was initially adjusted to 500 mg/L with dialysis buffer. This solution was further diluted 10-fold in a quartz cuvette with EDTA and chloramphenicol-free phosphate buffer at a final protein concentration of 50 μg/mL.

Oxidation was initiated by addition of $CuCl_2$ (10 μM, in cuvette). The formation of conjugated dienes was determined by monitoring the change of absorbance at 234 nm at 30°C. The optical density was recorded every 3 min during a 3-h period. From the absorbance curve the following parameters were derived: length of the lag phase (*T*lag); length of the time required to obtain maximum levels of conjugated dienes (*T*max); length of the propagation phase, oxidation rate, and maximal amount of conjugated dienes formed. *T*lag (expressed in minutes) or the length of the lag phase is defined as the time interval between the addition of $CuCl_2$ to initiate oxidation and the onset of rapid oxidation. *T*max (expressed in minutes) is the time at which the absorbance reaches a maximum. After reaching the maximum value, the conjugated dienes slowly decreased by decomposition. The length of the propagation phase (during which the absorbance rapidly increases to a maximum) is the difference between *T*max and *T*lag. The oxidation rate was calculated from the slope of the tangent to the curve during the propagation phase and is expressed as moles of dienes formed per minute per gram of LDL protein. The maximal amount of conjugated dienes formed (expressed as mol dienes/g LDL-protein) was calculated by means of the molar extinction coefficient for conjugated dienes (ε_{234} = 29,500 L/mol/cm). An actual experimental curve is given in Figure 1.

Plasma lipid analyses. Lipids were assayed using enzymatic-colorimetric methods based upon the technique of Allain *et al.* (17) for free and total cholesterol and that of Bucolo and David (18) for TG. LDL-cholesterol was calculated by difference between total cholesterol and cholesterol in the supernatant after precipitation of LDL with dextrane sulfate (QuantolipR, Immuno AG, Wien, Austria). HDL-cholesterol was determined in the supernatant after precipitation of the other lipoproteins with different concentrations of polyethylene glycol (QuantolipR, Immuno AG).

For the determination of the fatty acid composition of plasma PL, the following method was used. Lipids were extracted from 1 mL plasma according to Folch *et al* (19). The PL were prepared by thin-layer chromatography on rhodamine-impregnated silica gel plates using petroleum ether (bp 60–80°C; Merck Belgolab, Overijse, Belgium)/acetone 85:15 as mobile phase (20). The PL band was scraped off, and the fatty acids were converted into methyl esters by transesterifications with 2 mL of a mixture of methanol/benzene/HCl (80:20:5) (21). Fatty acid methyl esters were extracted with petroleum ether (bp 40–60°C), evaporated to dryness under a nitrogen flow at a temperature not exceeding 40°C, and analyzed by temperature-programmed capillary gas chromatography (Varian Model 3500) on a 25-m × 250 μm × 0.2 μm film thickness Silar 10C column (L. Restek, Interscience, Belgium) (21). The injection and detection temperatures were set at 285°C. The starting temperature of the column was 150°C, which was increased to 240°C after 3 min at a rate of 2°C/min. The carrier gas was nitrogen with a flow of 25 cm/s. Peak identification was performed by spiking with authentic standards (Sigma-Aldrich, Bornem, Belgium). Peak integration and calculation of the percentage composition were performed electronically with a Varian Model 4290 integrator.

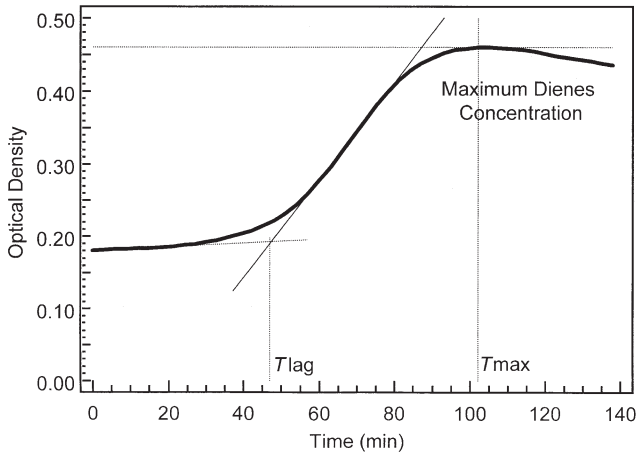

FIG. 1. Kinetics of density lipoprotein oxidation (actual experimental curve). *T*lag, length of the lag phase; *T*max, length of time required to obtain maximal levels of conjugated dienes.

Plasma levels of lipid-soluble vitamins with antioxidant activity. The concentrations of vitamin E, vitamin A, and β-carotene in plasma were measured by high-performance liquid chromatography as described by Catignani and Bieri (22). Peak identification was performed using the following standards: *d*-α-tocopherol, all-*trans*-retinol, and β-carotene (Sigma-Aldrich).

Statistical analysis. Normality of distribution was ascertained with the Kolmogorov-Smirnov test. Parameters related to LDL oxidative stability were log-transformed whereas the fatty acid fractions were arcsin transformed to reach normality of distribution. The vitamin levels (vitamin E, vitamin A, and β-carotene) had a normal distribution. Values are reported as mean (standard deviation). Differences in LDL oxidative stability-related parameters, in fatty acid composition of PL, and in vitamin levels between the first trimester and later stages in pregnancy or delivery were tested using the paired Student *t*-test. In order to avoid type 2 errors, due to multiple comparisons, a value of $P < 0.01$ was taken as the criterion of significance. Multiple regression and Spearman rank correlation coefficients were calculated to study the degree of association between LDL oxidative stability and antioxidant status-related parameters or plasma lipid concentrations. Trends during gestation were evaluated by computing Spearman's

rank correlation coefficients between gestational age and vitamin levels or plasma lipid concentrations. For the calculation of the correlation coefficients, samples from the same subject collected at different gestational ages were considered as independent samples. The data were analyzed using the MedCalc statistical program, version 6 (MedCalc Software, Mariakerke, Belgium) (23).

RESULTS

Clinical characteristics of the study population. The mean age of the mothers at delivery was 30 yr (range 25–41 yr). The mean Body Mass Index of the women before pregnancy was 24.2 kg/m^2 (range 17.6–35.6 kg/m^2), and mean weight gain at delivery was 15 kg (range 7–23 kg). All pregnant women were nullipara, all pregnancies were uncomplicated, and the infants were born healthy with a mean birth weight of 3365 g (range 2590–4200 g) and a mean birth length of 51.2 cm (range 47–55 cm). The median Apgar score 1 min after birth was 8 (range 4–10) and 5 min after birth 9 (range 6–10). The sex ratio of the infants was 13 males and 11 females.

Oxidative stability of LDL. The oxidizability-related parameters of LDL throughout gestation are summarized in Table 1. *T*lag was significantly higher at later stages of pregnancy compared to the value earlier than 18 wk of gestation. *T*lag reached a maximum in the third trimester, namely 129% (SD 41.1%) of the value at the first antenatal visit. The length of the propagation phase did not change significantly during pregnancy. The oxidation rate increased to reach a maximum in the third trimester of pregnancy: 147% (SD 64.6%) of the oxidation rate at the first antenatal visit. A similar pattern was found for the maximal amount of conjugated dienes: the value in the third trimester was 142% (SD 52.4%) of the value at the first antenatal visit.

Fatty acid composition of plasma PL. Table 2 summarizes the fatty acid composition of the plasma PL. The sum of the highly unsaturated fatty acids (HUFA; fatty acids with 20 or more carbon atoms and with at least 3 double bonds) of the plasma PL steadily decreased with progressing gestation whereas the sum of the saturated fatty acids was slightly higher at delivery compared to the first trimester.

No correlation was found between the fatty acid fractions of plasma PL and any of the LDL oxidative stability-related parameters studied (data not shown).

Plasma levels of cholesterol and TG (Table 3). Cholesterol and TG concentrations could not be determined in all the plasma samples (aliquots were too small) obtained from this study population. Significance calculations were based on paired values. The values obtained for each women from the first trimester were compared with those obtained at later stages during pregnancy. The TG concentration reached a maximum in the third trimester of pregnancy. Both total cholesterol and LDL-cholesterol increased significantly during pregnancy and reached a maximum in the third trimester. HDL-cholesterol, on the other hand, reached a maximum in the second trimester and then leveled off again. To test whether the length of the lag phase depends on the plasma TG or LDL-cholesterol concentration, multiple regression analysis was performed. No significant correlations were found between any of the above-mentioned parameters.

Plasma levels of lipid-soluble vitamins with antioxidant activity. As was the case for the determination of the choles-

TABLE 1
Oxidizability-Related Parameters of Low Density Lipoprotein (LDL)[a]

	<18 wk of gestation	20–26 wk of gestation	29–37 wk of gestation	Delivery
*T*lag (min)	41.6 (16.23)	49.2 (27.63)	51.1 (19.93)*	50.2 (28.88)
*T*max (min)	102.5 (22.00)	108.6 (32.28)	111.12 (24.95)*	113.75 (39.04)
Dienes (mol/g LDL-protein)	138.0 (61.59)	159.7 (54.99)*	179.1 (64.54)**	149.6 (63.54)
Oxidation rate (mol dienes/g LDL-protein/min)	2.33 (1.16)	2.72 (1.01)*	3.04 (1.19)*	2.48 (1.18)

[a]Paired Student *t*-test after log transformation. Values significantly different from values obtained before 18 wk of gestation: *: $P < 0.01$; **: $P < 0.001$(mean with standard deviation in parentheses throughout gestation (n = 24).

