nutrients

MDPI

Article

Association of *Fatty Acid Desaturase 1* rs174547 Polymorphism with the Composition of Long-Chain Polyunsaturated Fatty Acids in Serum Glycerophospholipids during Pregnancy

Terue Kawabata [1,*], Hideoki Fukuoka [2], Michiru Harada [1], Kumiko Shoji [1], Yoshinori Kubo [1], Chisato Mori [3,4], Kenichi Sakurai [5], Takeshi Ohkubo [6], Kyoichi Oshida [7] and Yuichiro Yamashiro [8]

1 Faculty of Nutrition, Kagawa Nutrition University, 3-9-21 Chiyoda, Sakado 350-0288, Japan
2 Department of Perinatal Mesenchymal Stem Cell Research, School of Medicine, Fukushima Medical University, 1 Hikarigaoka, Fukushima 960-1295, Japan
3 Department of Bioenvironmental Medicine, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan
4 Department of Sustainable Health Science, Center for Preventive Medical Sciences, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan
5 Department of Nutrition and Metabolic Medicine, Center for Preventive Medical Sciences, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan
6 Department of Health Nutrition, Faculty of Human Sciences, Sendai Shirayuri Women's College, 6-1 Honda-Cho, Izumi-ku, Sendai 981-3107, Japan
7 Department of Pediatrics, Juntendo University, 3-1-3 Hongo, Bunkyo-ku, Tokyo 113-8431, Japan
8 Probiotics Research Laboratory, Graduate School of Medicine, Juntendo University, 2-9-8-3F, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
* Correspondence: kawabata@eiyo.ac.jp; Tel.: +81-49-282-3705

Abstract: The increase in fetal requirements of long-chain polyunsaturated fatty acids (LCPUFAs) during pregnancy alters maternal fatty acid metabolism, and therefore, fatty acid desaturase (*FADS*) gene polymorphisms may change blood fatty acid composition or concentration differently during pregnancy. We investigated the relationship between a *FADS1* single-nucleotide polymorphism (SNP) and maternal serum LCPUFA levels in Japanese pregnant women during the first and third trimesters and at delivery. Two hundred and fifty-three pregnant women were included, and fatty acid compositions of glycerophospholipids in serum (weight %) and the *FADS1* SNP rs174547 (T/C) were analyzed. LCPUFAs, including arachidonic acid (ARA) and docosahexaenoic acid (DHA), significantly decreased from the first to the third trimester of pregnancy. Furthermore, DHA significantly decreased from the third trimester of pregnancy to delivery. At all gestational stages, linoleic acid (LA) and α-linolenic acid were significantly higher with the number of minor *FADS1* SNP alleles, whereas γ-linolenic acid and ARA and the ARA/LA ratio were significantly lower. DHA was significantly lower with the number of minor *FADS1* SNP alleles only in the third trimester and at delivery, suggesting that genotype effects become more obvious as pregnancy progresses.

Keywords: docosahexaenoic acids; trimester; pregnancy; *fatty acid desaturase 1*

Citation: Kawabata, T.; Fukuoka, H.; Harada, M.; Shoji, K.; Kubo, Y.; Mori, C.; Sakurai, K.; Ohkubo, T.; Oshida, K.; Yamashiro, Y. Association of *Fatty Acid Desaturase 1* rs174547 Polymorphism with the Composition of Long-Chain Polyunsaturated Fatty Acids in Serum Glycerophospholipids during Pregnancy. *Nutrients* **2023**, *15*, 722. https://doi.org/10.3390/nu15030722

Academic Editor: Hui-lian Zhu

Received: 4 January 2023
Revised: 25 January 2023
Accepted: 28 January 2023
Published: 31 January 2023

1. Introduction

The long-chain polyunsaturated fatty acids (LCPUFAs), arachidonic acid (ARA) (20:4n−6), and docosahexaenoic acid (DHA) (22:6n−3), are endogenously synthesized by Δ-5 and Δ-6 desaturases and elongase 2 and 5 enzymes from precursor PUFAs [1] (Figure 1). Single-nucleotide polymorphisms (SNPs) were identified in fatty acid desaturase genes 1 and 2 (*FADS1* and *FADS2*, respectively), encoding Δ-5 and Δ-6 desaturase, respectively, and the elongation of very long chain fatty acids genes 2 and 5 (*ELOVL2* and *ELOVL5*, respectively), encoding elongase 2 and 5, respectively [2]. The effects of *FADS1*, *FADS2*, and *ELOVL2* SNPs on blood fatty acid composition and concentration have been reported [3–5].

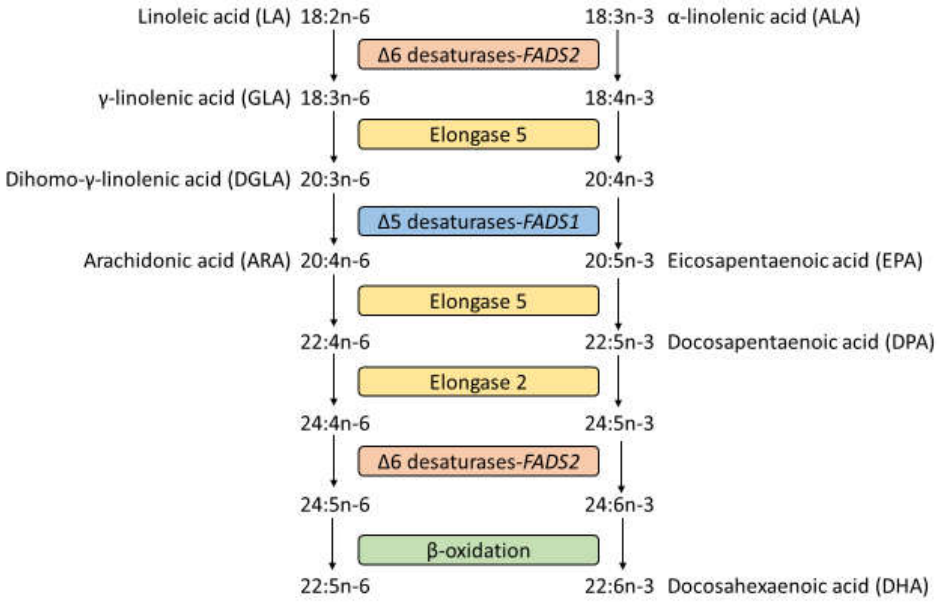

Figure 1. The n−6 and n−3 polyunsaturated fatty acid metabolism pathways.

