

Genetic characterisation of delta-5 and delta-6 fatty acid desaturases and associations with fatty acid status in pregnant women of African descent

Anika Prinsloo

 **orcid.org/0000-0003-3899-383X**

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Supervisor: Dr Lizelle Zandberg

Co-Supervisor: Dr Linda Malan

Co-Supervisor: Miss T.L. Chimhashu

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This would not have been possible without the love and support from my fiancé – Wesley, my family and friends. Thank you for your support and motivation even through times that I felt stuck with no will to continue.

“To raise new questions, new possibilities, to regard old problems from a new angle, requires creative imagination and marks a real advance in science.”

- Albert Einstein

Abstract

Introduction and aim

Globally, dietary habits have shifted over the years from a traditional rural diet to a more Westernised diet. This has caused a drastic change in the intake ratio of n-6:n-3 polyunsaturated fatty acids (PUFA) from 1:1 to 20:1. Biosynthesis of long-chain polyunsaturated fatty acids (LCPUFA) is associated with genetic variation within the fatty acid desaturase (*FADS*) gene cluster. The *FADS1/2* genes encode for the rate-limiting desaturation enzymes in the LCPUFA biosynthesis pathway. Thus far, genetic variations in the *FADS1/2* gene cluster and their effect on LCPUFA metabolism have been investigated in the United States and in Europe, but limited research exists for the African population. The aim of this study was to characterise the genetic variations of the *FADS1/2* genes and their effect on delta -5 and -6 desaturase (D5D and D6D) activity and study the associations with n-3 LCPUFA status at early pregnancy in women of African descent.

Methods

In this cross-sectional study, a total of 250 apparently healthy pregnant women of African descent were enrolled and 243 of these women gave consent for genetic testing. Participants were divided into two groups depending on their n-6:n-3 LCPUFA ratio: a higher and lower n-3 LCPUFA status group. Nine variants, located in the *FADS1/2* gene cluster, were investigated, and genotyping of these selected variants was done using the iPLEX® MassARRAY system from Agena Bioscience™. The genetic variants were investigated for association with D5D and D6D activity, estimating PUFA ratios and n-6:n-3 LCPUFA ratio. To determine D5D and D6D enzymatic activities, LCPUFA product to precursor ratios were determined. Univariate analyses were performed to assess differences between genotypes, adjusting for gestational age, age of the mother, and dietary FA intakes.

Results

The alternative allele carriers of *FADS1*-rs174546_(C/T) and *FADS2*-rs112425493_(G/A) associated with both lower AA:DGLA and higher DGLA:LA ratios that are respectively indicative of lower D5D and higher D6D activities. Alternative allele carriers for *FADS*-rs174547_(C/T), *FADS1*-rs174549_(A/G), *FADS1*-rs174555_(C/T) and *FADS2*-rs73489307_(T/C), were all associated with higher DGLA:LA and therefore were associated with higher estimated D6D activity in comparison to the homozygous ancestral alleles. Alternative allele carriers of *FADS2*-rs78678033_(G/A) were associated with higher AA:DGLA ratio, estimated D5D activity as well as lower DGLA:LA ratio and D6D activity. Alternative allele carriers of *FADS2*-

rs174606_(T/G) and *FADS2*-rs174616_(G/A) associated with both lower AA:LA and lower DGLA:LA ratios, lower combined D5D and D6D activity, and lower individual D6D activity. The carriers for the G allele for *FADS2*-rs112425493_(G/A), T allele for *FADS1*-rs174546_(C/T), T allele for *FADS1*-rs174547_(C/T), and the G allele for *FADS1*-rs174549_(A/G) all associated or tended to associate with a lower n-6:n-3 LCPUFA ratio and therefore with a higher n-3 LCPUFA status (P=0.033, P=0.0059, P=0.062 P=0.071, respectively). All associations were unaffected when adjusting for dietary intake of the individual n-6 or n-3 essential PUFA or LCPUFA.

Conclusion

This mini-dissertation sheds light on the unique and noteworthy African genome and the importance of conducting genetic research in a population of African descent. The current study contributes to the investigation of the *FADS1/2* gene cluster and the associations with LCPUFA metabolism in an African population. Nine genetic variants influenced estimated desaturase activities and four variants related to both increased desaturase activities and an improved n-3 LCPUFA status, and may thus be advantageous to improve n-3 LCPUFA status in pregnant women of African descent even in the context of low functional LCPUFA such as EPA and DHA dietary intake. This information may create an opportunity to alter the n-3 LCPUFA status of South Africans through dietary intervention which could in turn result in a decline in inflammatory diseases and an increase in optimal birth outcomes for the South African population.

Key Terms

Long chain polyunsaturated fatty acids, LCPUFA, Fatty acid desaturases; D5D, D6D, *FADS1*, *FADS2*; *FADS1/2*; Phospholipid fatty acids status, Pregnancy, African women

LIST OF ABBREVIATIONS

AA:	Arachidonic acid (20:4)
AdA:	Adrenic Acid (22:4)
ALA:	Alpha-linolenic acid (18:3)
ANC:	Antenatal care
hs-CRP:	High sensitivity C-reactive protein
CEN:	Centre of Excellence for Nutrition
CoJ:	City of Johannesburg
DDS:	Dietary Diversity Score
DGLA:	Dihomo-gamma-linolenic acid (20:3)
DoH:	Department of Health
DHA:	Docosahexaenoic acid (22:6)
DPA:	Docosapentaenoic acid (22:5)
EAR:	Estimated Average Requirement
EFA:	Essential Fatty Acid
ELOVL:	Fatty Acid Elongase
EPA:	Eicosapentaenoic acid (20:5)
EST:	Expressed Sequence Tags
FA:	Fatty Acid
FADS:	Fatty Acid Desaturase
FAME:	Fatty Acid Methyl Esters
FATP:	Fatty Acid Transfer Proteins
FBDG:	Food Based Dietary Guideline
FEN1:	Flap Endonuclease 1
GO:	Gene Ontology
GOED:	Global Organisation for EPA and DHA
GWAS:	Genome-Wide Association Studies
H-FABP:	Heart-Type Fatty Acid Binding Protein
HPCSA:	Health Professions Council of South Africa
HREC:	Health Research Ethics Committees of the North-West University

HIV:	Human immunodeficiency virus
LCPUFA:	Long Chain Polyunsaturated Fatty Acid(s) and Long Chain Polyunsaturated Fatty Acids
LA:	Linoleic acid (18:2)
LSM:	Living Standards Measure
MUAC:	Mid-Upper Arm Circumference
MUFA:	Mono-unsaturated Fatty Acid
n–3:	Omega-3
n–6:	Omega-6
NCD:	Non-communicable Disease
NHANES:	National Health and Nutrition Examination Survey
NuPED:	Nutrition during Pregnancy and Early Development
ORF:	Open Reading Frame
PUFA:	Polyunsaturated Fatty Acid
QFFQ:	Quantified Food Frequency Questionnaire
RBC:	Red Blood Cell
rs:	Reference sequence
SA:	South Africa
SANHANES:	South African National Health and Nutrition Examination Survey
SAMRC:	South African Medical Research Council
SFA:	Saturated Fatty Acid
SNPs	Single Nucleotide Polymorphisms
TFA:	Trans Fatty Acid