TABLE 2
Fatty Acid Composition (wt%) of Plasma Phospholipids[a]

	<18 wk of gestation	20–26 wk of gestation	29–37 wk of gestation	Delivery
SFA	44.6 (1.43)	45.6 (4.0)	45.0 (0.9)	46.5 (3.4)*
MUFA	12.1 (1.6)	11.7 (1.4)	12.8 (1.3)	12.7 (1.3)
PUFA	39.7 (2.3)	39.1 (3.8)	38.9 (1.8)	37.6 (3.6) *
HUFA	19.4 (2.5)	18.8 (2.8)	17.9 (2.5)*	17.8 (3.1)**

[a]Mean with standard deviation in parentheses throughout gestation ($n = 24$). Paired Student *t*-test after arcsin transformation. Values significantly different from values obtained before 18 wk of gestation: *: $P < 0.01$; **: $P < 0.001$; ***: $P < 0.0001$. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; HUFA, highly unsaturated fatty acids.

TABLE 3
Plasma Levels of Cholesterol and Triglycerides (TG)[a]

	<18 wk of gestation (n = 14)	20–26 wk of gestation (n = 15)	29–37 wk of gestation (n = 12)	Delivery (n = 14)
TG	110 (42.2)	135 (48.7)	186 (92.1)*	161 (55.0)
Total cholesterol	198.4 (30.8)	238.1 (41.5)***	258.6 (42.6)	**212.6 (73.4)
LDL-cholesterol	126.9 (29.9)	155.2 (38.5)**	171.6 (42.2)*	134.6 (55.6)
HDL-cholesterol	49.4 (13.5)	55.8 (16.5)*	49.7 (14.8)	45.6 (19.5)

[a] Expressed as (mg/dL) mean with standard deviation in parentheses throughout gestation. Paired Student *t*-test. Values significantly different from values obtained before 18 wk of gestation: *: $P < 0.01$; **: $P < 0.001$; ***: $P < 0.0001$. HDL, high density lipoprotein; for other abbreviation see Table 1.

TABLE 4
Plasma Concentrations of Lipid-Soluble Vitamins with Antioxidant Activity[a]

	<18 wk of gestation (n = 15)	20–26 wk of gestation (n = 17)	29–37 wk of gestation (n = 12)	Delivery (n = 13)
Vitamin A	1.40 (0.26)	1.24 (0.37)	1.03 (0.23)**	0.87 (0.24)***
Vitamin E	21.54 (7.61)	28.39 (13.13)	31.16 (8.56)*	30.19 (9.61)*
β-Carotene	0.29 (0.22)	0.26 (0.18)	0.24 (0.12)	0.23 (0.12)

[a]Expressed as (mg/dL) mean with standard deviation in parentheses) throughout gestation. Paired Student *t*-test. Values significantly different from values obtained before 18 wk of gestation: *: $P < 0.01$; **: $P < 0.001$; ***: $P < 0.0001$.

terol concentration, vitamin plasma levels (μmol/L) could not be determined in all the plasma samples (aliquots were too small) obtained from this study population. Calculations of significance were based on paired values. The values obtained for each woman from the first trimester were compared with those obtained at later stages during pregnancy. The results are summarized in Table 4. Plasma levels of vitamin E significantly increased from the first trimester and reached a maximum in the third trimester, whereas β-carotene remained stable throughout pregnancy. Vitamin A in maternal plasma was significantly lower at delivery compared to the beginning of pregnancy. When the vitamin status of all the women over all the visits was plotted vs. gestational age, the plasma vitamin E levels were found to increase and the vitamin A levels to decrease during gestation (Fig. 2). To test whether the oxidative stability-related parameters correlated with the vitamin plasma levels, multiple regression analysis was performed.

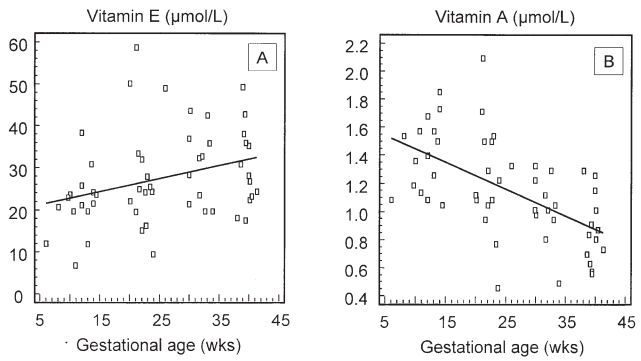

FIG. 2. Maternal plasma vitamin E (A) and vitamin A (B) levels (μmol/L) vs. gestational age. (Spearman's rank correlation coefficient, A: $r = 0.31$; $P = 0.025$; $n = 54$. B: $r = -0.62$; $P = 0.0001$; $n = 54$).

Only the vitamin E status correlated positively with *T*lag: $r = 0.54$; $P < 0.0001$ (Fig. 3) and with the amount of formed dienes: $r = 0.41$; $P < 0.01$. Neither vitamin A nor β-carotene correlated with the LDL oxidative stability-related parameters.

In our study population, no significant correlation was found between vitamin E plasma levels and total cholesterol or LDL-cholesterol concentration. On the contrary, vitamin E plasma levels correlated with the TG concentration during the course of pregnancy: $r = 0.41$; $P < 0.01$; $n = 49$.

DISCUSSION

To our knowledge this is the first report on maternal plasma lipid-soluble antioxidant vitamin levels (vitamins E, A, and β-carotene) and *in vitro* formed peroxidation products (con-

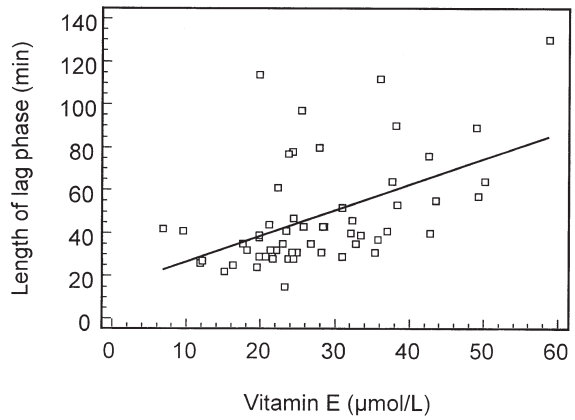

FIG. 3. Relation between the maternal plasma vitamin E concentration (μmol/L) and the length of the lag phase during *in vitro* oxidation of maternal low density lipoprotein (min). (Spearman's rank correlation coefficient, $r = 0.54$; $P = 0.0001$; $n = 57$).

jugated dienes of LDL), carried out in conjunction with determinations of the fatty acid compositions of maternal plasma PL at three times during normal uncomplicated pregnancy and at delivery.

Both the rate of formation and the amount of conjugated dienes formed reached a maximum in the third trimester. This could be due to a change in the composition of the LDL near the end of pregnancy. Indeed, structural changes in plasma lipoproteins during pregnancy have been described (1–7;24). An elevation of TG levels in all lipoprotein fractions during pregnancy compared to nonpregnant women was demonstrated previously (1,2,4,5). Longitudinal studies during various stages of pregnancy showed a steady rise of LDL-cholesterol and PL levels during pregnancy, which reached a maximum either at 36 wk of gestation (1) or at 2 wk postpartum (2). The observed increase in the concentration of plasma TG, cholesterol, and LDL-cholesterol in our study population is a well-known phenomenon during late pregnancy compared to early pregnancy or nonpregnant individuals (1,3–5). We observed an increase in HDL-cholesterol concentration that reached a maximum between 20 and 26 wk of gestation; others found a maximum of HDL-cholesterol at 28 wk of gestation (1).

The amount of conjugated dienes formed is a parameter for the concentration of substrate available for lipid peroxidation such as the amount of polyunsaturated fatty acids (PUFA) present in LDL. We did not determine the fatty acid composition of LDL, but it is conceivable that when the fatty acid composition of plasma PL changes there will be a related change in the LDL. In our study population the PUFA and HUFA status of plasma PL was significantly lower at delivery compared to the first trimester. No correlation was found between the fraction of PUFA or HUFA in PL and *T*lag or the amount of formed conjugated dienes.

It had been previously published that there is no correlation between the plasma α-tocopherol concentration and the α-tocopherol content of LDL (25). We found a positive correlation between *T*lag and plasma vitamin E concentrations during pregnancy. It is not clear how the higher plasma vitamin E concentrations can contribute to the higher resistance of LDL toward oxidation as measured by the prolonged lag phase. Vitamin E is tightly bound to LDL, and as LDL-cholesterol increases with progressing gestation it is expected that the vitamin E content will increase also. Our finding that vitamin E levels significantly rise during pregnancy is in line with reports by others (9,10,12–14). The observed increase in vitamin E levels during the course of pregnancy is probably not due to changes in dietary intake. Indeed, analysis of food frequency questionnaires surveyed at the beginning of pregnancy and in the third trimester of this study population revealed no significant differences in the vitamin E content of the diet, nor of the other vitamins, during the course of pregnancy (DeVriese, S.R., Matthys, C., De Henauw, S., Christophe, A.B., and Dhont, M., unpublished results). Our results suggest that when the amount of substrate available for lipid peroxidation in LDL increases (i.e., suggested by increased levels of conjugated dienes formed), the concentration of vitamin E increases also. Indeed, Esterbauer *et al*. (26) showed that the vitamin E content of LDL increases with the PUFA content of LDL. Vitamin A levels decreased with progressing gestation, as reported by others (14). We found no significant changes in maternal plasma β-carotene concentrations whereas others reported a decline in maternal β-carotene levels (14) or observed significantly higher β-carotene levels in pregnant women compared to nonpregnant women (27).