FADS1 resides on chromosome 11q12–q13.1, and the SNP rs174547 (T/C) located in intron 9 of *FADS1* regulates FADS1 (or Δ-5 desaturase) expression [2,6]. Nakayama et al. reported that the copy number of the minor allele for the SNP rs174547 was significantly associated with increased blood triglyceride levels and decreased high-density lipoprotein cholesterol levels in approximately 20,000 Japanese subjects [6]. We administered ^{13}C-linoleic acid (LA) (18:2n−6) to healthy young and elderly subjects and found that the area under the curve of the concentration of ^{13}C-ARA, the metabolite of LA, was 57% in heterozygotes (TC) and 37% in minor homozygotes (CC) compared with major homozygotes (TT) for rs174547 [7], directly indicating that the endogenous synthetic capacity of LCPUFAs is suppressed in the variant of the gene polymorphism. Furthermore, in an observational study involving an elderly Japanese population, we found that rs174547 C-minor allele carriers had significantly higher LA and lower ARA in erythrocyte membrane and plasma phospholipids (15% and 6% ARA reduction, respectively, per C-allele) [8]. A Dutch study using plasma cholesterol ester and a Korean study using plasma have shown results similar to ours [9,10]. An Australian study showed elevated erythrocyte LA in rs174547 C-allele carriers [11]. For the Japanese, Western, and Asian populations, *FADS1* rs174547 may be an important SNP affecting fatty acid metabolism.

The role of n−3LCPUFAs during gestation is to reduce the risk of preterm birth [12,13] and to contribute to the neural and visual development of the fetus [14]. The n−3LCPUFAs required for these roles are supplied either by direct uptake from the maternal diet or by endogenous synthesis from precursor PUFAs [1]. Therefore, the effect of *FADS* gene polymorphisms that affect the endogenous synthesis of LCPUFAs in women during pregnancy should be elucidated. We previously analyzed the *FADS1* SNP rs174547 in 383 pregnant Japanese women with gestational ages of 24–30 weeks and found higher LA and significantly lower ARA and DHA, converted products, in minor C-allele carriers than those in major T-allele homozygotes [15]. Thus, the rs174547 polymorphism is an important factor in determining blood PUFA composition in Japanese pregnant women.

The fetal LCPUFA requirements increase as pregnancy progresses, and maternal fatty acid metabolism changes markedly [16]. Therefore, the effect of *FADS* gene polymorphisms on blood fatty acid composition or concentration may change throughout the gestational period. Longitudinal gestational changes in the relationships between FADS gene polymorphisms and blood fatty acid compositions in mothers were investigated in a US study (at 14.5 weeks' gestation and birth) [17] and a Canadian study (at 16 and 36 weeks' gestation) [18]. In the US study, minor allele homozygotes for *FADS1* rs174533 had significantly

lower erythrocyte DHA and ARA status at 14.5 weeks' gestation (mean) and significantly lower maternal erythrocyte DHA at birth than major-allele carriers; however, no significant difference in maternal erythrocyte ARA was observed at delivery [17]. In the Canadian study, no difference in the 20 and 22 carbon chain n−3 fatty acids/ALA ratio in erythrocyte ethanolamine phosphoglyceride at 16 weeks' gestation was observed between the major-allele carriers and minor allele homozygotes of *FADS1* rs174553; however, this ratio was significantly lower in minor allele homozygotes at 36 weeks' gestation [18]. Thus, the effect of the *FADS* polymorphism on maternal blood fatty acid status probably differs between the first trimester and late pregnancy or at birth; however, data available to us are very limited and have not led to a unified view.

Therefore, we longitudinally examined how the *FADS1* gene polymorphism rs174547 affects LCPUFAs in maternal serum glycerophospholipids during pregnancy (12 weeks in the first, 32 weeks in the third trimester of pregnancy, and at delivery) in Japanese pregnant women living in areas of adjoining Tokyo.

2. Materials and Methods

2.1. Ethics Approval

The study protocol was approved by the Biomedical Research Ethics Committee of the Graduate School of Medicine, Chiba University (ID: 989, 20 September 2019), the Ethics Review Committee for Human Genome/Gene Analysis Research, Waseda University (ID: 2013-G002 (3), 13 November 2015), the Medical Ethics Committee of Kagawa Nutrition University (ID: 67, 20 July 2016; ID:284-G, 15 July 2020), and was conducted according to the Declaration of Helsinki. All participants gave informed consent for inclusion before participating in the study.

2.2. Study Population

This study was conducted as a part of the Chiba Study of Mother and Child Health (C-MACH) at the Center for Preventive Medical Sciences, Chiba University, and the Research Institute for Science and Engineering, Waseda University [19]. The C-MACH consists of three hospital-based cohorts from the Onodera Ladies Clinic and the Yamaguchi Women's Hospital, in the Chiba Prefecture and the Aiwa Hospital in Saitama Prefecture. The Chiba and Saitama prefectures are adjacent to Tokyo, the largest city in Japan. The participants were recruited between February 2014 and June 2015. The recruitment population consisted of pregnant women examined at <13 weeks of gestation in the three hospitals. The study population included children born to women who consented to participate. If a stillbirth occurred, the woman's participation in this study was terminated. Consent to participate in C-MACH was obtained from 433 women. Twenty-five women withdrew from the study after providing informed consent, resulting in a final cohort of 408 women [19]. The Onodera Ladies Clinic and Aiwa Hospital cohorts of the C-MACH were used for this study. Only the participants whose sera were collected at the time of recruitment and those who completed the maternal *FADS1* SNP analysis were included in the study, resulting in a final cohort of 242 pregnant women.

2.3. Maternal and Infant Information

Self-administered lifestyle questionnaires for the mothers were administered at the first (12 weeks) and third (32 weeks) trimesters of pregnancy. Maternal age and parity and non-pregnant height and weight were obtained from the early pregnancy questionnaire. Body mass index (BMI) was calculated from height and weight. The smoking status was obtained from the questionnaires administered during the first and third trimesters of pregnancy. Information on the sex of the child and gestational age was obtained from the medical records at delivery.

2.4. Dietary Survey

The mothers completed a brief-type self-administered diet history questionnaire (BDHQ) at 12- and 32-weeks' gestation. The validity of the BDHQ was confirmed previously using the 16-day weighted dietary record as the gold standard [20]. In the data analysis, various fatty acid intakes were used in the analysis as energy ratios. The exclusion criteria for reporting errors on the BDHQ were values < 0.5 times the energy requirement of Physical Activity Level I or >1.5 times the energy requirement of Physical Activity Level III for each subject.