TABLE OF CONTENTS

Acknowledgements.....	2
Abstract	3
LIST OF ABBREVIATIONS.....	5
CHAPTER 1: INTRODUCTION.....	10
1.1 Background and problem statement.....	10
1.2 Research aim and objectives.....	14
1.3 Research team.....	14
CHAPTER 2: LITERATURE REVIEW	16
2.1 South Africa today: Socio-economic and nutritional status.....	16
2.1.1 Population and ethnic diversity.....	16
2.1.2 Food security.....	17
2.1.3 Nutritional status and dietary diversity.....	18
2.2 Fatty acids in a South African context.....	18
2.3 Polyunsaturated fatty acids	20
2.4 Long chain polyunsaturated fatty acid metabolism	21
2.4.1 Long chain polyunsaturated fatty acid recommendations.....	22
2.4.2 Fatty acids during pregnancy	23
2.5 Fatty acid Desaturases	25
2.5.1 Molecular basis of the fatty acid desaturase gene cluster	25
2.5.2 Genetic variants and haplotypes of the <i>FADS</i> gene cluster.....	26
2.6 Conclusion.....	32
CHAPTER 3: MANUSCRIPT	33
4.1 Summary.....	76
4.2 Objective A: The characterisation of the genetic variations of the <i>FADS</i> gene cluster, which encodes for delta-5 and -6 fatty acid desaturases	76
4.3 Objective B: The associations of <i>FADS1/2</i> genetic variants with LCPUFA ratios and desaturase activity.....	77
4.4 Objective C: The impact of dietary intake on the associations of <i>FADS1/2</i> genetic variants with LCPUFA ratios and desaturase activity.....	78
4.5 Strengths	78
4.6 Limitations	78
4.7 Future research.....	79
4.8 Conclusion.....	79

REFERENCES.....	80
APPENDIXES	91
Appendix A.....	91
Appendix B.....	95
Appendix C.....	120

LIST OF FIGURES

Figure 2.1: Race distribution as a percentage of the total South African population.....	16
Figure 2.2: Population distribution across provinces as a percentage of the total South African Population. (StatsSA, 2019).....	17
Figure 2.3: Omega-3 and n-6 fatty acid elongation and desaturation metabolic pathways...	21
Figure 2.4: Schematic presentation of <i>FADS1</i> and <i>FADS2</i> genes.....	25
Figure 3.1: Metabolic pathway of n-6 and n-3 LCPUFA in humans.....	53
Figure 3.2: Schematic presentation of <i>FADS1</i> and <i>FADS2</i> genes located on chromosome 11 and location of genetic variants selected for this study.....	54

LIST OF TABLES

Table 1.1: Team members and their roles and expertise.....	14
Table 2.1: Common names, shorthand nomenclature and typical sources of fatty acids.....	19
Table 2.2: Summary of <i>FADS1</i> and <i>FADS2</i> genetic variants.....	29
Table 3.1: Participant characteristics.....	55
Table 3.2: Red blood cell total phospholipid fatty acid composition.....	56
Table 3.3: Characteristics of the nine selected genetic variants in the <i>FADS1/2</i> gene cluster of the NuPED study sample.....	57
Table 3.4: Distribution of plasma phospholipid LCPUFA ratios as classified by <i>FADS2</i> -112425493 _(G/A)	58
Table 3.5: Distribution of plasma phospholipid LCPUFA ratios as classified by <i>FADS1</i> -rs174546 _(C/T)	60
Table 3.6: Distribution of plasma phospholipid LCPUFA ratios as classified by <i>FADS2</i> -rs73489307 _(T/C)	62

Table 3.7: Distribution of plasma phospholipid LCPUFA ratios as classified by FADS1- rs174547 _(C/T)	64
Table 3.8: Distribution of plasma phospholipid LCPUFA ratios as classified by FADS1- rs174549 _(A/G)	66
Table 3.9: Distribution of plasma phospholipid LCPUFA ratios as classified by FADS1- rs174555 _(C/T)	68
Table 3.10: Distribution of plasma phospholipid LCPUFA ratios as classified by FADS2- rs78678033 _(G/A)	70
Table 3.11: Distribution of plasma phospholipid LCPUFA ratios as classified by FADS2- rs174606 _(T/G)	72
Table 3.12: Distribution of plasma phospholipid LCPUFA ratios as classified by FADS2- rs174616 _(G/A)	74

CHAPTER 1: INTRODUCTION

1.1 Background and problem statement

Fatty acids (FAs) are essential for life as they are a major source of energy and are structural components of cell membranes. They play an important role in inflammation, cell division and in the regulation of lipid metabolism. FA derivatives also serve as hormones and intracellular messengers (Berg *et al.*, 2002; Duplus & Forest, 2002). In pregnancy, essential FAs (EFAs) and long chain polyunsaturated fatty acids (LCPUFA) are crucial for normal foetal growth and development, and are selectively transferred from the mother to the foetus (Lauritzen *et al.*, 2001). Accordingly, women of childbearing age have a higher conversion of precursor to functional LCPUFA as an adaption to maintain higher LCPUFA levels as needed during pregnancy and lactation. This metabolic pathway of LCPUFA is associated with genetic variation within the fatty acid desaturase (*FADS*) gene cluster containing *FADS1* and *FADS2* genes. *FADS1* and *FADS2* encode for delta 5 desaturase (D5D) and delta 6 desaturase (D6D) enzymes in the LCPUFA biosynthesis pathway (Reynolds *et al.*, 2018).

Maternal nutritional status is a strong predictor of foetal growth and development (Abu-Saad & Fraser, 2010). The maternal metabolism is modified and altered from the beginning of pregnancy in order to support the nutritional requirements of the foetus. The first trimester of pregnancy can be considered as an anabolic period during which the mother's body weight increases *via* hyperphagia and storing in adipose tissue (Herrera & Ortega-Senovilla, 2010). Fat storage is facilitated by increasing insulin responsiveness of maternal adipose tissue, which reduces lipoprotein lipase activity (Cetin *et al.*, 2009). The adipose tissue formed during the first period of pregnancy becomes the source of LCPUFA during the third trimester, when the maternal lipid metabolism switches to a catabolic condition and docosahexaenoic acid (DHA 22:6n-3) increases in the foetal circulation (Cetin *et al.*, 2009). The maternal fatty acid status at entry of pregnancy, therefore, gives the mother a better adiposity makeup for the recruitment of DHA during the third trimester (Cetin *et al.*, 2009; Herrera & Ortega-Senovilla, 2010).

The LCPUFA are selectively transferred from the mother to the foetus and are accumulated in the foetal nervous system as early as the second trimester of pregnancy. Poor foetal and infancy nutrition is a risk factor for future chronic diseases (Delisle, 2008). The foetus relies on the maternal supply of fatty acids *in utero*. Essential fatty acids (EFAs) and LCPUFA are needed for foetal growth and development, and must be obtained from maternal circulation (Herrera & Ortega-Senovilla, 2010). The LCPUFA are especially important early in pregnancy because this is a vulnerable period for neuro- and immune development. In particular, DHA is

the predominant fatty acid in the brain tissue (particularly in the synaptic membranes), and retina (Lauritzen *et al.*, 2001; Martinez, 1994). Moreover, omega-3 (n-3) PUFA have been shown to play an important role in a child's development in many other ways: n-3 PUFA supplementation has been positively correlated with placental weight and birth weight (Magnusardottir *et al.*, 2009); lower n-6 (omega-6): n-3 ratio during early pregnancy has shown a negative association with behavioural problems in children (Loomans *et al.*, 2014); and lower n-6: n-3 ratio has shown a positive association with increased heart rate variability and lowered heart rate (Drewery *et al.*, 2017). Maternal DHA status has also been associated with immune function of infants (Calder *et al.*, 2008); and n-3 LCPUFA play a role in primary prevention of asthma and allergies in early childhood (Kremmyda *et al.*, 2011b).