In conclusion, we report an increase in maternal plasma vitamin E levels, a decrease in vitamin A levels, and unchanged β-carotene levels during pregnancy. Furthermore, this study showed an increase in the oxidative stability of LDL with progressing gestation as measured by a prolonged lag phase after *in vitro* oxidation of isolated LDL with Cu^{2+}.

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Chapter 11

Seasonal variation in long-chain polyunsaturated fatty acids

Stephanie R. De Vriese, Armand B. Christophe and Michael Maes

Chapter 11: Seasonal variation in long-chain polyunsaturated fatty acids

In this dissertation we studied the fatty acid composition of plasma phospholipids (PL) during the course of pregnancy. When performing this kind of long-term studies it is important to consider the possibility of seasonal variations. Therefore we determined whether there exits seasonal variation in the fatty acid composition of plasma PL obtained from 23 healthy individuals during one calendar year. The results described in this chapter have been used as part of a larger study dealing with seasonal variation in the number of suicide deaths and serotonergic markers of suicide. In that study it was determined whether these seasonal variations are related to a seasonal variation in the PUFA fractions of serum PL. The results of that study have been described in a manuscript entitled "In humans, the seasonal variation in n-3 poly-unsaturated fatty acids is related to the seasonal variation in violent suicide and serotonergic markers of violent suicide" by SR De Vriese, AB Christophe and M Maes, submitted to Prostaglandins, Leukotrienes and Essential Fatty acids.

1. Introduction

Seasonal variation in plasma total cholesterol and high density lipoprotein cholesterol (HDL) have been repeatedly reported [1;2]. However, no data are available on the seasonal variation in PUFA. In this dissertation we study the fatty acid composition of plasma PL during the course of pregnancy. When performing this kind of long-term studies it is important to consider seasonal variations in plasma fatty acid compositions. This study was conducted in order to examine whether there is seasonal variation in the PUFA fractions of PL in the serum of healthy volunteers.

2. Subjects and Methods

2.1. Study population

Twenty-three healthy Caucasians (12 men and 11 women, mean age 38.5 years, range 23 – 69 years) volunteered to participate in this study. Inclusion and exclusion criteria for subjects are described somewhere else [3]. The geographical coordinates for this study are 51.2°N and 4.5°E around the city of Antwerp, Belgium. The subjects gave oral informed consent to participate in the study in accordance with the ethical standards of the Ethical Committee of the University of Antwerp. The study period extended from December 11, 1991 until December 25, 1992. Seasons were defined by their respective solstices and equinoxes, that is, winter: December 21 to March 20; spring: March 21 to June 20; summer: June 21 to September 20; and fall: September 21 to December 20.

2.2. Methods

Blood collections were performed under standardized conditions to minimize sources of pre-analytical variation [4]. Blood samples were taken after an overnight fast at 8:00 am (± 30 minutes). Each subject had 12 consecutive monthly blood samplings carried out by the same investigator. Blood samplings in men and postmenopausal women were evenly spaced at monthly intervals. Blood samplings in premenopausal females were always carried out 5 to 10 days after the first day of the menstrual cycle. Serum was stored in plastic tubes under nitrogen at –80°C until thawed for fatty acid analysis. All serum samples from one subject are analysed simultaneously using the same batch of solvents and the same capillary GC column [4]. Lipids were extracted from 1 mL serum according to a modified Folch extraction with methanol:chloroform (1:2) [5]. The lipids were separated by thin layer chromatography on rhodamine-impregnated silica gel plates using petroleum ether (bp 60-80°C; Merck Belgolab, Overijse, Belgium)/acetone 85:15 as mobile phase [6]. The PL fraction of serum lipids was scraped off and the fatty acids converted into methyl esters by transesterification with 2 mL of a mixture of methanol:benzene:HCl (aqueous, 12N) (80:20:5) [7]. After cooling and adding 2 mL of water, fatty acid methyl esters were extracted with petroleum ether (bp 40-60°C), evaporated to dryness under a nitrogen flow at a temperature not exceeding 40°C, and analysed by temperature programmed capillary gas chromatography (Varian Model 3500, Walnut Creek, CA, USA) on a 25m x 250µm

(LxID) x 0.2µm df Silar 10C column [7]. The injection and detection temperature were set at 285°C. The starting temperature of the column was 150°C, which was increased to 240°C after 3 min at a rate of 2°C/min. The carrier gas was nitrogen with a flow of 25 cm/s. Peak identification was performed by spiking with authentic standards (Sigma-Aldrich, Bornem, Belgium). Peak integration and calculation of the percent composition was performed electronically with a Varian Model 4290 integrator. The coefficient of variation of intra-assay samples of the entire method of fatty acid analysis for peaks bigger than 1 w% is less than 5% and for peaks smaller than 1 w% is less than 10%. The results are expressed as weight percent of total fatty acids.

2.3. Statistical analysis

Seasonal variation has been ascertained by means of analysis of variance (ANOVA) [8;9]. Values are reported as means with standard deviation.

3. Results

Table 1 shows the mean PUFA values in the seasons. AA was significantly lower in winter than in the other seasons. EPA was significantly lower in winter and spring than in summer. DHA was significantly lower in winter than in the other seasons. Total n-3 was significantly lower in winter than in autumn and summer. There were no significant seasonal differences in any of the other PUFA fractions, including 18:2n-6 and 18:3n-3.

Figure 11.1 shows the seasonal variation in 22:6n-3 and figure 11.2 shows the monthly variation in 22:6n-3. These figures illustrate a significant annual rhythm in 22:6n-3 with peaks around August - September and lows in December – February.

Table 1: Seasonal differences in serum phospholipid PUFA fractions in 23 healthy volunteers who had monthly blood samplings during one calendar year

	Winter	Spring	Summer	Fall	F	df	P
18:2n-6	21.59 (2.80)	21.42 (3.28)	21.08 (3.03)	21.09 (2.61)	1.8	3/171	0.2
18:3n-3	0.27 (0.16)	0.29 (0.19)	0.27 (0.14)	0.27 (0.16)	0.5	3/171	0.7
20:4n-6	7.38 (1.30)	7.89 (1.57)	8.38 (1.55)	8.20 (1.65)	4.9	3/171	0.003
20:5n-3	0.53 (0.28)	0.67 (0.44)	0.76 (0.56)	0.68 (0.43)	3.1	3/171	0.02
22:5n-6	0.57 (0.77)	0.51 (0.64)	0.54 (0.64)	0.52 (0.67)	0.6	3/171	0.6
22:6n-3	2.37 (0.53)	2.85 (0.75)	3.08 (0.72)	2.89 (0.77)	8.1	3/169	0.0001
total n-6	32.5 (2.18)	32.6 (2.52)	33.0 (2.08)	32.8 (2.27)	0.6	3/171	0.6
total n-3	3.96 (0.86)	4.65 (1.25)	5.02 (1.23)	4.71 (1.75)	3.3	3/171	0.02

All results are expressed as mean (SD). All results of ANOVAs with seasons and subjects as factors.

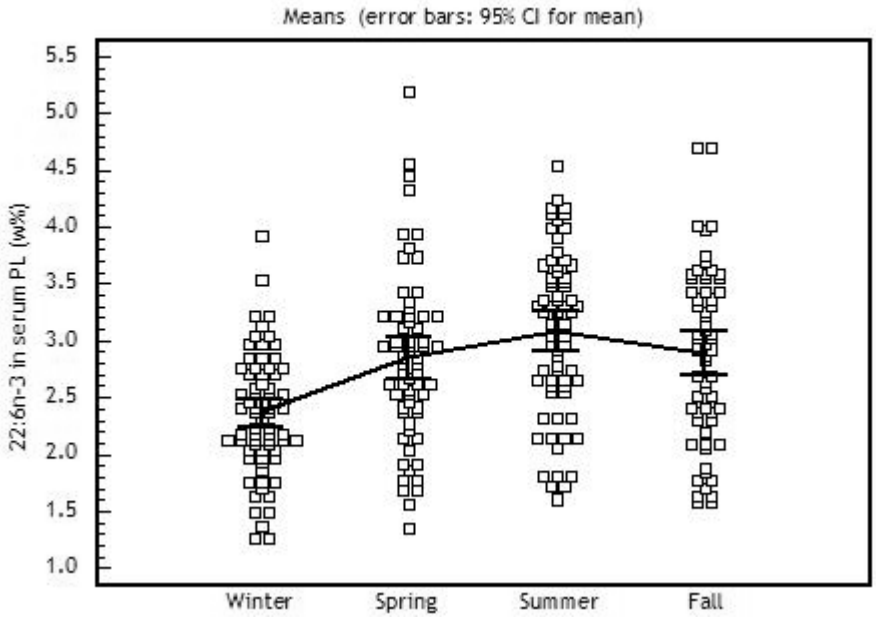

Figure 11.1: Seasonal variation of 22:6n-3 (w%).