2.5. Fatty Acid Analysis in Serum Glycerophospholipids

Blood samples were collected in the first and third trimesters of pregnancy (12- and 32-weeks' gestation, respectively) and at delivery. After collection, blood was separated into serum and clots, and the serum was frozen at −80 °C. The serum stored at the Center for Preventive Medical Sciences, Chiba University, was then transferred to Kagawa Nutrition University using a cold storage box containing dry ice and continued to be frozen at −80 °C until fatty acid analysis. Serum fatty acid analysis was performed following the method of Glaser et al. [21]. Proteins were removed from the serum using methanol, followed by methyl ester exchange with sodium methoxide solution to produce fatty acid methyl esters in glycerophospholipids, and then, fatty acid analysis was performed by gas chromatography. The details are described in a previous report [22]. The ratio of the peak area of each fatty acid to the total peak area (weight %) was calculated and used as the value of fatty acids in serum glycerophospholipids.

2.6. Genotyping

For DNA extraction, we used concentrated blood. Concentrated blood, which is the remaining plasma dispensed, was stored at −80 °C at Chiba University and transferred to Kagawa Nutrition University. A fully automated nucleic acid extraction system (MagLEAD 12gC; Precision System Science, Matsudo-shi, Chiba, Japan) was used with the MagDEA Dx SV reagent (Precision System Science Co., Ltd., Matsudo-shi, Chiba, Japan). The rs174547 (an intronic T/C polymorphism of *FADS1*) genotypes of all participants were determined using a TaqMan probe with the 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) at Kagawa Nutrition University.

2.7. Statistical Analysis

The data were checked for normality using normal distribution point plots. Variables determined to have non-normal distribution were transformed into the natural logarithm and subsequently used in the analysis. The agreement of genotype frequencies with Hardy–Weinberg equilibrium expectations was tested by the Chi-square test. Comparisons of fatty acid composition in serum glycerophospholipids according to stages of pregnancy were performed using repeated one-way analysis of variance, followed by the Tukey–Kramer honestly significant difference test for multiple comparisons between groups. To explore the association between fatty acid composition in maternal serum glycerophospholipids and genetic polymorphisms, multiple regression analysis was performed using the fatty acid composition as the objective variable and rs174547 as the explanatory variable, according to the stages of pregnancy. Genotypes were pre-transformed as an additive model according to the number of minor alleles (0 for major homozygotes and 1 for carriers of at least one minor allele). The corresponding fatty acid intake, mother's age, mother's non-pregnant BMI, and smoking were included in the analysis as covariates. Data on fatty acid intake and smoking status in the first trimester of pregnancy were used to analyze the fatty acid composition in the first trimester of pregnancy, and data on fatty acid intake and smoking status in the third trimester of pregnancy were used to analyze the fatty acid composition in the third trimester of pregnancy and at delivery.

All statistical analyses were performed using the JMP (SAS Institute Inc., Cary, NC, USA). Differences with p-values of less than 0.05 were considered statistically significant.

3. Results

3.1. Participant Characteristics

The characteristics of the subjects are shown in Table 1. For mothers, information on age at 12 weeks' gestation, non-pregnant body size, parity, smoking status, and the *FADS1* genotype were listed, and for children, the gestational period, sex, and *FADS1* genotype were listed. Both genotypes for mother and child were in Hardy–Weinberg equilibrium (all $p > 0.45$).

Table 1. Subject characteristics.

Variables	n	(%)	Median	(25th–75th)
Mothers				
Age at participation				
<30 years old	63	(27.9%)		
30–39 years old	148	(65.5%)		
≧40 years old	15	(6.6%)		
Missing	16	-		
Height, cm	226		159	(155–163)
Prepregnancy weight, kg	226		52	(48–58)
Prepregnancy body mass index (kg/cm^2)	226		20.7	(19.1–22.9)
Parity				
0	91	(40.8%)		
1	96	(43.1%)		
≧2	36	(16.1%)		
Missing	19	-		
Smoking status in the first trimester of pregnancy				
Smoker or quit	48	(21.3%)		
Never	177	(78.7%)		
Missing	17	-		
Smoking status in the third trimester of pregnancy				
Smoker or quit	52	(22.7%)		
Never	177	(77.3%)		
Missing	13	-		
FADS1; rs174547 genotype				
TT	97	(40.1%)		
TC	108	(44.6%)		
CC	37	(15.3%)		
Infants				
Gestational period, day	226		278	(271–284)
Sex				
Male	117	(52.2%)		
Female	107	(47.8%)		
Missing	18			
FADS1; rs174547 genotype				
TT	80	(35.2%)		
TC	113	(49.8%)		
CC	34	(15.0%)		
Missing	15	-		

-, Percent is not calculated for missing.

3.2. Maternal Serum Fatty Acid Changes during Gestation

Fatty acid compositions of maternal plasma glycerophospholipids (weight % of each fatty acid to the total fatty acids) according to gestational age are shown in Table 2. From the first trimester to the third trimester of pregnancy, saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), dihomo-γ-linolenic acid (DGLA) (20:3n−6), and α-linolenic acid (ALA) (18:3n−3) were significantly elevated, and PUFA, γ-linolenic acid (GLA) (18:3n−6), ARA, eicosapentaenoic acid (EPA) (20:5n−3), docosapentaenoic acid (DPA) (22:5n−3), DHA, ARA/LA ratio, and ARA/DGLA ratio were significantly decreased. Furthermore, from the third trimester of the pregnancy to delivery, DPA and DHA significantly decreased.

Table 2. Fatty acid compositions of maternal plasma glycerophospholipids according to gestational age [1].