Fatty acids (FA) play a major role in the development of inflammatory diseases (Duchen & Bjorksten, 2001), as n-6 LCPUFA are metabolised to powerful promoters of inflammation called lipid mediators or eicosanoids (Calder, 2008), while n-3 LCPUFA promote the synthesis of pro-resolving lipid mediators and decrease the release of pro-inflammatory cytokines (Annettta *et al.*, 2015; Calder, 2010; Calder, 2013). LCPUFA can be obtained from the diet, or can be synthesised endogenously from essential n-3 and n-6 LCPUFA precursors, alpha-linolenic acid (ALA 18:3n-3) and linoleic acid (LA 18:2n-6), respectively (Di Stasi *et al.*, 2004). A series of elongation and desaturation steps are necessary for this synthesis process, which is catalysed by the fatty acid desaturase – and elongase enzymes (Schaeffer *et al.*, 2006; Sprecher *et al.*, 1995). Diets rich in n-6 LCPUFA, due to high intake of vegetable oils rich in LA lead to predominance of arachidonic acid (AA 20:4n-6) in tissues. AA increases eicosanoid levels (prostaglandin E₂), which can enhance the synthesis of T helper type 2 cytokines and immunoglobulin E antibodies. When diets are high in n-3 LCPUFA, these FAs are incorporated into cellular phospholipids. The higher n-3 LCPUFA content lowers production of pro-inflammatory prostaglandins and reduces pro-inflammatory signalling in comparison to lower n-3 LCPUFA consumption (Calder *et al.*, 2007; Gottrand, 2008).

When FAs are consumed in too high quantities, or in non-ideal ratios of saturated fatty acids (SFAs), mono-unsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFA), it can increase risk for chronic- and inflammatory diseases (Glew *et al.*, 2004; Van Doormaal *et al.*, 1984). Globally, the consumption of n-6 LCPUFA (arachidonic acid [AA], dihomo-gamma-linolenic acid [DGLA], linoleic acid [LA]) rich foods has increased, whereas consumption of foods high in n-3 LCPUFA, (alpha-linolenic acid [ALA], eicosapentaenoic acid [EPA], docosahexaenoic acid [DHA]) has decreased over the past few decades (Blasbalg *et al.*, 2011; Kremmyda *et al.*, 2011b). The dietary shift over the years to a Western diet has caused a drastic change in the intake ratio of n-6 to n-3 FAs from 1:1 to 20 – 30:1 (Gómez Candela

et al., 2011). In African countries, rapid urbanisation and ongoing behavioural transitions have resulted in a double burden of over- and under nutrition (Delisle, 2008). The amount and ratio of the types of FAs consumed in the South African population is a concern since it directly affects the morbidity risk of the population (Glew *et al.*, 2004; Van Doormaal *et al.*, 1984).

Vorster *et al.* (2011) reported on how total fat intake increased from 21% to 30% of energy in urban South African women, and from 15.5% to 21% of energy in rural African women from 1975 to 1996 and from 1996 to 2005 (Vorster *et al.*, 2011). An increased intake of energy from total fat was observed in the urban areas, with percentages approaching or already at the upper dietary goal. In 2005, however, the percentage of energy from total fat in the rural areas was still below the lower dietary goal, at 21% of total energy (Smuts & Wolmarans, 2013; Vorster *et al.*, 2011). The intake of SFAs varied at 3.9% to 9.1% of energy, MUFAs at 4.2% to 10.4% of energy, and PUFA at 5.5% to 8.3% of total energy intake. Mean PUFA intakes were <10% of energy, with some studies showing that the mean intake was <6% of energy, the lower end of the range recommended for PUFA intake. The low PUFA intakes were especially applicable to those whose total fat intake was low (17.6% – 23.8% of energy from fat). Therefore, PUFA intake could be compromised if the intake of total fat is too low.

The original South African food-based dietary guideline (FBDG) for fat intake reads: “Eat fats sparingly” (Vorster *et al.*, 2013). Several organisations world-wide recommend a total fat intake of 30% – 35% of total energy (FAO, 2010; WHO/FAO, 2003). Even though the quantitative dietary goals have always recommended that the consumption of SFA should be reduced, and that MUFA and PUFA should replace SFA, the focus of the guidelines were on the total amount of fat intake, and not on the quality and type of fatty acids (Smuts & Wolmarans, 2013). The impact of different FAs on health and disease is now better understood than it was previously and, therefore, better recommendations can now be made (Smuts & Wolmarans, 2013).

Based on the current body of scientific evidence, the global organisation for EPA and DHA (GOED) omega-3 website has recommended 700 – 1000 mg/day n-3 PUFA for pregnant and lactating women, of which the optimal intake is 700 mg/day of EPA + DHA (at least 300 mg should be DHA) (EFSA, 2012; GOED, 2017; Koletzko *et al.*, 2014; Kris-Etherton *et al.*, 2002). In South Africa, the population is obligatorily tethered to expensive marine sources of n-3 PUFA, and need more affordable, plant based sources of n-3 PUFA (Mathias *et al.*, 2012). Plant based n-3 PUFA are relatively inexpensive, easier accessible to the population, and can be obtained in South Africa from canola oil and canola oil-based products. Currently, plant oils account for most of the natural oils and fats in the world. Changing the South African population’s preferred cooking oils to plant oils rich in n-3 PUFA should be a sustainable

change for the population, and an easier way to increase n-3 LCPUFA status. However, these shorter chain n-3 PUFA still need to be metabolised to n-3 LCPUFA.

As mentioned earlier, biosynthesis pathway for LCPUFA is modified by genetic variation within the *FADS* gene cluster, containing both *FADS1* and *FADS2* genes that encode the rate-limiting desaturation enzymes D5D and D6D respectively (Reynolds *et al.*, 2018). The hypothesis that both D5D- and D6D enzymes play a key role in inflammatory diseases is strengthened by functional studies in mice, where selective D5D- and D6D inhibitors showed an altered pro and anti-inflammatory response (Obukowicz *et al.*, 1998).

Recent studies on the association between LCPUFA levels and genetics, suggest that there is a dramatic difference in LCPUFA levels and product-precursor ratios (pathway efficiency) in total plasma lipids between African Americans and European Americans in the United States. Evidence points to enhanced *FADS1* efficiency as being a pivotal component to these differences (Mathias *et al.*, 2011; Sergeant *et al.*, 2012). Based on previous publications, Mathias *et al.* selected seven genetic variants which map to the *FADS* gene cluster (rs174537, rs102275, rs174546, rs174556, rs1535, rs174576, rs174579) and reported that African Americans had significantly higher circulating levels of plasma AA and lower DGLA levels than European Americans (Mathias *et al.*, 2011). The study concluded that the impact of these *FADS* genetic variants on PUFA metabolism (especially AA levels) is likely more pronounced in African Americans due to the larger proportion of individuals carrying the genotype associated with increased *FADS1* enzymatic conversion of DGLA to AA in this group (Mathias *et al.*, 2011).

Ford *et al.* (2016) investigated the dietary fat intake in three different communities in South Africa and compared their FA intake with their red blood cell (RBC) FA composition. They concluded that rural children from the Limpopo Province had the lowest EFA intake (n-3 and n-6 FAs), but reflected the highest RBC DHA profile, probably suggesting a more efficient conversion from ALA to DHA (Ford *et al.*, 2016) and adds to the body of evidence that genetics may play a pivotal role in the LCPUFA biosynthesis pathway.

Thus far, limited research has attempted to identify the genetic variants that are involved in LCPUFA metabolism within the African population. This is because most of the research has been done in Western Caucasians or African Americans. It is important to understand the *FADS* metabolism and underlying genetic predictors, as well as gain knowledge of the n-6 and n-3 LCPUFA intake and status at entry of pregnancy to be able to tailor strategies and dietary recommendations to optimise FA status and its associated health benefits in pregnant women in South Africa.