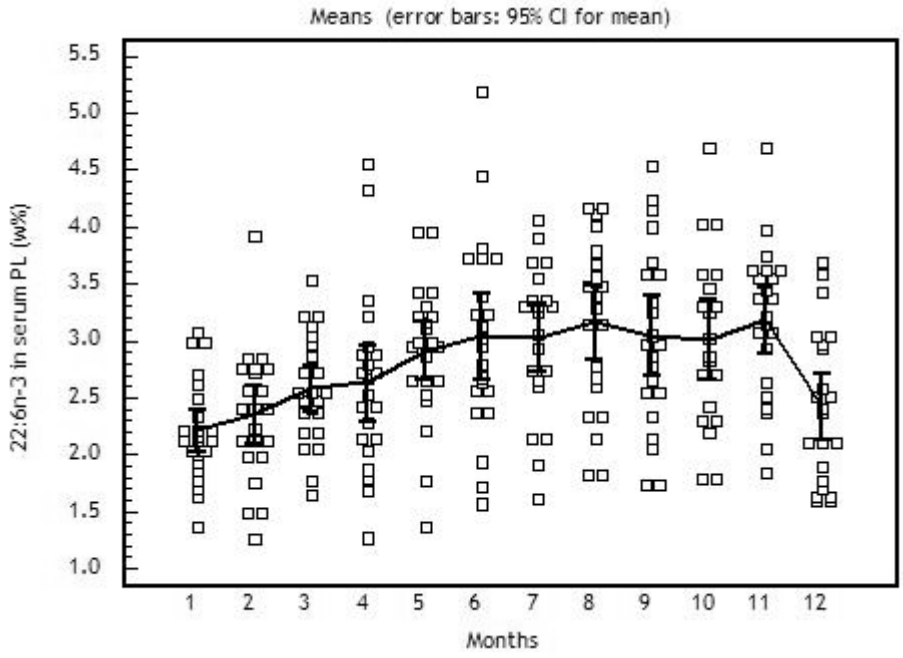

Figure 11.2: Monthly variation of 22:6n-3 (w%).

4. Discussion

The major finding of this present study is that 20:4n-6, 20:5n-3, 22:6n-3 and total n-3 fractions, show - in a normal population - statistically significant annual rhythms with peaks around August - September and lows in winter. It is interesting to note that linoleic acid and α-linolenic acid (the parent EFA) showed no significant seasonal variation whereas there is a true seasonality in the delta-5-desaturase products (20:4n-6 and 20:5n-3) and in the elongation-desaturation product of the latter (22:6n-3). This could suggest that there is a seasonal variation in the delta-5-desaturase. The origin of the above seasonal rhythms has remained elusive, but the seasonal variation in many human physiological functions is related to genetically determined processes (endogenous rhythms) which may be adjusted in time (or "entrained" or "synchronized") by cycles in light-dark span or ambient temperature.

Recently Bluher *et al* [10] studied whether seasonal variations could be due to changes in nutrition or changes in physical activity depending on the season. They

found annual rhythms in plasma total cholesterol and HDL concentrations with a maximum in winter and a minimum in summer. This rhythm was independent of age, gender, BMI, diet or physical activity. They concluded that the annual rhythm of total cholesterol and HDL was not primarily induced by seasonal differences in dietary intake or physical activity. The annual rhythm in cholesterol levels is most likely determined by endogenous factors or factors directly related to seasonal changes in the environment [10].

It is possible that some changes found in the fatty acid composition during the course of pregnancy were enlarged or reduced by this seasonal variation. However this was impossible to determine in our study population of pregnant women because (i) our study population was too small; (ii) the delivery date of our group of pregnant women was stretched over different calendar years and (iii) no more than 4 women delivered in the same month. In retrospective, it was determined whether DHA level in plasma PL of women who delivered in winter (n=8) was significantly lower ($P<0.05$; one-sided test) compared to that of women who delivered in other seasons (n=26). This was not the case suggesting that seasonal effects, if any are small relative to the effects of pregnancy (but once again our study population is too small to make this kind of statement). It would be interesting to correct for seasonal variation in a larger population of pregnant women.

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Chapter 12

General conclusions

Chapter 12: General conclusions

1. Main findings

1.1. The maternal diet.

During gestation there is no change in dietary intake of energy, macronutrients and micronutrients (**chapter 3).** In this study population most pregnant women had an adequate caloric intake (2148 (SD 386) kcal/d). Protein intake compared favourably with the Belgian dietary guidelines. The average carbohydrate intake was too low whereas the average total fat intake was too high compared to the Belgian recommendations. High fat intake was due to an excessive intake of saturated fat. Only one quarter of this pregnant population met the recommendations for Ca intake. We advise pregnant women to increase the intake of milk and milk products (yoghurt and cheese) to obtain an adequate supply of calcium, vitamin B2 and vitamin D. Increasing the intake of cheese and milk products will however increase the intake of saturated fat. Therefore we advise to reduce the intake of fatty meat products, sauces, chips, sweets and pastry. None of the women reached the recommended intake for magnesium and selenium entailing the risk that they could become deficient for these micronutrients with progressing pregnancy. Magnesium deficiency during pregnancy can cause fatigue and increases the risk of premature birth and maternal hypertension. Low serum selenium levels have been associated with miscarriage. Nuts, seeds and chocolate are good sources of magnesium and seafood is a good source of selenium. In general, the intake of some vitamins and minerals in this study population is below the recommendations. Some women could benefit from a multivitamin/mineral supplement.

Dietary intake of long-chain PUFA during pregnancy was determined by the validated food frequency questionnaire (FFQ) **(chapter 2 & 4).** The most dominant

n-6 fatty acid in the diet is 18:2n-6, with a mean intake of 12.9 (SD 5.3) g/day and the dominant n-3 fatty acid in the diet is 18:3n-3 with an average intake of 1.3 (SD 0.5) g/day. The dietary intake of 20:4n-6 is 0.13 (SD 0.03) g/d and that of the longer chain n-3 fatty acids is 0.46 (SD 0.21) g/d. Meat and poultry are the major sources of 20:4n-6 in the diet, whereas fish and fish products are the main sources of 20:5n-3 and 22:6n-3. In 2003 the Belgian recommendations for fat intake have been adapted and are identical to the recommendations for adequate intake of long-chain PUFA by Simopoulos *et al* [1]. According to these recommendations, for pregnant women, the sum of 20:5n-3 and 22:6n-3 should be 0.65 g/day, the 20:5n-3 intake at least 0.22 g/day and the 22:6n-3 intake at least 0.3 g/day. In only 7 out of 30 women of this study population the intake of 20:5n-3 and 22:6n-3 was higher than the thus defined adequate intake. The intake of linoleic acid is rather high compared to the current Belgian recommendations. A high maternal intake of linoleic acid may negatively affect the maternal and neonatal n-3 fatty acid status. Therefore it would be advisable to increase the dietary intake of long chain n-3 PUFA and reduce that of linoleic acid during pregnancy.

In maternal plasma PL, the fractions of 18:2n-6, 20:5n-3 and 22:6n-3 are positively correlated with their respective dietary intakes (g/kg BW/day) in this study population. For the latter fatty acid, a positive correlation was also found between intake and level in maternal plasma CE. In umbilical plasma PL, 20:5n-3 and the sum of n-6 fatty acids are positively associated with the intake of these (groups of) fatty acids during pregnancy.

1.2. Maternal and neonatal EFA status

The lower content of long-chain PUFA in maternal plasma PL results in a higher mean melting point of the fatty acid mixture. Long-chain PUFA were preferentially replaced by shorter chain SFA, especially palmitic acid. These shorter chain fatty acids are more fluid than their longer chain homologues because they have lower melting points. Moreover, the high content of long-chain PUFA in umbilical plasma PL is accompanied with more longer-chain, less fluid SFA. Thus the fatty acid composition of the SFA changes in a way to counteract changes in the mean melting point induced by changed long-chain PUFA composition **(chapter 5)**.

During pregnancy we found changes in the fatty acid composition of PL and CE (**Chapter 6).** There is a decrease in linoleic acid (in CE), arachidonic acid (in PL

and CE) and the sum of PUFA (in PL and CE) and an increase in saturated (in PL) and mono-unsaturated fatty acids (in PL and CE).

Linoleic acid in cord plasma PL is half of that in maternal plasma PL but arachidonic acid (AA) in cord plasma is twice that observed in the mother. Similarly, the α-linolenic acid concentration in newborns is half of that in the mother, whereas the DHA concentration is almost double. This situation, in which the relative plasma concentrations of the n-3 and n-6 long-chain PUFA exceed those of their precursors has only been observed in newborns and does not exist in adults. It is obviously an extremely favourable situation for the development of the newborn, especially at a time when large quantities of AA and DHA are required for the development of the brain and retina.

During early puerperium (from 6 days before until 3 days after delivery) we found significant changes in the fatty acid composition of maternal plasma PL and CE **(chapter 7)**. The previously reported gradual changes in the fatty acid composition of PL and CE which occur with the progression of normal pregnancy diminish shortly after delivery.

The wall of the umbilical artery (efferent blood vessel) contains less 18:2n-6 and 20:4n-6 and significantly more Mead acid than the wall of the umbilical vein (afferent blood vessel) **(chapter 8)**. Mead acid, a desaturation and elongation product of oleic acid, only accumulates in tissues if insufficient amounts of the parent EFA are available. This study confirmed previous findings in which evidence was obtained for a marginal EFA status of the newborn.

We found significant differences in the postpartum fatty acid status between women who developed a postpartum depression compared to mothers who did not **(chapter 9)**. We observed a significant association between the ratio of Σn-6/Σn-3 in PL and the occurrence of postpartum depression. Women who became depressed after delivery had a significantly lower status of 22:6n-3 and of Σn-3 fatty acids in PL and CE compared to women who did not.

This observed an increase in the oxidative stability of LDL with progressing gestation measured by a prolonged lag-phase after in vitro oxidation of isolated LDL with Cu^{2+}. Both the rate of formation and the amount of conjugated dienes formed reached a maximum in the third trimester. This could be due to a change in the composition of the LDL near the end of pregnancy (structural changes in

plasma lipoproteins during pregnancy have been described). Additionally we observed significant increases in the vitamin E levels during pregnancy **(chapter 10)**.