	The 1st Trimester		The 3rd Trimester		At Delivery		p [2]
	n = 242		n = 237		n = 213		
SFA (%)	40.14	(39.01–41.20) [a]	41.78	(40.28–42.76) [b]	41.89	(40.88–42.82) [b]	<0.001
MUFA (%)	18.18	(16.55–19.50) [a]	18.48	(17.17–19.82) [b]	18.60	(17.51–19.96) [b]	0.001
PUFA (%)	41.90	(40.6–43.32) [a]	40.02	(38.75–41.06) [b]	39.46	(38.33–40.80) [b]	<0.001
n−6 (%)							
18:2n−6 (LA)	22.91	(21.01–24.74)	22.99	(21.16–24.60)	22.93	(21.26–24.85)	0.943
18:3n−6 (GLA) [3]	0.07	(0.05–0.11) [a]	0.05	(0.03–0.07) [b]	0.05	(0.04–0.08) [c]	<0.001
20:3n−6 (DGLA)	2.13	(1.74–2.55) [a]	2.40	(2.12–2.75) [b]	2.44	(2.08–2.74) [b]	<0.001
20:4n−6 (ARA)	8.76	(7.73–9.72) [a]	7.07	(6.32–7.85) [b]	6.92	(6.14–7.84) [b]	<0.001
n−3 (%)							
18:3n−3 (ALA) [3]	0.51	(0.40–0.64) [a]	0.58	(0.47–0.70) [b]	0.55	(0.47–0.64) [b]	<0.001
20:5n−3 (EPA) [3]	0.72	(0.52–1.08) [a]	0.58	(0.38–0.95) [b]	0.65	(0.39–1.00) [b]	<0.001
22:5n−3 (DPA)	0.63	(0.54–0.76) [a]	0.52	(0.42–0.62) [b]	0.47	(0.37–0.56) [c]	<0.001
22:6n−3 (DHA)	4.84	(4.16–5.30) [a]	4.47	(3.75–5.23) [b]	4.28	(3.50–5.01) [c]	<0.001
Ratios							
ARA/LA ratio	0.37	(0.32–0.45) [a]	0.31	(0.27–0.37) [b]	0.30	(0.26–0.36) [b]	<0.001
ARA/DGLA ratio [3]	4.10	(3.31–4.93) [a]	2.91	(2.56–3.38) [b]	2.91	(2.57–3.30) [b]	<0.001

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. [1] Values are presented as median (25th%–75th%). [2] Comparisons of fatty acid composition in serum glycerophospholipids by stages of pregnancy were performed using repeated one-way analysis of variance, followed by the Tukey–Kramer HSD test for multiple comparisons between groups. Values within a row with unlike superscript letters were significantly different ($p < 0.05$). [3] Data were converted to natural logarithms and then used for statistical analysis.

3.3. Associations between Serum Glycerophospholipid Fatty Acids and rs174547 Polymorphism

Table 3 shows the results of the multiple regression analysis with maternal serum glycerophospholipid fatty acid composition (%) as the objective variable and maternal rs174547 polymorphism as the explanatory variable. In all stages of pregnancy, LA and ALA were significantly positively associated with the number of minor rs174547 polymorphism alleles, whereas GLA and ARA and the ARA/LA ratio were significantly negatively associated. The standard beta values (Std β) for LA, ARA, and the ARA/LA ratio were particularly high (all >0.3 in absolute value; $p < 0.001$), indicating a strong association with the rs174547 genotype. DHA and the ARA/DGLA ratio showed no association in the first trimester of pregnancy; however, a significant negative association with the number of minor rs174547 polymorphism alleles was observed in the third trimester of pregnancy and at delivery. DGLA and EPA showed significant negative correlations or negative

correlation trends with the number of alleles of the minor rs174547 polymorphism at all gestational stages.

Table 3. Associations of maternal FADS1 gene polymorphisms with fatty acid compositions of maternal serum glycerophospholipids [1].

Objective Variable [2]	The 1st Trimester			The 3rd Trimester			At Delivery		
	n = 185			n = 180			n = 165		
	B	Std β	*p*	B	Std β	*p*	B	Std β	*p*
SFA	−0.216	−0.090	0.233	−0.050	−0.019	0.801	−0.342	−0.165	0.036
MUFA	0.369	0.122	0.091	0.333	0.132	0.079	0.566	0.215	0.005
PUFA	−0.159	−0.052	0.469	−0.249	−0.094	0.207	−0.186	−0.064	0.410
				n−6					
18:2n−6 (LA)	1.316	0.351	<0.001	1.179	0.348	<0.001	1.289	0.395	<0.001
18:3n−6 (GLA)	−0.449	−0.466	<0.001	−0.232	−0.271	<0.001	−0.326	−0.360	<0.001
20:3n−6 (DGLA)	−0.176	−0.216	0.003	−0.144	−0.212	0.004	−0.098	−0.154	0.051
20:4n−6 (ARA)	−1.089	−0.491	<0.001	−0.953	−0.636	<0.001	−0.926	−0.562	<0.001
				n−3					
18:3n−3 (ALA)	0.116	0.223	0.003	0.098	0.231	0.002	0.036	0.157	0.047
20:5n−3 (EPA)	−0.159	−0.222	0.002	−0.127	−0.142	0.052	−0.133	−0.141	0.063
22:5n−3 (DPA)	−0.015	−0.061	0.414	−0.001	−0.003	0.970	0.005	0.025	0.745
22:6n−3 (DHA)	−0.108	−0.074	0.321	−0.278	−0.193	0.008	−0.307	−0.208	0.006
				Ratios					
ARA/LA ratio	−0.069	−0.498	<0.001	−0.059	−0.626	<0.001	−0.057	−0.581	<0.001
ARA/DGLA ratio	−0.116	−0.063	0.374	−0.204	−0.219	0.003	−0.228	−0.254	0.001

[1] Statistical analysis was performed with multiple regression analysis using fatty acid composition as the objective variable and rs174547 polymorphism as the explanatory variable. The covariates were corresponding fatty acid intake, mother's age, mother's non-pregnant BMI, and smoking. [2] The following variables were used in the analysis after natural log transformation: ALA, EPA, and GLA at the 1st trimester; ALA, EPA, and GLA at the 3rd trimester; and EPA and GLA at delivery.

4. Discussion

This study evaluated the association between fatty acid Δ5 desaturase gene polymorphism (*FADS1* rs174547) and fatty acid composition in maternal serum glycerophospholipids (weight % of each fatty acid to the total fatty acids) in the first and third trimester, and at delivery. As a result, minor allele carriers of the rs174547 polymorphism had significantly higher maternal serum n−6 LA and lower ARA than major homozygotes, throughout pregnancy. That is, minor allele carriers have elevated precursor fatty acids and lower product fatty acids, which are consistent with the previous observational studies, indicating that fatty acid desaturation is suppressed due to the decreased expression or activity of Δ5 desaturase of minor allele [8,15,23].