1.2 Research aim and objectives

The aim of this study was to determine and characterise the genetic variations of the delta-5 and -6 fatty acid desaturases (*FADS*) gene cluster and to study associations with LCPUFA status in pregnant women of African descent.

The objectives of this study were:

- A.) To characterise the genetic variations of the *FADS* gene cluster, which encodes for delta-5 and -6 fatty acid desaturases
- B.) To study the associations of selected *FADS* gene cluster variations with the ratios of the respective fatty acid estimated desaturase enzyme activities
- C.) To assess whether the associations in B are dependent on ALA, LA, DHA, EPA and AA intake, respectively

1.3 Research team

The members of the research team and their roles and expertise are listed in **Table 1.1**. None of the team members have a conflict of interest.

Table 1. 1: Team members and their roles and expertise

Affiliation	Name	Qualification, knowledge, experience, skills	Professional registration	Role
North-West University	Anika Prinsloo	BSc Dietetics	HPCSA-Dietitian	Masters student of this sub study imbedded within the larger NUPED study. Responsible for protocol development, laboratory analysis of which samples, genotyping of all variants as reported on, statistical analyses, data interpretation and presentation, manuscript preparation and writing of the mini-dissertation
North-West University	Dr Lizelle Zandberg	Molecular nutrition expert with a special interest in the underlying genetics of fatty acids and micronutrient	-	Supervisor

		metabolism		
North-West University	Dr Linda Malan	Fatty acid and micronutrient nutrition; Community-based clinical trials; gas chromatography mass spectrometry specialist	HPCSA-Medical Biological Scientist	Co-supervisor
North-West University	Miss T.L. Chimhashu	Nutritionist	-	Assistant-supervisor
North-West University	Prof C. Marius Smuts	Essential fatty acid and micronutrient nutrition; Nutrition during pregnancy and infancy; Community-based clinical trials	HPCSA Nutritionist -	Principal investigator
North-West University	Dr Maryke Schoonen	Molecular and computational biologist	-	Bioinformatist
University of South Africa	Dr Elize Symington	Nutrition during pregnancy expert	HPCSA - Dietetics	Project leader (NUPED)
North-West University	Dr Jeannine Baumgartner	Essential fatty acid and micronutrient nutrition; expert	-	Statistician and expert advisor

CHAPTER 2: LITERATURE REVIEW

The following literature review will describe the elongation and desaturation metabolism of LCPUFA in pregnancy, and how genetics may influence this metabolic process.

2.1 South Africa today: Socio-economic and nutritional status

South Africa is classified as a developing country and has a high poverty and food insecurity rate. According to StatsSA a low-income population is defined as one with an income of between R1 and R19 000 per month. In 2011 29% of households in South Africa fell into this category. A mid-income population is defined as a population that receives between R19 201 – R307 200 per month; 48% of households in South Africa (most of the population) fell into this category during the 2011 survey. The upper income category is defined as a population that receives R307 201 and above per month, and this category only makes out 7.3% of South Africa's households (StatsSA, 2011). In South Africa, the unemployment rate is 27.1%. Health care is expensive and the health burden for families is high. Therefore, any way to lower the prevalence of morbidity, and so also the cost of health care, should be pursued.

2.1.1 Population and ethnic diversity

In South Africa, the population is estimated to be 56.5 million. The black African population is in the majority as it represents 80.8% of the total South African population (**Figure 2.1**). South Africa has 9 provinces, of which the highest percentage of the population lives in Gauteng (**Figure 2.2**) (StatsSA, 2019).

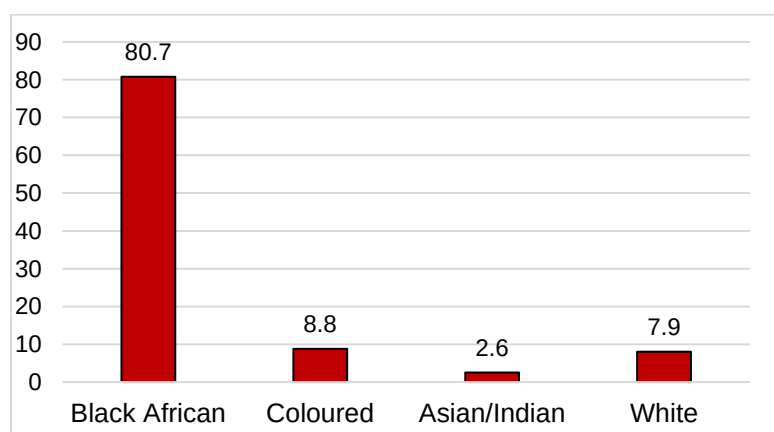


Figure 2.1: Race distribution as a percentage of the total South African population (StatsSA, 2019)

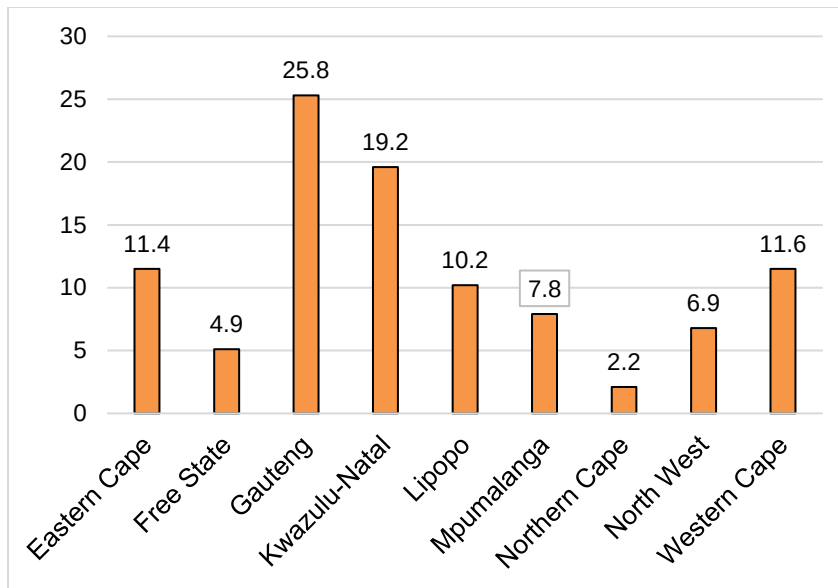


Figure 2.2: Population distribution across provinces as a percentage of the total South African Population. (StatsSA, 2019)

In 2018, a total of 1 009 065 births were registered in South Africa. Mothers in the age group of 25 – 29 years accounted for the highest number of births, followed by the 20 – 24 years age group, and then 30 – 34 years age group (StatsSA, 2018).

2.1.2 Food security

A healthy diet could lower the risk for non-communicable disease, and thus the risk for morbidity. To be able to follow a healthy and balanced diet, food security is important. Food security exists when people have physical and economic access to safe, sufficient, and nutritious food at all times, to meet their dietary needs as well as food preferences to live an active life (FAO, 1996).

A community survey done in South Africa (2016) determined that a little less than a fifth (19.9%) of households in the country reported that they had run out of money to afford food in the past year (StatsSA, 2016). Thirteen percent (13.3%) of South African households reported that it had been necessary for them to skip a meal in the year preceding the survey (StatsSA, 2018). Even though food insecurity has lowered since the previous survey done in 2015, there is still some insecurity (StatsSA, 2016). The survey required households to indicate whether they have run out of money to buy food or whether they have skipped a meal, and how often this would occur. This does not indicate whether the food that they do consume meets their dietary needs to prevent deficiencies. According to the definition as stated by the FAO, food security is not only about the amount of food that an individual consumes, but also about the

quality of the food (FAO, 2006; StatsSA, 2016). Our concern is regarding the nutritional intake of the population, especially fatty acids in pregnant women.