It the last experiment of this thesis we found a true seasonality in long-chain PUFA, such as 20:4n-6, 20:5n-3 and 22:6n-3 **(chapter 11).** Significant annual rhythms were detected in the long-chain PUFA: 20:4n-6, 20:5n-3 and 22:6n-3. These three fatty acids and the sum of total n-3 fatty acids were significantly lower in winter than in the other seasons.

2. Dietary recommendations for pregnant women

2.1. General

In our study population important differences between mean intake and dietary recommendations were found. Although this does not mean that for the individual woman there are deficiencies, it seems prudent to give individuals dietary advice to meet the recommendations. Thus it is recommended that expecting mothers are seen by a dietician to get individualised advice.

2.2. Essential fatty acids

2.2.1 General

It is now well established that adequate supply of long-chain PUFA is critical and essential for a normal neurological development of the foetus [2;3]. The degree to which the foetus is capable of fatty acid desaturation and elongation is not clear [4;5]. In addition, it is known that the long-chain PUFA concentration in umbilical plasma PL is lower when gestational age at birth is lower, in children born from multiple pregnancies and when birth order is higher. The nutritional status of the mother during gestation has been related to foetal growth. Significant linear correlations between the mother and the newborn have been found for long-chain n-3 and n-6 PUFA [6;7].

From the literature and the results of our studies it may be felt necessary to increase the dietary EFA and/or long-chain PUFA intake of pregnant women in order to prevent the decrease of their long-chain PUFA status during pregnancy and to optimise the foetal long-chain PUFA status.

This may be of particular importance for infants born preterm, because they have a significantly lower PUFA status than term neonates [8]. In addition, their long-chain PUFA status drops considerably during the first postnatal weeks, even when given breast milk [9;10], whereas during intra-uterine life the foetal EFA status increases considerably during the same postconceptional period [11]. Consequently, during the growth spurt of the brain, the availability of LCPUFA is much lower for infants born preterm than for the intra-uterine foetus of comparable postconceptional age. Whether or not this contributes to the well known developmental disadvantage of preterm *vs.* term infants needs careful consideration.

Many supplementation studies (mainly with fish oil) have shown that it is possible to alter the maternal fatty acid status as well as that of their neonates. Supplementation of pregnant women with long-chain PUFA has been shown to improve neonatal long-chain PUFA status [12;13]. However it is not yet elucidated whether these biochemical differences have functional consequences. If supplementation with essential PUFA during pregnancy is considered, it should be recalled that the two PUFA families compete for the same metabolic enzymes. Therefore, the supplement of choice should contain a mixture of n-6 and n-3 (long-chain) PUFA.

2.2.2 Requirements

In 2000 ISSFAL (International Society for the Study of Fatty Acids and Lipids) published new recommendations for fatty acid intake, namely: 2 – 3 en% linoleic acid, 1 en% 18:3n-3, more than 0.3 en % EPA + DHA. Recently, in 2003 the Belgian recommendations for fat intake have been adapted in a similar way: n-3 fatty acid intake should range between 1.3 and 2 en%, the 18:3n-3 intake at least 1 en% and the intake of EPA + DHA at least 0.3 en%. The n-6 fatty acid intake should range between 4 and 8 en% and that of 18:2n-6 should be at least 2 en%. Based on a 2,000 kcal diet this means for 18:2n-6 between 4.4 and 6.6 g/day, for 18:3n-3 more than 2.22 g/day and for EPA + DHA more than 667 mg/day.

Pregnant Spanish women having more than 4 fatty fish meals per month have an average fish, EPA and DHA intake of 54.5 g/d, 320 mg/d and 650 mg/d respectively. Two to four fish meals per month accounted for an average fish, EPA and DHA intake of 8.9 g/d, 100 and 200 mg/d respectively [14]. Thus when the

Belgian and the ISSFAL recommendations are translated into fish intake pregnant women should consume at least 4 fatty fish meals per month.

2.2.3 Optimum timing of supplementation

Sattar *et al* [15] suggested that the optimum timing for dietary supplementation with long-chain PUFA during pregnancy is the first trimester. Arguments for this suggestion were: (i) programmes in developed countries of supplementation during the second and third trimester with high protein and high carbohydrate diets have not improved the outcome for the infant; (ii) pre-eclampsia is associated with pathological changes in the placenta during the first trimester. As the placenta is rich in cell membranes it is possible that its development depends on the supply of EFA just after conception or that the endothelium requires EFA to maintain the integrity of endothelial cell membranes in pre-eclampsia; and iii) epidemiological observations of a protective effect of fish intake on pregnancy complications relate to women who had a lifelong exposure to diets rich in fish and seafood.

2.3. Fatty acid content of the Westernised diets

The Westernised diet is rich in linoleic acid and relatively poor in α-linolenic acid. Linoleic acid is found in highest concentrations in vegetable oils and thus in PUFA-rich margarines but it is also present in a wide range of other foods. Lean meat, organ meats and eggs contain linoleic acid and also small quantities of arachidonic acid, which is readily incorporated into cell membranes [16]. α-Linolenic acid is found in high concentrations in linseed, canola, soybean, and wheat germ oil and in walnuts [17]. Vegetables are poor sources of n-3 fatty acids. The long-chain n-3 PUFA are found in highest concentrations in fatty fish and other marine foods such as shellfish, molluscs and fish oil. EPA and DHA originate in phytoplankton and are concentrated in fish and shellfish through the aquatic food chain. Recently, eggs rich in α-linolenic acid or in DHA have been introduced in the market. DHA and AA are also available as dietary supplements, such as fish oil concentrates (mainly DHA and its precursors EPA and 22:5n-3) and single cell oils (DHA and AA).

In fatty fish, breast milk, fish oils and single cell oils, the long-chain PUFA are mainly present as triacylglycerols. In lean fish, meat, and egg yolk, phospholipids are the major long-chain PUFA carriers, whereas in certain supplements n-3 long-chain PUFA are present as ethyl-esters. Studies comparing the bio-availability of

these various forms of long-chain PUFA indicated considerable differences but are still incomplete [18-25]. Therefore, further research on this issue is needed.

2.4. Maternal seafood diet: balance between beneficial and adverse effects

Seafood is rich in n-3 PUFA (mainly EPA and DHA) and selenium. The beneficial effects of n-3 PUFA during gestation have been described in detail in this dissertation. In summary, n-3 PUFA are related to a reduced incidence of pre-eclampsia, reduced incidence of postpartum depression, increased length of gestation and increased birth weights.

However seafood may also contain toxic contaminants, including polychlorinated biphenyls (PCB's) and methyl mercury. PCB's have been associated with decreased birth weight and delays in neurobehavioral development [26-29]. Methyl mercury has been linked with lower birth weight in Greenland populations [30] while in Faroe populations birth weights showed an increase at higher exposure to mercury [31].

Thus seafood contains both beneficial and potentially toxic components. Few studies have evaluated the relative importance of and possible interactions between these seafood components. In a birth cohort from the Faroe Islands it was found that after adjustment for PUFA and other covariates, mercury and PCB were poorly associated with birth weight [32].

Selenium, an important micronutrient present in seafood, may offer some protection against mercury toxicity.

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Appendices

Voedingsfrequentievragenlijst

Algemene voedingsvragen

1. Volgt u speciale voedingsrichtlijnen of een dieet ?

☐ nee
☐ ja, vermageringsdieet/ zoutarm dieet (*)
☐ ja, vegetarisch/ macrobiotisch (*)
☐ ja, andere nl.....

2. Met hoeveel personen eet u de warme maaltijd ?

☐ ik eet alleen
☐ ik eet met nog ……perso(o)n(en), m.a.w. totaal aantal personen: ……
☐ indien elders een warme maaltijd gebruikt wordt: …… x per week elders gegeten.

3.

Heeft u de afgelopen maand thuis ……. gegeten ?	ik eet niets	ik eet .....deel	ik eet alles	afgelopen maand niet bereid
Gekookte groente				
Rauwe groente				
Pasta (macaroni, spaghetti, e.d.)				
Rijst (niet van de Chinees, maar zelfgemaakt)				
Vlees in een saus (goulasch, vol au vent, e.d.)				
Gebakken aardappelen				
Aardappelpuree				
Gerechten met peulvruchten (chili, witte bonen in tomatensaus)				

* schrappen wat niet juist is a.u.b.

Vragen over productgebruik

I. Oliën en vetten bij de broodmaaltijd

4. Wat smeert u meestal op uw brood ?

Noteer het merk én de benaming (*vb. niet enkel "Buttella" maar "Buttella plantaardig" of "Buttella super", ...*)

- ☐ Niets
- ☐ Minarine merk(en):..... x per week
- ☐ margarine in vlootje merk(en):..... x per week
- ☐ margarine in papierwikkel merk(en):..... x per week
- ☐ (room)boter ..... x per week
- ☐ halfvolle boter merk(en):..... x per week
- ☐ andere, nl. ..... x per week

5. Hoe dik besmeert u uw boterham daarmee ?

- ☐ …… g per snede van een rond/ vierkant/ groot/ klein brood (*)
- ☐ …… g per .....

6. Indien u wel eens broodjes eet, besmeert u dan beide kapjes ?

- ☐ ja
- ☐ nee

7. Hoeveel brood eet u gemiddeld op een volledige (ontbijt + andere broodmaaltijd)

- ☐ weekdag (ma-vr): ..... sneetjes van een rond/ vierkant/ groot/ klein brood (*)
  en ..... broodjes (welke?.....)
- ☐ weekenddag: - zaterdag: .....sneetjes van een rond/ vierkant/ groot/ klein brood (*)
  en ..... broodjes (welke?.....)
  - zondag: .....sneetjes van een rond/ vierkant/ groot/ klein brood (*)
  en ..... broodjes (welke?.....)