Several studies have examined the association between maternal *FADS* polymorphisms and blood PUFA composition or concentration during pregnancy [17,18,24–31]. Among them, one study examined the same *FADS1* rs174547 polymorphism as our study and reported the plasma phospholipid fatty acid composition in 180 pregnant women at 24 weeks' gestation who participated in a Spanish birth cohort [24]. The results showed that the rs174547 C-minor allele count tended to be negatively correlated with the ARA/DGLA ratio, an index of Δ5 desaturase activity; however, the relationship was not significant, and the authors did not find a significant effect of the aforementioned SNP on pregnant women. In our study, we found a significant negative association between rs174547 C-minor allele counts and the ARA/DGLA ratio in late pregnancy (32 weeks) and at delivery; however, no

association was observed in early pregnancy (12 weeks). Although the Spanish study and our study are not definitive because of the different timings of the analysis of the fatty acid composition in blood, our results agree with those of the Spanish study, demonstrating no association between the rs174547 polymorphism and the ARA/DGLA ratio at the relatively early stage of pregnancy.

In our study, the ARA/LA ratio showed a stronger correlation with the rs174547 genotype than the ARA/DGLA ratio. The Δ5 desaturase encoded by the *FADS1* gene converts DGLA to ARA. However, in this study, serum LA was higher, and fatty acids downstream from GLA were consistently lower in minor allele carriers than in major-allele homozygotes. This suggests that the metabolism of LA to GLA, which occurs upstream of the Δ5 desaturase point of action, is already suppressed. Similar results have been observed in our previous studies [7,8]. The rs174547 polymorphism is in linkage disequilibrium with many other *FADS* polymorphisms, including *FADS2* polymorphism [6,32]. Wang et al. also conducted a meta-analysis of the association between the rs174547 polymorphism and blood PUFAs and found that minor C-allele carriers were associated not only with reduced D5D activity index (ARA/DGLA) but also with reduced Δ6 desaturase activity index (GLA/LA) [33]. Therefore, regarding the effects of the *FADS1*/rs174547 SNP on plasma fatty acid composition, we believe that the ARA/LA ratio, which is an indicator of the activity of the entire fatty acid metabolic pathway, would more clearly confirm the association of plasma fatty acid composition with the *FADS1*/rs174547 SNP.

Several studies have reported a decrease in maternal blood DHA composition from approximately 18 weeks' gestation to delivery [34–37]. We also found that the DHA composition in maternal erythrocytes during pregnancy was significantly lower at delivery than at 27 weeks' gestation [38]. In this study, serum n−3LCPUFA composition, including DHA, also decreased from the first to delivery. In general, this change in maternal blood DHA composition in late pregnancy has been attributed to increased placental transfer of DHA from mother to fetus [16,34,39]. As a result, the demand for DHA in the mother increased from late pregnancy to delivery. The metabolism of ALA to EPA and DHA is regulated by negative feedback with the products of n−3LCPUFA [40], endogenous synthesis of DHA may be enhanced in late pregnancy and beyond, when demand for n−3LCPUFA increases. Notably, in this study, the association between *FADS1* polymorphisms and maternal serum DHA composition was found only in the third trimester and at delivery. We hypothesize that increased maternal demand for DHA may have increased endogenous synthesis of EPA and DHA from ALA, which would have resulted in more pronounced effects of genetic polymorphisms later in the pregnancy and beyond.

Another factor may contribute to the change in the relationship between *FADS1* polymorphisms and maternal serum DHA composition as pregnancy progresses. The total plasma lipid DHA composition is higher in women than in men [41], and the endogenous conversion of ALA to DHA has been reported to be greater in women than in men of the same age [42,43]. Furthermore, women taking oral contraceptives had approximately 10% higher DHA concentrations than those not taking them, and oral ethinyl estradiol, but not transdermal 17β-estradiol, increased DHA by 42% [44]. Oestrogen presumably upregulates DHA synthesis from ALA. Thus, it is more likely that the effects of SNPs affecting desaturase activity will be detectable after the second half of the pregnancy when oestrogen secretion increases.

In this study, we analyzed the association between fatty acid composition and genetic polymorphisms from as early as 12 weeks until delivery and examined the association between the fatty acid composition in the mother's serum glycerophospholipids and genetic polymorphisms, including dietary fatty acid intake. However, this study has several limitations. In accordance with the previous study, we entered as covariates in multiple regression analysis only the fatty acid intake corresponding to each of the fatty acids as the objective variable, with the aim of excluding the effect of diet [45]. Because fatty acids are interrelated, it cannot be ruled out that fatty acids other than the corresponding fatty acid intake and blood fatty acids are related and that this may be a confounding factor.

Furthermore, this is a hospital-based cohort study conducted in an urban setting within Japan, which limits the generalization of the results to areas with other different lifestyles.

Another limitation of this study was the evaluation of fatty acids in serum glycerophospholipids using relative composition (weight % of each fatty acid to the total fatty acids), not absolute concentration (mmol/mL or mg/mL). The plasma pool volume of pregnant women physiologically increases as pregnancy progresses. A meta-analysis showed that plasma volume increased beginning in the first week of pregnancy, with the steepest increase in the second trimester. Furthermore, plasma volume continued to increase during the third trimester. The pooled maximum increase in plasma volume was 1.13 L, resulting in a 45.6% increase in pregnant women compared with nonpregnant women [46]. Such changes in the plasma pool may significantly impact the interpretation of the results. For example, if the absolute amount of most fatty acids increases with increasing plasma pool volume, but the absolute amount of some fatty acids remains unchanged, the relative composition of these fatty acids will decrease. In other words, if there is an increase in the plasma pool volume during pregnancy, but the absolute amount of LCPUFA does not change while the absolute amount of other fatty acids increases, the relative composition of LCPUFA will decrease. Therefore, the absolute amounts may be misinterpreted as decreasing. The results of our study show a decrease in most LCPUFA compositions and fatty acid ratios (ARA/LA and ARA/DGLA) during pregnancy. However, this does not imply that the absolute amount of LCPUFA decreased. At this stage, we cannot discuss changes in absolute amounts of LCPUFA because we have not examined absolute concentrations or plasma-pooled amounts of fatty acids during pregnancy. Stark et al. demonstrated that fatty acid data vary depending on the relative composition, absolute concentration, and total amount in the plasma pool [45]. Therefore, fatty acid status during pregnancy is best reported in both relative composition and absolute concentration for proper interpretation. The results of this study are based on relative compositions. Thus, the use of this data is limited.