2.1.3 Nutritional status and dietary diversity

In African countries, rapid urbanisation and on-going behavioural transitions have resulted in a double burden of over- and under nutrition (Delisle, 2008). High-fibre foods are substituted for processed food high in refined carbohydrates and saturated fatty acids (SFAs), and diets are becoming more energy dense (Popkin, 2006). Women are at higher risk for malnutrition, due to high nutritional needs during pregnancy and lactation, gender inequalities, as well as poverty (Delisle, 2008). In South Africa particularly, the prevalence of overweight and obesity is starting to predominate, physical inactivity is rising, and under nutrition is becoming less frequent (Prioreschi *et al.*, 2017). The South African National Health and Nutrition Examination Survey (SANHANES) found in 2013 that the mean dietary diversity score (DDS) across all age categories at the national level was 4.2. This is extremely close to the cut-off level (DDS score of 4.0) to be considered nutritionally adequate (SANHANES, 2013). Four out of ten participants in the age groups 15 – 24 years and 35 – 44 years had the lowest DDS. The black African participants had the lowest DDS as well as the highest number (44.9%) of participants that had low dietary diversity (SANHANES, 2013).

There is no single food that contains all the required nutrients needed for optimal health, and thus a diet that is sufficiently diverse reflects nutrient adequacy. Dietary diversity is used as an outcome measure of food security at individual and/or household level (Kennedy *et al.*, 2009). Apart from reflecting food security, a low DDS is also associated with stunted growth and lowered weight (Rah *et al.*, 2010), dyslipidaemia, cardiovascular disease (Azadbakht *et al.*, 2006), along with metabolic syndrome (Azadbakht *et al.*, 2005).

Fatty acid (FA) intake and the ratio of n-6:n-3 LCPUFA intake in the South African population is a concern, because it directly affects the morbidity risk of the population (Glew *et al.*, 2004; Van Doormaal *et al.*, 1984).

2.2 Fatty acids in a South African context

FAs are crucial for life as they are vital sources of energy and are structural elements of cell membranes. They play an important role in cell division, inflammation and regulation of lipid metabolism. Fatty acid derivatives also perform as hormones and intracellular messengers (Berg *et al.*, 2002; Duplus & Forest, 2002; Kremmyda *et al.*, 2011a; Tvrzicka *et al.*, 2011). When FAs are consumed in excessive – or disproportionate quantities and ratios of saturated

FAs (SFAs), mono-unsaturated FAs (MUFAs) and polyunsaturated FA (PUFA), risk for chronic- and inflammatory diseases can be increased in humans (Glew *et al.*, 2004; Van Doormaal *et al.*, 1984).

Vorster *et al.*, (2011) reported how total fat intake has increased from 15.5% to 21% of total energy in rural South African women, and from 21% to 30% of total energy in urban African women from the years 1975 – 1996, to 2005 (Vorster *et al.*, 2011). In urban areas, it was observed that there was a rise in energy consumption from total fat, with figures at the upper dietary goal or quickly approaching the upper dietary goal for fat. In 2005, however, the percentage of energy intake from total fat in rural areas was still too low, at only 21% of energy (Smuts & Wolmarans, 2013; Vorster *et al.*, 2011). The intake of PUFA varied at 5.5% – 8.3% of energy, MUFAs at 4.2% – 10.4% of energy, and SFAs at 3.9% – 9.1% of energy. The mean PUFA consumption was <10% of energy, with a few studies indicating that the mean consumption was <6% of energy, which is the lower end of the range recommended for PUFA intake. The low PUFA intakes were particularly applicable to those in whom the total fat intake was also low (ranging from 17.6% – 23.8% of energy from fat). PUFA intake could therefore be compromised if the intake of total fat is too low.

FAs are hydrocarbon chains that contain a carboxyl group at one end with a methyl group at the other end of the chain (Calder & Burdge, 2004). Carboxyl groups are reactive and can readily form ester links with alcohol groups, which in turn forms acylglycerols (e.g. phospholipids) as well as cholesteryl esters. FA chain lengths may range from two to 30 or more and may also contain double bonds. FAs that contain double bonds in the hydrocarbon chain are referred to as unsaturated FAs. A FA that contains one double bond in the chain is called a MUFA, where a FA containing two or more double bonds is called a PUFA (Calder, 2010). FAs can have common names (**Table 2.1**) or systematic names.

Table 2. 1: Common names, shorthand nomenclature and typical sources of fatty acids

Common name	Shorthand nomenclature	Typical source
Oleic acid	18:1 n-6	Olive oil, soybean oil and fish oil, oily/fatty fish
Linoleic acid (LA)	18:2 n-6	Soybean oil
Dihomo-gamma-linolenic acid (DGLA)	20:3 n-6	Only trace amounts in animal products
Arachidonic acid (AA)	20:4 n-6	Animal products
Alpha-linolenic acid (ALA)	18:3 n-3	Soybean oil, linseed oil, canola oil, chia seeds
Eicosapentaenoic acid (EPA)	20:5 n-3	Fish oil, oily/fatty fish
Docosapentaenoic acid (DPA)	22:5 n-3	Fish oil oily/fatty fish
Docosahexaenoic acid (DHA)	22:6 n-3	Fish oil oily/fatty fish

Modified from Calder and Burdge, 2004 (Calder & Burdge, 2004)

2.3 Polyunsaturated fatty acids

Omega-3 FAs (n-3) are PUFA with a double bond in-between the third and the fourth carbon in the chain. Mammals cannot synthesise carbon-carbon double bonds and therefore these compounds are essential to their diet. Omega-6 FAs (n-6) are also PUFA and promote the synthesis of highly pro-inflammatory compounds such as prostaglandin E₂ and leukotriene B₄, while omega-3 FAs promote the synthesis of less inflammatory eicosanoids such as prostaglandin E₃ and leukotriene B₅, and decrease the release of pro-inflammatory cytokines IL-1 and TNF- α (Annettta *et al.*, 2015; Calder, 2010; Calder, 2013).

Worldwide, the consumption of n-6 PUFA (arachidonic acid [AA], dihomo-gamma-linolenic acid [DGLA], linoleic acid [LA]), rich foods has increased, whereas that of n-3 (alpha-linolenic acid [ALA]; eicosapentaenoic acid [EPA]; docosahexaenoic acid [DHA]) PUFA have declined over the past few decades (Blasbalg *et al.*, 2011; Kremmyda *et al.*, 2011b).

A number of complex human diseases such as the metabolic syndrome (Van Doormaal *et al.*, 1984; Vessby, 2003), cardiovascular diseases (Glew *et al.*, 2004) and immune related diseases such as osteoarthritis and chronic obstructive pulmonary disease have been shown to be associated with increased PUFA composition in phospholipids (Goldring & Berenbaum, 2004). FAs have been thought to play a major role in the development of inflammatory diseases such as allergies (Duchen & Bjorksten, 2001), since PUFA are processed to powerful inflammatory promoters, namely eicosanoids, such as leukotrienes and prostaglandins (Calder, 2008). The levels of PUFA in phospholipids are determined by both nutrition (Di Stasi *et al.*, 2004) and metabolism. Diets rich in n-6 PUFA due to high intake of vegetable oils (rich in LA) lead to predominance of AA in tissues. AA increases eicosanoids (e.g. prostaglandin E₂), which can amplify the synthesis of T helper type 2 cytokines and immunoglobulin E antibodies. When diets are higher in n-3 PUFA, these n-3 PUFA are incorporated into cellular phospholipids, displacing AA in the process. This in return lowers reduction of prostaglandin E₂ synthesis, alteration of receptor expression and activity, and reduced pro-inflammatory cytokine responses (Calder *et al.*, 2007; Gottrand, 2008).