II. Oliën en vetten bij de warme maaltijd

8. Heeft u de afgelopen maand de volgende gerechten gegeten ?

Indien ja, noteer bij de "vetsoort": de soort vetstof, alsook het merk én de benaming (*vb. margarine Fama in vlootje, margarine Becel bakken en braden, frituurolie Vandemoortele,...*)

8.1. Pasta (macaroni, spaghetti, e.d.): blanke/ volkoren (*)

a. In totaal wordt er ..... gram/ pak(ken)/ tas(sen) (*) pasta gekookt
b. Vetsoort (bv. in (vlees)saus die bij de pasta gemaakt wordt)
   .....
c. Hoeveel vet in totaal: ..... gram
d. Hoe vaak: ..... x per week/ 2 weken / 3 weken/ maand (*)

8.2. Rijst (zelfgemaakte, niet van de Chinees): witte/ zilvervlies (*)

a. In totaal wordt er ..... gram/ builtje(s)/ tas(sen) (*) rijst gekookt
b. Vetsoort (bv. in (vlees)saus die bij de rijst gemaakt wordt)
   .....….
c. Hoeveel vet in totaal: ..... gram
d. Hoe vaak: ..... x per week/ 2 weken / 3 weken/ maand (*)

8.3. Zelf gefrituurde frieten

a. Ik eet een kleine/ middel/ grote portie frieten (*)
b. Vetsoort: .....
c. Hoe vaak: ..... x per week/ 2 weken / 3 weken/ maand (*)

8.4. Gebakken aardappelen

a. Ik eet ..... eetlepel(s)
b. Vetsoort: .....
c. Hoeveel vet in totaal: ..... gram
d. Hoe vaak: ..... x per week/ 2 weken / 3 weken/ maand (*)

8.5. Aardappelpuree

a. Ik eet ..... eetlepel(s)
b. Vetsoort: .....
c. Hoeveel vet in totaal: ..... gram
d. Hoe vaak: ..... x per week/ 2 weken/ 3 weken/ maand (*)

8.6. Gekookte aardappelen

a. Ik eet ..... stuks aardappelen, ter grootte van een ei
b. Hoe vaak: ..... x per week/ 2 weken/ 3 weken/ maand (*)

8.7. Peulvruchten: bruine bonen/kapucijners/witte bonen in tomatensaus (*)

a. In totaal wordt er ..... blik of pot à 1 liter/ blik of pot à ½ liter/gram bereid (*)
b. Vetsoort: .....
c. Hoeveel vet in totaal: ..... gram
d. Hoe vaak: ..... x per week/ 2 weken/ 3 weken/ maand (*)

8.8. Gekookte groente, de meest gegeten groentesoorten in de afgelopen maand zijn:

..... vers gekookt/ diepvries/ blik of glas (*)
..... vers gekookt/ diepvries/ blik of glas (*)
..... vers gekookt/ diepvries/ blik of glas (*)
..... vers gekookt/ diepvries/ blik of glas (*)
..... vers gekookt/ diepvries/ blik of glas (*)

a. Ik eet gemiddeld ..... eetlepel(s) (de evt. witte saus niet meerekenen)
b. Vetsoort: .....
c. Hoeveel vet in totaal: ..... gram
d. Ik eet ..... x per week/ 2 weken/ 3 weken/ maand (*) gekookte groente
   en er komt ..... x per week/ 2 weken/ 3 weken/ maand (*) het bovengenoemde vet bij (vergeet het vet in witte saus niet)

8.9. Stukjes vlees in een saus (goulash, vol au vent)

a. Vetsoort: .....
b. Hoeveel vet in totaal: ..... gram
c. Hoe vaak: ..... x per week/ 2 weken/ 3 weken/ maand (*)

8.10. Vlees of vis (lapjes of een stuk)

a. Vetsoort: .....……
b. Hoeveel vet in totaal: ..... gram
c. Hoe vaak: ..... x per week/ 2 weken/ 3 weken/ maand (*)
d. Gebruikt u jus of saus, gemaakt van het braadvet van het vlees ? (stukjes vlees in een saus, zoals goulash, hier niet meerekenen!)
   ☐ nee
   ☐ ja, ... x per week/ maand (*)
e. Voegt u water toe bij de bereiding van de jus ?
   ☐ nee
   ☐ ja, ... eetlepel(s)/ tas(sen) (*)
f. Wordt de jus wel eens ontvet (het gestolde vet eraf geschept) ?
   ☐ nee
   ☐ ja, ... x per week/ maand (*)
g. Het hoeveelste deel van de totale hoeveelheid bereide saus neemt u ?
   .....
   (evt. hoeveel eetlepels neemt u? ..... eetlepels)

III. Sausen

Vetpercentages staan in het algemeen vermeld op de verpakking. Indien u het niet weet, gelieve het merk en evt. de benaming te vermelden.

8.11. Heeft u de afgelopen maand rauwe groenten gegeten ?

☐ nee
☐ ja, de meest gegeten rauwe groentesoorten waren:
.....……
.....……

Ik eet gemiddeld ..... gram/ eetlepel(s)/ schaaltje(s) (*)

rauwe groente ..... x per week/ 2 weken/ 3 weken/ maand (*)

Gebruikt u een saus bij de rauwe groente?

☐ nee
☐ ja, in totaal (dus voor de bereiding van het hele gerecht) wordt gebruikt: .....
☐ ja, ik neem voor mezelf:
   ☐ slasaus ..... …..... % vet; ..... …..... koffielepel(s)/ eetlepel(s) (*)
   .....x per week/ 2 weken / 3 weken/ maand (*)
   ☐ fritessaus ..... …..... % vet; ..... …..... koffielepel(s)/ eetlepel(s) (*)
   ..…..... x per week/ 2 weken / 3 weken/ maand (*)
   ☐ mayonaise ..... …..... % vet; ..... …..... koffielepel(s)/ eetlepel(s) (*)
   ..…..... x per week/ 2 weken / 3 weken/ maand (*)
   ☐ Becel dressing... …..... % vet; ..... …..... koffielepel(s)/ eetlepel(s) (*)
   ..…..... x per week/ 2 weken / 3 weken/ maand (*)
   ☐ yoghurt, mager/ halfvol/ vol (*)…….…….....….. koffielepel(s)/ eetlepel(s) (*)
   ..…..... x per week/ 2 weken / 3 weken/ maand (*)
   ☐ olie, soort ....koffielepel(s)/eetlepel(s) (*)
   ..…..... x per week/ 2 weken / 3 weken/ maand (*)
   ☐ overige, nl. ....koffielepel(s)/eetlepel(s) (*)
   ..…..... x per week/ 2 weken / 3 weken/ maand (*)

8.12. Gebruikt u een saus bij frieten?

☐ Nee

☐ ja, nl. ..... % vet;..... koffielepel(s)/ eetlepel(s) (*)
.. ……..... x per week/ 2 weken / 3 weken/ maand (*)

8.13. Heeft u de afgelopen maand nog andere (dus naast de bij vraag 11 en 12 al genoemde) sausjes gebruikt? *(bv. knoflooksaus, cocktailsaus, ….)*

☐ nee

☐ ja, nl. ..... % vet;..... koffielepel(s)/ eetlepel(s) (*)
..... x per week/ 2 weken / 3 weken/ maand (*)

IV. Zuivel

Heeft u afgelopen maand de volgende producten gebruikt?

9. Melk:

☐ nee

☐ ja, nl. magere/ halfvolle/ volle (*)
ik gebruik ..... tas(sen)/ glas(zen)/ liter
- ☐ per dag
- ☐ per week
- ☐ per 2 weken
- ☐ per 3 weken
- ☐ per maand

Denk ook aan het gebruik van melk in koffie, sausen, zelfgemaakte pudding, aardappelpuree ...

10. Kant & klare chocolademelk:

☐ nee

☐ ja, nl. magere/ halfvolle/ volle (*)
ik gebruik ..... tas(sen)/ glas(zen)/ liter
- ☐ per dag
- ☐ per week
- ☐ per 2 weken
- ☐ per 3 weken
- ☐ per maand

11. Koffiemelk:

☐ nee

☐ ja, nl. magere/ halfvolle/ volle/ koffiemelkpoeder (*)
ik gebruik ..... tas(sen) koffie
- ☐ per dag
- ☐ per week
- ☐ per 2 weken
- ☐ per 3 weken
- ☐ per maand

12. Yoghurt:

☐ nee
☐ ja, nl. magere/ volle/ halfvolle (*) en met/ zonder fruit (*)
ik gebruik ..... schaaltje(s)/ liter/ eenpersoonsportie(s) van 125 g/150 g/200 g (*)
☐ per dag
☐ per week
☐ per 2 weken
☐ per 3 weken
☐ per maand

13. Kant & klare vla/ pudding:

☐ nee
☐ ja, nl. magere/ volle/ halfvolle (*)
ik gebruik ..... schaaltje(s)/liter (*)
☐ per dag
☐ per week
☐ per 2 weken
☐ per 3 weken
☐ per maand

14. Ijs:

☐ nee
☐ ja, nl. waterijs/ consumptie-ijs/ roomijs (*) .....(merk noteren)
ik gebruik ..... ijsjes/ bolletjes ijs (*)
☐ per dag
☐ per week
☐ per 2 weken
☐ per 3 weken
☐ per maand

15. (slag)Room: (room in sausen, op ijs, …) (room op gebak niet meerekenen: zie VI)

☐ nee
☐ ja, nl. light room/ verse room/ slagroom (*) .....(merk noteren)
ik gebruik ..... eetlepel(s)/ liter (*)
☐ per dag
☐ per week
☐ per 2 weken
☐ per 3 weken
☐ per maand

16. Platte kaas:

☐ nee
☐ ja, nl. mager/ halfvolle/ volle (*) en met/ zonder fruit (*)
ik gebruik .....… eetlepel(s)/ gram/ eenpersoonsportie(s) van ..... gram (*)
☐ per dag
☐ per week
☐ per 2 weken
☐ per 3 weken
☐ per maand

17. Smeerkaas/ fonduesneetje kaas (vb. Ziz, e.d.) (*):

☐ nee
☐ ja, nl. magere/ volvette/ dubbelroom (*)
ik gebruik .....….. sneetje(s)/ gram/ driehoekje(s) (*)
☐ per dag
☐ per week
☐ per 2 weken
☐ per 3 weken
☐ per maand

18. Franse kaas, Belgische halfharde kaas:

☐ nee
☐ ja, nl. soort(en) met % vet;
..... ik gebruik ..... gram per .....
..... ik gebruik ..... gram per .....
..... ik gebruik ..... gram per .....
..... ik gebruik ..... gram per .....
..... ik gebruik ..... gram per .....
..... ik gebruik ..... gram per .....
..... ik gebruik ..... gram per .....
..... ik gebruik ..... gram per .....