5. Conclusions

LCPUFAs, including ARA, DPA, and DHA, significantly decreased from the first to the third trimester of pregnancy. Furthermore, DPA and DHA significantly decreased from the third trimester of pregnancy to delivery. The association between n−6 PUFAs and the rs174547 genotype was strong at all gestational stages. DHA, an n−3 fatty acid, was associated with the rs174547 genotype only in the third trimester and at delivery, suggesting that genotype effects become more obvious as pregnancy progresses.

Author Contributions: Conceptualization, T.K., H.F. and Y.Y.; methodology, T.K., M.H. and K.S. (Kumiko Shoji); validation, T.K., M.H. and K.S. (Kumiko Shoji); formal analysis, T.K., M.H., K.S. (Kumiko Shoji) and H.F.; investigation, T.K., H.F., C.M. and K.S. (Kenichi Sakurai); resources, H.F, C.M. and K.S. (Kenichi Sakurai); data curation, H.F., C.M. and K.S. (Kenichi Sakurai); writing—original draft preparation, T.K.; writing—review and editing, H.F., K.S. (Kenichi Sakurai), C.M., K.S. (Kumiko Shoji), Y.K., T.O., K.O. and Y.Y.; visualization, T.K.; supervision, H.F. and Y.Y.; project administration, H.F. and Y.Y.; funding acquisition, K.O. and Y.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the Biomedical Research Ethics Committee of the Graduate School of Medicine, Chiba University (ID: 989, 20 September 2019); the Ethics Review Committee for Human Genome/Gene Analysis Research, Waseda University (ID: 2013-G002 (3), 13 November 2015); and the Medical Ethics Committee of Kagawa Nutrition University (ID: 67, 20 July 2016; ID:284-G, 15 July 2020).

Informed Consent Statement: Informed consent was obtained from all participants involved in the study.

Data Availability Statement: The data sets used and analyzed in this study are available from the C-MACH research committee upon reasonable request.

Acknowledgments: We thank the participants in the study and are grateful for the cooperation and support of C-MACH members.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ratnayake, W.M.; Galli, C. Fat and fatty acid terminology, methods of analysis and fat digestion and metabolism: A background review paper. *Ann. Nutr. Metab.* **2009**, *55*, 8–43. [CrossRef] [PubMed]
2. Schaeffer, L.; Gohlke, H.; Müller, M.; Heid, I.M.; Palmer, L.J.; Kompauer, I.; Demmelmair, H.; Illig, T.; Koletzko, B.; Heinrich, J. Common genetic variants of the FADS1 FADS2 gene cluster and their reconstructed haplotypes are associated with the fatty acid composition in phospholipids. *Hum. Mol. Genet.* **2006**, *15*, 1745–1756. [CrossRef] [PubMed]
3. Koletzko, B.; Reischl, E.; Tanjung, C.; Gonzalez-Casanova, I.; Ramakrishnan, U.; Meldrum, S.; Simmer, K.; Heinrich, J.; Demmelmair, H. FADS1 and FADS2 polymorphisms modulate fatty acid metabolism and dietary impact on health. *Annu. Rev. Nutr.* **2019**, *39*, 21–44. [CrossRef] [PubMed]
4. Alsaleh, A.; Maniou, Z.; Lewis, F.J.; Hall, W.L.; Sanders, T.A.; O'Dell, S.D. ELOVL2 gene polymorphisms are associated with increases in plasma eicosapentaenoic and docosahexaenoic acid proportions after fish oil supplement. *Genes Nutr.* **2014**, *9*, 362. [CrossRef]
5. Lemaitre, R.N.; Tanaka, T.; Tang, W.; Manichaikul, A.; Foy, M.; Kabagambe, E.K.; Nettleton, J.A.; King, I.B.; Weng, L.C.; Bhattacharya, S.; et al. Genetic loci associated with plasma phospholipid n−3 fatty acids: A meta-analysis of genome-wide association studies from the CHARGE Consortium. *PLoS Genet.* **2011**, *7*, e1002193. [CrossRef]
6. Nakayama, K.; Bayasgalan, T.; Tazoe, F.; Yanagisawa, Y.; Gotoh, T.; Yamanaka, K.; Ogawa, A.; Munkhtulga, L.; Chimedregze, U.; Kagawa, Y.; et al. A single nucleotide polymorphism in the FADS1/FADS2 gene is associated with plasma lipid profiles in two genetically similar Asian ethnic groups with distinctive differences in lifestyle. *Hum. Genet* **2010**, *127*, 685–690. [CrossRef]
7. Sasaki, H.; Sueyasu, T.; Tokuda, H.; Ito, M.; Kaneda, Y.; Rogi, T.; Kawashima, H.; Horiguchi, S.; Kawabata, T.; Shibata, H. Aging and FADS1 polymorphisms decrease the biosynthetic capacity of long-chain PUFAs: A human trial using [U-^{13}C]linoleic acid. *Prostaglandins Leukot. Essent. Fat Acids* **2019**, *148*, 3. [CrossRef]
8. Horiguchi, S.; Nakayama, K.; Iwamoto, S.; Ishijima, A.; Minezaki, T.; Baba, M.; Kontai, Y.; Horikawa, C.; Kawashima, H.; Shibata, H.; et al. Associations between a fatty acid desaturase gene polymorphism and blood arachidonic acid compositions in Japanese elderly. *Prostaglandins Leukot. Essent. Fat Acids* **2016**, *105*, 9–14. [CrossRef]
9. Lu, Y.; Vaarhorst, A.; Merry, A.H.; Dollé, M.E.; Hovenier, R.; Imholz, S.; Schouten, L.J.; Heijmans, B.T.; Müller, M.; Slagboom, P.E.; et al. Markers of endogenous desaturase activity and risk of coronary heart disease in the CAREMA cohort study. *PLoS ONE* **2012**, *7*, e41681. [CrossRef]
10. Kim, M.; Yoo, H.J.; Lee, A.; Jeong, S.; Lee, J.H. Associations among FADS1 rs174547, eicosapentaenoic acid/arachidonic acid ratio, and arterial stiffness in overweight subjects. *Prostaglandins Leukot. Essent. Fat Acids* **2018**, *130*, 11–18. [CrossRef]
11. Cribb, L.; Murphy, J.; Froud, A.; Oliver, G.; Bousman, C.A.; Ng, C.H.; Sarris, J. Erythrocyte polyunsaturated fatty acid composition is associated with depression and FADS genotype in Caucasians. *Nutr. Neurosci.* **2018**, *21*, 589–601. [CrossRef] [PubMed]
12. Middleton, P.; Gomersall, J.C.; Gould, J.F.; Shepherd, E.; Olsen, S.F.; Makrides, M. Omega-3 fatty acid addition during pregnancy. *Cochrane Database Syst. Rev.* **2018**, *11*, CD003402. [CrossRef]
13. Ciesielski, T.H.; Bartlett, J.; Williams, S.M. Omega-3 polyunsaturated fatty acid intake norms and preterm birth rate: A cross-sectional analysis of 184 countries. *BMJ Open* **2019**, *9*, e027249. [CrossRef] [PubMed]
14. Mun, J.G.; Legette, L.L.; Ikonte, C.J.; Mitmesser, S.H. Choline and DHA in Maternal and Infant Nutrition: Synergistic Implications in Brain and Eye Health. *Nutrients* **2019**, *11*, 1125. [CrossRef] [PubMed]
15. Nita, R.; Kawabata, T.; Kagawa, Y.; Nakayama, K.; Yanagisawa, Y.; Iwamoto, S.; Kimura, F.; Miyazawa, T.; Tatsuta, N.; Arima, T.; et al. Associations of erythrocyte fatty acid compositions with FADS1 gene polymorphism in Japanese mothers and infants. *Prostaglandins Leukot. Essent. Fat Acids* **2020**, *152*, 102031. [CrossRef]
16. Haggarty, P. Effect of placental function on fatty acid requirements during pregnancy. *Eur. J. Clin. Nutr.* **2004**, *58*, 1559–1570. [CrossRef]
17. Scholtz, S.A.; Kerling, E.H.; Shaddy, D.J.; Li, S.; Thodosoff, J.M.; Colombo, J.; Carlson, S.E. Docosahexaenoic acid (DHA) supplementation in pregnancy differentially modulates arachidonic acid and DHA status across FADS genotypes in pregnancy. *Prostaglandins Leukot. Essent Fat. Acids* **2015**, *94*, 29–33. [CrossRef]
18. Xie, L.; Innis, S.M. Genetic variants of the FADS1 FADS2 gene cluster are associated with altered (n−6) and (n−3) essential fatty acids in plasma and erythrocyte phospholipids in women during pregnancy and in breast milk during lactation. *J. Nutr.* **2008**, *138*, 2222–2228. [CrossRef]
19. Sakurai, K.; Miyaso, H.; Eguchi, A.; Matsuno, Y.; Yamamoto, M.; Todaka, E.; Fukuoka, H.; Hata, A.; Mori, C.; On behalf of the Chiba study of Mother and Children's Health group. Chiba study of Mother and Children's Health (C-MACH): Cohort study with omics analyses. *BMJ Open* **2016**, *6*, e010531. [CrossRef]