Desaturase and elongase enzymes are responsible for the catalytic conversion of PUFA in humans (Schaeffer *et al.*, 2006). The hypothesis that both D5D and D6D enzymes play an important role in inflammatory diseases is strengthened by functional studies performed in mice, where selective D5D and D6D inhibitors expressed an anti-inflammatory response (Obukowicz *et al.*, 1998).

2.4 Long chain polyunsaturated fatty acid metabolism

As seen in figure 2.3, two essential fatty acids namely linoleic acid (LA; 18:2 n-6) and alpha-linolenic acid (ALA; 18:3 n-3) obtained from the diet produce LCPUFA through a series of desaturation and elongation steps (Di Stasi *et al.*, 2004; Jump, 2002). The initial step in PUFA synthesis is desaturation of LA and ALA *via* the enzyme D6D. Once consumed from the diet, LA is metabolised by desaturases and elongases to form GLA and DGLA, which eventually produces AA, a key intermediate product that is metabolised further to form DPA. To produce EPA, the n-3 fatty acid, ALA must firstly be metabolised into stearidonic acid and eicosatetraenoic acid (ETA). The same enzymes (desaturases and elongases) used to synthesise AA are also used to produce EPA. EPA is then converted further to form either DHA or eicosanoids (Brenna *et al.*, 2009; Schmitz & Ecker, 2008).

Omega-3 and n-6 LCPUFA therefore compete for the same metabolic pathway and produce eicosanoids with different properties. It has thus been theorised that the balance of the two classes of LCPUFA may play an important role in the development of inflammatory diseases (Tanaka *et al.*, 2009).

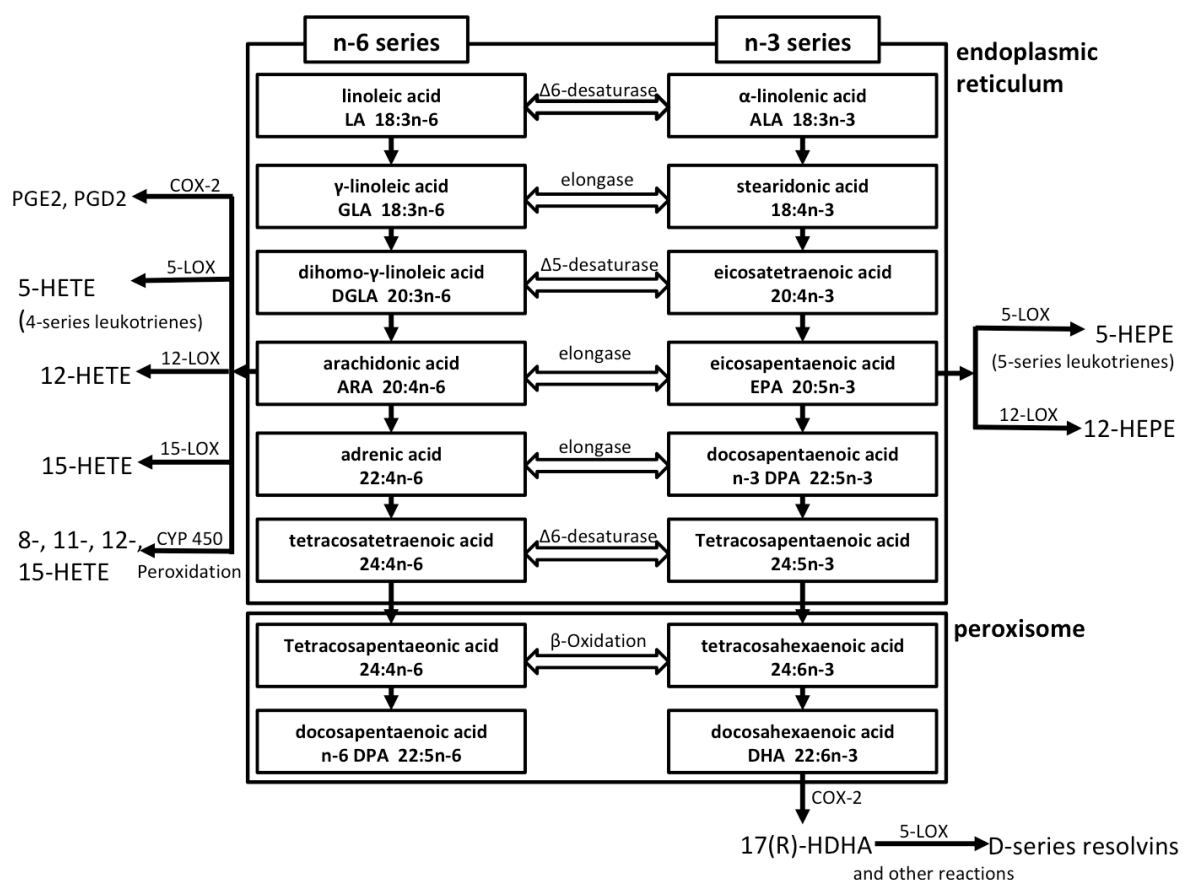


Figure 2.3: Omega-3 and n-6 fatty acid elongation and desaturation metabolic pathways

Δ indicates delta

Desaturase activity of the D5D and D6D enzymes can be measured in humans by use of product-to-precursor ratios (Warensjö *et al.*, 2009). Enzymatic activity can also be measured directly by measuring the rate at which precursor FAs (which are radiolabelled) are converted to their end products (Narce *et al.*, 1994). This practice is, however, prevented in humans due to practical and ethical reasons, because for example, if an investigated gene is expressed in liver cells, a liver biopsy would be required to measure the activity of the gene, which makes this approach unethical and therefore impossible to measure desaturase activity directly from humans in large studies. Therefore, an indirect method of measuring estimated enzyme activities, by means of product-to-precursor ratios such as AA/DGLA and DGLA/LA ratios are therefore the preferred method to measure D5D and D6D activity (Vessby *et al.*, 2006).

2.4.1 Long chain polyunsaturated fatty acid recommendations

The original South African food-based dietary guideline (FBDG) for fat intake reads: “Eat fats sparingly”. Based on new research, several organisations world-wide recommend a total fat intake that ranges from 30% to 35% of total energy consumption (FAO, 2010; WHO/FAO, 2003). Even though the quantitative dietary goals have recommended in the past that the consumption of SFA should be decreased, and that MUFA and PUFA consumption should increase and replace these SFAs, the focus has always been on the total amount of fat intake, and not on the type and quality of FAs (Smuts & Wolmarans, 2013). The impact of different FAs on health and disease is now better understood than it was in the past, and therefore better recommendations can now be made to promote n-6 and especially n-3 LCPUFA, as they are essential nutrients that are involved in crucial physiological processes (Smuts & Wolmarans, 2013).

The GOED Omega-3 website has established the following intake recommendations based on current scientific evidence: 500 mg/day EPA + DHA for the general population with the goal of lowering the risk of coronary heart disease, and 700 – 1000 mg/day for pregnant and lactating women, of which the optimal intake is 700 mg/day of EPA + DHA (a minimum of 300 mg should be DHA) (EFSA, 2012; GOED, 2017; Koletzko *et al.*, 2014; Kris-Etherton *et al.*, 2002). In South Africa, the population is obligatorily tethered to expensive marine sources of n-3 LCPUFA, and need more affordable, plant based sources (Mathias *et al.*, 2012). Plant based LCPUFA are relatively inexpensive, sustainable, easier accessible to the population, and can be obtained from sources such as flaxseed oil, walnut oil or most importantly canola oil (Ursin, 2003). Currently, plant oils comprise the majority of natural fats and oils in the world, around 150 million metric tons of vegetable oils are consumed annually, and it is expected that this amount will increase in the future (OECD/FAO, 2012). If the South African population can change their preferred cooking oil to a plant oil rich in LCPUFA such as canola oil, it should

be a sustainable change and an easier way to increase the intake of n-3 LCPUFA. Other fish options such as sardines/pilchards can also be considered as they serve as an affordable alternative to increase EPA and DHA consumption.