19. Andere kaas (Hollandse kaas, andere vaste kaas, e.d.):

☐ nee
☐ ja, nl. soort(en):.....
.....…….....……..
ik gebruik ..... (voorgesneden) plak(ken)/ gram (*)
☐ per dag
☐ per week
☐ per 2 weken
☐ per 3 weken
☐ per maand

V. Vlees, gevogelte en vis

20. Vleeswaren

Hoeveel keer per week eet u vleeswaren ? ……. keer

		gemiddeld aantal sneetjes				
Heeft u de afgelopen maand ……. gegeten ?	niet gebruikt	per dag	per week	per 2 weken	per 3 weken	per maand
gekookte ham						
casselerrib/filet de Saxe (*)						
gerookt paarden- of rundvlees (filet d'Anvers) (*)						
rauwe ham/ gerookte ham/ bacon (*)						
Kippenwit/ kalkoenham/ kalfskop (*)						
salami/ boterhamworst/ hespeworst/ kalfsworst/ Parijse worst/ champignonworst (*)						
Frankfurtworst/ Weense worst/ cervelaat/ lookworst (*)						
pâté (aantal gram aanduiden)/ vleesbrood/ hoofdvlees/ corned beef (*)						
Filet américain puur/ préparé (*) (aantal gram aanduiden)						
Vleessalade/ vissalade/ zeevruchten-salade (*) (aantal gram aanduiden)						

21. Vlees, vis en gevogelte.

Hoeveel keer per week eet u een warme maaltijd ? …… keer

Hoeveel keer per week eet u vlees, vis of gevogelte bij de warme maaltijd? ……..keer

		gemiddeld aantal gram of aantal stuks				
Heeft u de afgelopen maand ……. gegeten ?	niet gebruikt	per dag	per week	per 2 weken	per 3 weken	per maand
Varkenshaasje/ varkensmignonette (*)						
Varkensfiletgebraad/filetcotelet/ ribcotelet/ varkensbrochette/mager varkenslapje/ varkensschnitzel (*)						
Spieringcotelet/ vet varkenslapje/ varkensschoudergebraad/ varkensstoofvlees (*)						
Varkensgehakt/ varkensbraadworst/ ribbetjes of spareribs (*)						
Vers spek						
Gemengd gehakt/ braadworsten van gemengd gehakt/ blinde vink (*)						
Bloedworst/ witte worst (*)						
Nier: varkens-/rund-/kalfs- (*)						
Lever: varkens-/rund- (*)						
Biefstuk/ rumsteak/ châteaubriand/ ossehaas/ tournedos/ rosbief (*)						
Filet américain/ rundbrochetten (*)						

		gemiddeld aantal gram of aantal stuks				
Heeft u de afgelopen maand ……. gegeten ?	niet gebruikt	per dag	per week	per 2 weken	per 3 weken	per maand
Entrecôte zonder vet Entrecôte met vet (*)						
Rundstoofvlees/ soepvlees (*)						
Hamburger						
Kalfslap/kalfsblanquette/kalfsgehakt (*)						
Lamsvlees, schapevlees (*)						
Paardevlees						
Kalkoenfilet/ kalkoenbout (*) Kippefilet, enkele of dubbele (*) Kippebout, met of zonder vel (*)						
Konijn: rugstuk/voorbout/achterbout (*)						
Ander vlees of gevogelte						
Vis: vers/ diepvries (*) kabeljauw/, koolvis, schelvis, wijting, leng, rog, tarbot, zeeduivel (*)						
Vis: vers/ diepvries (*) pladijs, tong, heilbot, zeepaling (*)						
Vis: vers/ diepvries (*) zalm, zalmforel, makreel, haring, paling						

		gemiddeld aantal gram of aantal stuks				
Heeft u de afgelopen maand ……. gegeten ?	niet gebruikt	per dag	per week	per 2 weken	per 3 weken	per maand
Vis: gerookt sprot, heilbot, makreel, forel, paling, zalm (*)						
Vis: in blik of bokaal sardine, ansjovis, tonijn, zalm, makreel, pilchards, opgelegde haring (*)						
Garnalen						
Mosselen (gewicht: met schelp)						

VI. Koek, snoep en gebak

Heeft u de afgelopen maand de volgende producten gebruikt?

22. Cake:

- ☐ nee
- ☐ ja, ik eet gemiddeld …… plakjes
  - ☐ per dag
  - ☐ per week
  - ☐ per 2 weken
  - ☐ per 3 weken
  - ☐ per maand

23. Peperkoek:

- ☐ nee
- ☐ ja, ik eet gemiddeld …… plakjes
  - ☐ per dag
  - ☐ per week
  - ☐ per 2 weken
  - ☐ per 3 weken
  - ☐ per maand

24. Koekjes: welke? .....(evt. merknaam noteren)

☐ nee
☐ ja, ik eet gemiddeld …… stuks
  ☐ per dag
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

25. Wafels: Luikse/ vanille-/ chocolade (*)

☐ nee
☐ ja, ik eet gemiddeld …… stuks
  ☐ per dag
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

26. Slagroomtaart/ crème au beurre taart (*)

☐ nee
☐ ja, ik eet gemiddeld …… stukken
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

27. Taart: rijst-/fruit-/confituur-/mattetaart (*)

☐ nee
☐ ja, ik eet gemiddeld …… stukken
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

28. Tompoes/ éclair of soes, met gele crème/met slagroom (*)

☐ nee
☐ ja, ik eet gemiddeld …… stuks
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

29. Oliebollen/ appelbeignets/ pannenkoeken (*)

☐ nee
☐ ja, ik eet gemiddeld …… stuks
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

30. Chocolade: melk/ puur/ met noten (*)

☐ nee
☐ ja, ik eet gemiddeld …… repen van …… gram
  ☐ per dag
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

31. Pralines: met crèmevulling/likeurvulling (*)

☐ nee
☐ ja, ik eet gemiddeld …… stuks
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

32. Mars/ Twix/ Snicker/ overige, nl. .....

☐ nee
☐ ja, ik eet gemiddeld …… mini stuks / …… gewone stuks (*)
  ☐ per dag
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

33. Toffees (met/zonder chocolade) / smarties (*)

☐ nee
☐ ja, ik eet gemiddeld …… stuks
  ☐ per dag
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

34. Marsepein

☐ nee
☐ ja, ik eet gemiddeld …… gram
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

VII. Diversen, hartig

Heeft u de afgelopen maand de volgende producten gebruikt ?

35. Pinda's: gezouten/ in chocoladelaagje (*)

☐ nee
☐ ja, ik eet gemiddeld …… handjes/ zakje(s)/ gram (*)
  ☐ per dag
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

36. Borrelnootjes

☐ nee
☐ ja, ik eet gemiddeld …… handjes/ zakje(s)/ gram (*)
  ☐ per dag
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

37. Overige noten, nl. .....

☐ nee
☐ ja, ik eet gemiddeld …… handjes/ zakje(s)/ gram (*)
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

38. Chips, nl. .....

☐ nee
☐ ja, ik eet gemiddeld …… handjes/ zakje(s)/ gram (*)
  ☐ per dag
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

39. Kroepoek

☐ nee
☐ ja, ik eet gemiddeld …… handjes
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

40. Olijven

☐ nee
☐ ja, ik eet gemiddeld …… stuks
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

41. Bitterbal: frituur/ oven/ zelf gefrituurd (*) in .....

☐ nee
☐ ja, ik eet gemiddeld …… stuks
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

42. Frikandel

☐ nee
☐ ja, ik eet gemiddeld …… stuks
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

43. Loempia

☐ nee
☐ ja, ik eet gemiddeld …… stuks
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

44. Saucijzenbroodje

☐ nee
☐ ja, ik eet gemiddeld …… stuks
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

45. Pizza: diepvries/ pizzeria/ zelfgemaakt (*) zo groot als een diepvriespizza/ als bij de pizzeria (*)

☐ nee
☐ ja, ik eet gemiddeld ……. stuks
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

46. Frieten van de frituur

☐ nee
☐ ja, ik eet gemiddeld ……. porties
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

47. Frieten: zelf gefrit. aardappelen/ diepvries frieten/ ovenfrieten (*)

☐ nee
☐ ja, ik eet gemiddeld ……. porties
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

48. Aardappelkroketten: oven/ zelf gefrituurd (*) in .....

☐ nee
☐ ja, ik eet gemiddeld ……. stuks
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

49. Nasi van de Chinees/kant en klaar (*)

☐ nee
☐ ja, ik eet gemiddeld ……. Chinees bakje(s)/ eetlepel(s)
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

50. Bami van de Chinees/kant en klaar (*)

☐ nee
☐ ja, ik eet gemiddeld ……. Chinees bakje(s)/ eetlepel(s)
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

51. Varkenssaté/kippensaté (*)

☐ nee
☐ ja, ik eet gemiddeld …… stuk(s)
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

VIII. Overigen

Heeft u de afgelopen 2 maanden de volgende producten gebruikt?