20. Kobayashi, S.; Honda, S.; Murakami, K.; Sasaki, S.; Okubo, H.; Hirota, N.; Notsu, A.; Fukui, M.; Date, C. Both comprehensive and brief self-administered diet history questionnaires satisfactorily rank nutrient intakes in Japanese adults. *J. Epidemiol.* **2012**, *22*, 151–159. [CrossRef]
21. Glaser, C.; Demmelmair, H.; Koletzko, B. High-throughput analysis of fatty acid composition of plasma glycerophospholipids. *J. Lipid Res.* **2010**, *51*, 216–221. [CrossRef] [PubMed]
22. Matsumoto, A.; Kawabata, T.; Kagawa, Y.; Shoji, K.; Kimura, F.; Miyazawa, T.; Tatsuta, N.; Arima, T.; Yaegashi, N.; Nakai, K. Associations of umbilical cord fatty acid profiles and desaturase enzyme indices with birth weight for gestational age in Japanese infants. *Prostaglandins Leukot. Essent. Fat Acids* **2021**, *165*, 102233. [CrossRef] [PubMed]
23. Niwa, S.; Kawabata, T.; Shoji, K.; Ogata, H.; Kagawa, Y.; Nakayama, K.; Yanagisawa, Y.; Iwamoto, S.; Tatsuta, N.; Asato, K.; et al. Investigation of maternal diet and FADS1 polymorphism associated with long-chain polyunsaturated fatty acid compositions in human milk. *Nutrients* **2022**, *14*, 2160. [CrossRef] [PubMed]
24. de la Garza Puentes, A.; Montes Goyanes, R.; Chisaguano Tonato, A.M.; Torres-Espínola, F.J.; Arias García, M.; de Almeida, L.; Bonilla Aguirre, M.; Guerendiain, M.; Castellote Bargalló, A.I.; Segura Moreno, M.; et al. Association of maternal weight with FADS and ELOVL genetic variants and fatty acid levels- The PREOBE follow-up. *PLoS ONE* **2017**, *12*, e0179135. [CrossRef] [PubMed]
25. Koletzko, B.; Lattka, E.; Zeilinger, S.; Illig, T.; Steer, C. Genetic variants of the fatty acid desaturase gene cluster predict amounts of red blood cell docosahexaenoic and other polyunsaturated fatty acids in pregnant women: Findings from the Avon Longitudinal Study of Parents and Children. *Am. J. Clin. Nutr.* **2011**, *93*, 211–219. [CrossRef]
26. Gonzalez-Casanova, I.; Rzehak, P.; Stein, A.D.; Garcia Feregrino, R.; Rivera Dommarco, J.A.; Barraza-Villarreal, A.; Demmelmair, H.; Romieu, I.; Villalpando, S.; Martorell, R.; et al. Maternal single nucleotide polymorphisms in the fatty acid desaturase 1 and 2 coding regions modify the impact of prenatal supplementation with DHA on birth weight. *Am. J. Clin. Nutr.* **2016**, *103*, 1171–1178. [CrossRef]
27. Carvalho, G.Q.; Pereira-Santos, M.; Marcon, L.D.; Louro, I.D.; Peluzio, M.C.G.; Santos, D.B. Maternal polymorphisms in the FADS1 and FADS2 genes modify the association between PUFA ingestion and plasma concentrations of omega-3 polyunsaturated fatty acids. *Prostaglandins Leukot. Essent. Fat Acids* **2019**, *150*, 38–46. [CrossRef]
28. Moltó-Puigmartí, C.; Plat, J.; Mensink, R.P.; Müller, A.; Jansen, E.; Zeegers, M.P.; Thijs, C. FADS1 FADS2 gene variants modify the association between fish intake and the docosahexaenoic acid proportions in human milk. *Am. J. Clin. Nutr.* **2010**, *91*, 1368–1376. [CrossRef]
29. Steer, C.D.; Hibbeln, J.R.; Golding, J.; Davey Smith, G. Polyunsaturated fatty acid levels in blood during pregnancy, at birth and at 7 years: Their associations with two common FADS2 polymorphisms. *Hum. Mol. Genet* **2012**, *21*, 1504–1512. [CrossRef]
30. Santana, J.D.M.; Pereira, M.; Carvalho, G.Q.; Gouveia Peluzio, M.D.C.; Drumond Louro, I.; Santos, D.B.D.; Oliveira, A.M. FADS1 and FADS2 Gene Polymorphisms Modulate the Relationship of Omega-3 and Omega-6 Fatty Acid Plasma Concentrations in Gestational Weight Gain: A NISAMI Cohort Study. *Nutrients* **2022**, *14*, 1056. [CrossRef]
31. Yeates, A.J.; Love, T.M.; Engström, K.; Mulhern, M.S.; McSorley, E.M.; Grzesik, K.; Alhamdow, A.; Wahlberg, K.; Thurston, S.W.; Davidson, P.W.; et al. Genetic variation in FADS genes is associated with maternal long-chain PUFA status but not with cognitive development of infants in a high fish-eating observational study. *Prostaglandins Leukot. Essent. Fat Acids* **2015**, *102–103*, 13–20. [CrossRef] [PubMed]
32. Huang, M.C.; Chang, W.T.; Chang, H.Y.; Chung, H.F.; Chen, F.P.; Huang, Y.F.; Hsu, C.C.; Hwang, S.J. FADS Gene Polymorphisms, Fatty Acid Desaturase Activities, and HDL-C in Type 2 Diabetes. *Int. J. Environ. Res. Public Health* **2017**, *14*, 572. [CrossRef] [PubMed]
33. Wang, Y.; Tang, Y.; Ji, Y.; Xu, W.; Ullah, N.; Yu, H.; Wu, Y.; Xie, L. Association between FADS1 rs174547 and levels of long-chain PUFA: A meta-analysis. *Br. J. Nutr.* **2021**, *126*, 1121–1129. [CrossRef] [PubMed]
34. Al, M.D.; van Houwelingen, A.C.; Kester, A.D.; Hasaart, T.H.; de Jong, A.E.; Hornstra, G. Maternal essential fatty acid patterns during normal pregnancy and their relationship to the neonatal essential fatty acid status. *Br. J. Nutr.* **1995**, *74*, 55–68. [CrossRef] [PubMed]
35. Zhou, Y.B.; Li, H.T.; Trasande, L.; Wang, L.L.; Zhang, Y.L.; Si, K.Y.; Bai, M.X.; Liu, J.M. A Correlation Study of DHA Intake Estimated by a FFQ and Concentrations in Plasma and Erythrocytes in Mid- and Late Pregnancy. *Nutrients* **2017**, *9*, 1256. [CrossRef] [PubMed]
36. Montgomery, C.; Speake, B.K.; Cameron, A.; Sattar, N.; Weaver, L.T. Maternal docosahexaenoic acid supplementation and fetal accretion. *Br. J. Nutr.* **2003**, *90*, 135–145. [CrossRef]
37. Vlaardingerbroek, H.; Hornstra, G. Essential fatty acids in erythrocyte phospholipids during pregnancy and at delivery in mothers and their neonates: Comparison with plasma phospholipids. *Prostaglandins Leukot. Essent. Fat Acids* **2004**, *71*, 363–374. [CrossRef]
38. Kawabata, T.; Kagawa, Y.; Kimura, F.; Miyazawa, T.; Saito, S.; Arima, T.; Nakai, K.; Yaegashi, N. Polyunsaturated Fatty Acid Levels in Maternal Erythrocytes of Japanese Women during Pregnancy and after Childbirth. *Nutrients* **2017**, *9*, 245. [CrossRef]
39. Kuipers, R.S.; Luxwolda, M.F.; Offringa, P.J.; Boersma, E.R.; Dijck-Brouwer, D.A.; Muskiet, F.A. Fetal intrauterine whole body linoleic, arachidonic and docosahexaenoic acid contents and accretion rates. *Prostaglandins Leukot. Essent. Fat Acids* **2012**, *86*, 13–20. [CrossRef]