2.4.2 Fatty acids during pregnancy

Maternal nutritional status is a powerful predictor of foetal growth and development, with excessive gestational weight gain, obesity, and adiposity being associated with adverse birth outcomes (Abu-Saad & Fraser, 2010). Poor foetal and infancy nutrition is a risk factor for future chronic diseases (Delisle, 2008) and the foetus relies on the maternal supply of fatty acids *in utero*. As previously mentioned, higher LCPUFA intake is recommended during pregnancy with a recommended dosage of 700 – 1000 mg/day, of which 300 mg or more should be DHA (EFSA, 2012; GOED, 2017; Koletzko *et al.*, 2014; Kris-Etherton *et al.*, 2002). Multiple pregnancies, as well as closely spaced pregnancies, create a demand for maternal LCPUFA even greater than that of singleton pregnancies (Szajewska *et al.*, 2006; Zeijner *et al.*, 1997).

Hyperlipidaemia and accumulation of fat depots are the two major changes that occur during pregnancy in lipid metabolism (Herrera & Ortega-Senovilla, 2010). Maternal metabolism is modified and altered since the start of the pregnancy in order to support the nutritional requirements of the foetus. The first trimester can be seen as an anabolic period during which the mother's body weight increases *via* hyperphagia and substance storing in adipose tissue (Herrera & Ortega-Senovilla, 2010). Fat storage is enabled by increased insulin responsiveness of maternal adipose tissue, which in turn lowers lipoprotein lipase activity (Cetin *et al.*, 2009). The conversion of ALA to DHA is affected by numerous factors. Studies have shown that this process is inclined to be more affective in children as well as young women in comparison to young men (Calder, 2016). It has been suggested that these higher rates of ALA conversion to DHA amongst women may be because of their higher requirements throughout pregnancy and lactation (Carnielli *et al.*, 2007; Derbyshire, 2019).

Essential FAs and LCPUFA are necessary for foetal growth and development, and must be obtained from maternal circulation (Herrera & Ortega-Senovilla, 2010). LCPUFA are essential early in pregnancy because this is a vulnerable period for neuro- and immune development (Lauritzen *et al.*, 2001). LCPUFA are selectively carried over from the mother to the foetus and are accumulated in the foetal nervous system as soon as the second trimester of pregnancy. In particular, DHA is the predominant FA in brain tissue, especially in the synaptic membranes and the retina (Lauritzen *et al.*, 2001; Martinez, 1994). The physiological importance of DHA during pregnancy is justified by its active maternal-to-foetal placental transfer. This process is assisted by particular FA transfer proteins (FATP-1 and FATP-4) as

well as membrane binding proteins (H-FABP), that favour the placental transport of DHA over other FAs such as LA (Koletzko *et al.*, 2007b; Koletzko *et al.*, 2008; Larque *et al.*, 2006).

The results of a large, community-based cohort study conducted by Van Eijsden *et al.* (2008) suggest that the maternal n-3 and n-6 LCPUFA status in early stages of pregnancy affects foetal growth. This study concluded that both low maternal plasma concentrations of n-3 eicosatetraenoic acid, DGLA, and DPA and EPA, as well as high concentrations of AA were associated with lower foetal growth, with a decrease of an estimated 50 – 60 g in birth weight and an increase of 40% – 50% in risk of being born small for gestational age (SGA) (Van Eijsden *et al.*, 2008). Magnusardottir and colleagues detected a similar result in which n-3 LCPUFA supplementation in the first trimester of pregnancy was associated with placental weight as well as birth weight (Magnusardottir *et al.*, 2009).

Several studies have also indicated that consumption of fish, fish oil or n-3 LCPUFA oil during pregnancy associate with longer gestation and reduced risk of preterm delivery (Horvath *et al.*, 2007; Koletzko *et al.*, 2007a; Makrides *et al.*, 2006; Szajewska *et al.*, 2006). LCPUFA consumption plays a part in primary prevention of asthma and allergies in early childhood and throughout childhood (Kremmyda *et al.*, 2011b). Regarding maternal health, higher seafood consumption and greater DHA concentrations in maternal breast milk was associated with lower prevalence of postpartum depression, of where the lowest prevalence was found in Japan (2%) and the highest in South Africa (24.5%) (Hibbeln, 2002).

When n-6 and n-3 LCPUFA ratios are considered, a lower n-6:n-3 ratio during early pregnancy has indicated a negative association with behavioural problems in children (Loomans *et al.*, 2014), and has indicated a positive association with increased heart rate variability and decreased heart rate (Drewery *et al.*, 2017). Maternal LCPUFA status is also associated with the immune function of infants leading to a decreased risk for inflammatory diseases (e.g. allergies and asthma), and increased defence against pathogenic invaders (Calder, 2008; Klemens *et al.*, 2011).

It is evident that a lower maternal n-6:n-3 LCPUFA ratio in pregnant women is associated with better birth outcomes and offspring health, and it can be assumed that it would be beneficial to go into the pregnancy with an adequate LCPUFA status (lower n-6:n-3 LCPUFA ratio). Studies on the optimal n-6:n-3 LCPUFA ratio at entry of pregnancy have not yet been done, and therefore it is not known what an adequate fatty acid status during entry of pregnancy should be.

2.5 Fatty acid Desaturases

Fatty acid desaturases (*FADS*) are enzymes that function to add double bonds into fatty acyl chains, and their key function is to maintain a proper structure as well as function of biological cell membranes (Los & Murata, 1998). The human desaturase cDNAs were cloned for the first time in 1999 (Cho *et al.*, 1999). In 2000 it was identified as fatty acid desaturase 1 (*FADS1*) (encoding D5D) and fatty acid desaturase 2 (*FADS2*) (encoding D6D) in the human genome (Marquardt *et al.*, 2000). By means of genome-wide association studies (GWASs) multiple genetic loci have been identified that contribute to inter-individual variations in MUFAs, PUFA, SFAs, and trans fatty acids (TFAs) in whole plasma, plasma phospholipids, as well as erythrocyte membrane phospholipids in populations of European ancestry (He *et al.*, 2018; Tintle *et al.*, 2015; Wu *et al.*, 2013). Biosynthesis of LCPUFA is affiliated with genetic variation within the *FADS* gene cluster, containing *FADS1* and *FADS2* genes which encode the rate-limiting desaturation enzymes within the LCPUFA biosynthesis pathway (Reynolds *et al.*, 2018). These two genes are located on chromosome 11 in a cluster with a head-to-head orientation (Schaeffer *et al.*, 2006).