52. Vers fruit

☐ nee
☐ ja, nl. (soorten) .....
  Ik eet gemiddeld ..... stuks vers fruit
  ☐ per dag
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

53. Fruit in blik of glas

☐ nee
☐ ja, nl. (soorten) .....
  Ik eet gemiddeld ..... schaaltje(s)/ eetlepel(s)/ ½ stuks /schijven (*)
  ☐ per dag
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

54. Vruchtesappen

☐ nee
☐ ja, nl. (soorten) .....
  Ik drink gemiddeld ..... glazen/ liter (*)
  ☐ per dag
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

55. Eieren: gekookt/gebakken (*) in ..... gram ..... (vetsoort) per stuk

- ☐ nee
- ☐ ja, ik eet gemiddeld ..... stuks
  - ☐ per dag
  - ☐ per week
  - ☐ per 2 weken
  - ☐ per 3 weken
  - ☐ per maand

56. Pindakaas

- ☐ nee
- ☐ ja, ik besmeer hiermee gemiddeld ..... sneetje(s) en gebruik ..... gram per snede
  - ☐ per dag
  - ☐ per week
  - ☐ per 2 weken
  - ☐ per 3 weken
  - ☐ per maand

57. Pasta: chocolade-/chocoladehazelnoot (*)

- ☐ nee
- ☐ ja, ik besmeer hiermee gemiddeld ..... sneetje(s) en gebruik ..... gram per snede
  - ☐ per dag
  - ☐ per week
  - ☐ per 2 weken
  - ☐ per 3 weken
  - ☐ per maand

58. Hagelslag of vlokken: melk of puur (*)

- ☐ nee
- ☐ ja, ik bestrooi hiermee gemiddeld .....…... sneetje(s) en gebruik ..... gram per snede
  - ☐ per dag
  - ☐ per week
  - ☐ per 2 weken
  - ☐ per 3 weken
  - ☐ per maand

59. Ontbijtgranen: cornflakes/ muesli/ overige (*) nl. .....

- ☐ nee
- ☐ ja, ik eet gemiddeld .....…..... eetlepel(s)/ tas(sen) (*)
  - ☐ per dag
  - ☐ per week
  - ☐ per 2 weken
  - ☐ per 3 weken
  - ☐ per maand

60. Bier, nl .....

☐ nee
☐ ja, ik drink gemiddeld .....…..... glas(zen)
  ☐ per dag
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

61. Wijn: witte/rode (*)

☐ nee
☐ ja, ik drink gemiddeld .....…..... glas(zen)
  ☐ per dag
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

62. Overige alcoholische dranken, nl. .....

☐ nee
☐ ja, ik drink gemiddeld .....…..... glas(zen)
  ☐ per dag
  ☐ per week
  ☐ per 2 weken
  ☐ per 3 weken
  ☐ per maand

Questionnaire postpartum mood syndromes

SCID-P (version 1.0):

I. Current major depressive episode

1. **Tenminste 5 van de volgende symptomen waren aanwezig tijdens dezelfde twee weken. Tenminste één van de symptomen was ofwel (i) depressieve gemoedstoestand, of (ii) verlies van interesse en plezier**
   - Absent
   - Subtreshold
   - Treshold or true
2. **Was u heel wat minder geïnteresseerd in de meeste dingen waar u vroeger wel plezier in had? (Hoe was dat?). Indien ja, was dat bijna elke dag? Hoelang duurde het? (Twee weken?)**
   - Absent
   - Subtreshold
   - Treshold or true
3. **Gedurende deze periode: vermagerde of verdikte u? (hoeveel? Trachtte u te vermageren?). Indien nee: hoe was uw eetlust. (hoe was uw eetlust in vergelijking met uw gebruikelijke eetlust? Moest u uzelf dwingen om te eten?)**
   - Absent
   - Subtreshold
   - Treshold or true
4. **Gedurende deze periode: hoe sliep u? (moeilijk inslapen, moeilijk doorslapen, frequent wakker worden, teveel slapen?). Hoeveel uren per nacht ivgl met gewoonlijk? (was dat bijna elke nacht?).**
   - Absent
   - Subtreshold
   - Treshold or true
5. **Was u zo zenuwachtig of rusteloos dat u niet kon stilzitten? (was het zo erg dat het anderen opviel? Was dat bijna elke dag?). Indien nee: wat met het tegenovergestelde: langzamer spreken of bewegen dan normaal is voor u. (was het zo erg dat het anderen opviel? Was dat bijna elke dag?)**
   - Absent
   - Subtreshold
   - Treshold or true

6. **Hoe stond het met uw energie? (bijna elke dag voortdurend vermoeid).**
   - Absent or false
   - Subtreshold
   - Treshold or true

7. **Wat vond u van uzelf? (waardeloos? Bijna elke dag?). Indien nee: voelde u zich schuldig over dingen die u gedaan had of niet gedaan had? (Bijna elke dag?)**
   - Absent or false
   - Subtreshold
   - Treshold or true

   Nota: code 1 of 2 if only low self-esteem

8. **Had u moeite met denken of om u te concentreren? (welke zaken verstoorden u? bijna elke dag?). Indien nee: was het moeilijk beslissingen te nemen over alledaagse dingen?**
   - Absent
   - Subtreshold
   - Treshold or true

9. **Ging het zo slecht dat u vaak dacht over de dood of dat u beter dood had kunnen zijn? Indien ja: hebt u iets gedaan om uzelf te verwonden?**
   - Absent
   - Subtreshold or inadequate information
   - Treshold or true

10. **Was u vlak voor dit begon lichamelijk ziek? (wat vond uw dokter ervan?) Gebruikte u drugs of geneesmiddelen? (was er verandering in de hoeveelheid die u gebruikte?). Indien ja voor één van deze vragen: ga na of de depressieve episode werd geïnitieerd en onderhouden door een organische factor.**
    - Organic mood syndrome
    - No organic etiology

II. PAST MAJOR DEPRESSIVE SYNDROME

11. **Indien momenteel niet depressief: Is er ooit een periode geweest waarin u zich bijna elke dag het grootste gedeelte van de tijd depressief of neerslachtig voelde? (hoe was dat?)**
    - Absent
    - Subtreshold
    - Treshold or true

12. **Indien past depressed mood: Gedurende die periode, was u dan heel wat minder geinteresseerd in de meeste dingen of kon u niet meer genieten van de dingen waar u vroeger wel plezier in had?**
    - Absent
    - Treshold or true

Curriculum vitae

The author of this thesis was born on September 19, 1974 in Ghent, Belgium. She started the study of Nutrition and Dietetics at the Ghent University in October 1994. She obtained the degree of Licentiate in Nutrition and Dietetics with great distinction in July 1998. In October 1998, she started the research work described in this thesis at the Department of Endocrinology of Ghent University Hospital, under the promotorship of Prof. Dr. A. Christophe. In June 2002 she finished the doctoral training in Nutrition and Dietetics. As a part of this training she followed the course 'Laboratory animal sciences' and obtained the degree of FELASA category C in June 2001. Since December 2002 she works at the Institute for Public Health in Brussels where she coordinates the Belgian Food Consumption Survey.

The author is the recipient of the Honoured Student Award of the American Oil Chemists' Society in 1998. She attended the European Nutrition Leadership Programme in March 2002 which was subsidised by the European Commission. Recently she received the decennial award "Alumnus with outstanding merit in Nutrition and Dietetics, period 1992 – 2002 from the Faculty of Medicine and Health Sciences.

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Errata

Chapter 6: Fatty acid composition of cholesteryl esters and phospholipids in maternal plasma during pregnancy and at delivery and in cord plasma at birth. (2003). *Lipids, 38, 1-7.*

1/L/8-9: "18:3n-3 decreased" should be replaced by "18:3n-3 increased"

2/R/15: "SPPS" should be replaced by "SPSS"

3/R/5: "extremely" should be replaced by "relatively"

Table 2: The RM ANOVA P-values for 18:1n-7 and 18:1n-9 are switched. It has to be: for 18:1n-7; P=0.54 and for 18:1n-9; P<0.005.

5/L/17: "(7,10)" should be replaced by "(6,7,10)"

5/L/30-31: "Thus this study supports our hypothesis that …" should be replaced by "Thus this study confirms our earlier observation that …"

5/L/38: "(26,27)" should be replaced by "(27,28)".

5/L/40-41: "as has been confirmed by others (26-28)" should be replaced by "as was found by others before (26)."

Chapter 7: Fatty acid composition of phospholipids and cholesteryl esters in maternal serum in the early puerperium. (2003). *Prostaglandins Leukot Essent Fatty Acids, 68, 331-335.*

332/R/45: "SPPS" should be replaced by "SPSS"

334/L/8: "This is in contrast to the previous findings of Al *(Chapter 2, p19).*" Chapter 2, p19 should be added to clarify the reference.

Chapter 10: Oxidative stability of Low Density Lipoproteins and vitamin E levels increase in maternal blood during normal pregnancy. (2001) *Lipids, 36, 361-366.*

Table 4: Expressed as "(mg/dL)" should be replaced by "(µmol/L)"

Cover picture: Dick Hoette "Zwanger 3" 2002.