40. Henderson, R.J.; Burkow, I.C.; Buzzi, M.; Bayer, A. Effects of docosahexaenoic (22:6n−3), tetracosapentaenoic (24:5n−3) and tetracosahexaenoic (24:6n−3) acids on the desaturation and elongation of n−3 polyunsaturated fatty acids in trout liver microsomes. *Biochim. Biophys Acta* **1998**, *1392*, 309–319. [CrossRef]
41. Bakewell, L.; Burdge, G.C.; Calder, P.C. Polyunsaturated fatty acid concentrations in young men and women consuming their habitual diets. *Br. J. Nutr.* **2006**, *96*, 93–99. [CrossRef] [PubMed]
42. Burdge, G.C.; Wootton, S.A. Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. *Br. J. Nutr.* **2002**, *88*, 411–420. [CrossRef] [PubMed]
43. Burdge, G.C.; Jones, A.E.; Wootton, S.A. Eicosapentaenoic and docosapentaenoic acids are the principal products of alpha-linolenic acid metabolism in young men. *Br. J. Nutr.* **2002**, *88*, 355–363. [CrossRef] [PubMed]
44. Giltay, E.J.; Gooren, L.J.; Toorians, A.W.; Katan, M.B.; Zock, P.L. Docosahexaenoic acid concentrations are higher in women than in men because of estrogenic effects. *Am. J. Clin. Nutr.* **2004**, *80*, 1167–1174. [CrossRef]
45. Stark, K.D.; Beblo, S.; Murthy, M.; Buda-Abela, M.; Janisse, J.; Rockett, H.; Whitty, J.E.; Martier, S.S.; Sokol, R.J.; Hannigan, J.H.; et al. Comparison of bloodstream fatty acid composition from African-American women at gestation, delivery, and postpartum. *J. Lipid. Res.* **2005**, *46*, 516–525. [CrossRef]
46. De Haas, S.; Ghossein-Doha, C.; van Kuijk, S.M.; van Drongelen, J.; Spaanderman, M.E. Physiological adaptation of maternal plasma volume during pregnancy: A systematic review and meta-analysis. *Ultrasound Obstet. Gynecol.* **2017**, *49*, 177–187. [CrossRef]