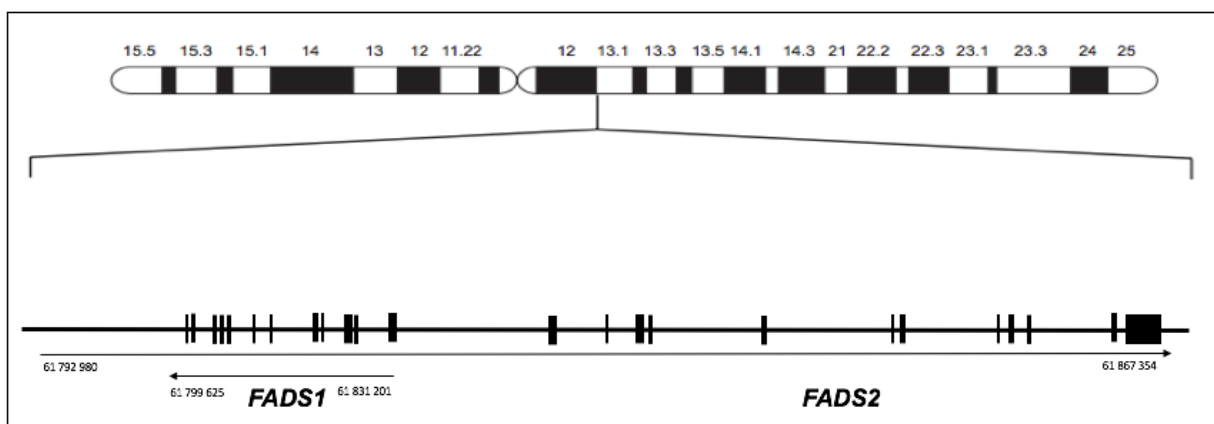


Figure 2.4: Schematic presentation of *FADS1* and *FADS2* genes

FADS = Fatty acid desaturase: = exon coding region

2.5.1 Molecular basis of the fatty acid desaturase gene cluster

The *FADS1* complementary DNA (cDNA) is a 4205-bp long transcript including an open reading frame (ORF) that is 1410-bp long. The *FADS2* cDNA is 3149-bp long with an ORF that is 1485-bp long, beginning 151-bp downstream of the 5'-end. Expressed Sequence Tags (EST) were assembled and when achieved, overlapping segments with more than 98% similarity gave rise to a third transcript that was distinct and was classified as *FADS3*. *FADS3*'s cDNA sequence is 1757-bp long with an ORF that is 1468-bp long (Marquardt *et al.*, 2000).

The *FADS* gene cluster comprises of *FADS1*, *FADS2* and *FADS3* genes and makes up a 100 kilo base (kb) cluster that is located on the long arm of chromosome 11 (11q12 - 13.1). In this region of chromosome 11(11q12 - 13.1), the *FADS1* gene is located 2.4 kb in a telomeric position and is oriented tail-to-tail with an endonuclease gene (namely flap endonuclease 1 or *FEN1*). *FADS2* follows in a downstream position 11.3 kb away from the *FADS1* gene and is oriented in a head-to-head position with the *FADS1* gene. *FADS3* follows in a tail-to-tail orientation, 6.0 kb away, telomeric to the *FADS2* gene (Marquardt *et al.*, 2000).

To establish the exon/intron organisation of the *FADS1* gene cluster, cDNA of the three *FADS* genes in the cluster were aligned to their genomic sequences. ATG (an in-frame translation initiation codon) was present in all transcripts. A probable polyadenylation signal, situated at the 3' end position of all three of the *FADS* genes, was found when the sequence was further explored. It was found that each of the genes contains 12 exons spanning at different sizes across genomic DNA. There is a substantial variability in the lengths of the intervening sequences of the three *FADS* genes, yet the structure of the exon and intron regions of the genes do not differ significantly. All three genes have identical lengths at exons 2 to 4, exons 6 to 11 as well as the coding section of exon 12 (the final exon). These three genes, did however, differ in length for exons 1 and -5. All the exon coding sequences of the genes were interrupted by the exon/intron boundaries at the exact same nucleotide positions that are within highly conserved codons (Marquardt *et al.*, 2000). A high degree of similarity (62% – 70%) in the nucleotide sequence was detected in the coding sequences of these genes. The EST clones (which represent the *FADS1*, *FADS2*, and *FADS3* genes) were extracted from human brain cells, which is an indication that these three genes are greatly expressed in human brain tissue (Marquardt *et al.*, 2000).

Translation of the transcripts for *FADS1*- and *FADS2* genes lead to an outcome of 444 amino acid peptides with molecular masses of approximately 52.0 kDa for *FADS1* and 52.3 kDa for the *FADS2* gene. The *FADS3* transcript translated into a 445 amino acid peptide with a 51.1 kDa estimated molecular mass. When *FADS1* and *FADS2* polypeptide sequences are aligned, a 61% sequence identity is found. A sequence identity of 52% is found when *FADS1* and *FADS3* are aligned, and *FADS2* has a sequence identity of 62% when compared with the *FADS3* gene. The sequence of amino acids for the *FADS* gene cluster, when compared to sequences of multiple other proteins that are known and registered in protein databases, has a remarkable homology to that of the desaturase enzymes (Marquardt *et al.*, 2000).

2.5.2 Genetic variants and haplotypes of the *FADS* gene cluster

Variations in the *FADS1* and 2 genes, that encode for D5D and D6D respectively, have been shown to be associated with several plasma and red blood cell n-6 and n-3 FA levels; more

especially with AA concentration (Barman *et al.*, 2015; Qin *et al.*, 2011). In previous studies the AA (C20:4 n-6):LA(C18:2 n-6) ratio has been used as an indicator of overall desaturase activity, without classifying D6D and D5D activities (Bokor *et al.*, 2010; Martinelli *et al.*, 2008; Xie & Innis, 2008). D5D and D6D activities can separately be determined by using the ratios DGLA (C20:3n-6):LA(C18:2n-6) as well as AA (C20:4n-6):DGLA (C20:3n-6) (Bokor *et al.*, 2010; Warensjö *et al.*, 2009).

The association of *FADS1* and *FADS2* genetic variations with altered estimated desaturase activity and accompanying health implications has not yet been explored in a lot of different ethnic groups and populations (Merino *et al.*, 2011). Recent studies that have been done on the association between plasma LCPUFA concentrations (AA, EPA, DGLA) and the *FADS* gene cluster (rs174537, rs102274, rs174546, rs1535, rs174576, rs174597) suggest that there is a dramatic difference in LCPUFA levels and product to precursor ratios (implicating pathway efficiency) in total plasma lipids between African Americans and Caucasian Americans in the United States, with most evidence pointing to “enhanced *FADS1* efficiency as being a pivotal component to these differences” (Mathias *et al.*, 2011; Sergeant *et al.*, 2012). The study done by Mathias *et al.*, in 2011 reported that African Americans have significantly higher circulating levels of plasma AA with lower plasma DGLA levels in comparison to Caucasian Americans. The study concluded that the impact of genetic variants of the *FADS* gene cluster on PUFA metabolism (especially AA levels) is probably more pronounced in African Americans attributable to the larger proportion of individuals from this population that are carriers of the genotype associated with higher *FADS1* enzymatic conversion of DGLA to AA (Mathias *et al.*, 2011).

When Mathias *et al.*, (2011) selected seven genetic variants (rs174537, rs102275, rs174546, rs174556, rs1535, rs174576, rs174579) which mapped to the *FADS* gene cluster based on previous publications (Schaeffer *et al.*, 2006; Tanaka *et al.*, 2009) ancestry-related differences were observed by an upstream marker previously associated with AA levels (rs174537) (**Table 2.2**). Regarding this specific genetic variant (rs174537), 79% – 82% of African Americans homozygous for the G allele in comparison to only 42% – 45% of Caucasian Americans. It is important to note that the allelic effect of the G allele (which is associated with increased conversion of DGLA to AA) on enzymatic efficiency was similar in both the African and Caucasian groups (**Table 2.2**) (Mathias *et al.*, 2011; Schaeffer *et al.*, 2006; Tanaka *et al.*, 2009).

Ford *et al.*, (2016) investigated the dietary fat intake in three different communities in South Africa, and compared their fatty acid intake with their red blood cell (RBC) fatty acid composition. They concluded that rural children from the Limpopo province had the lowest

EFA consumption but still reflected the highest RBC DHA profile, indicating a better n-3 LCPUFA status than expected (Ford *et al.*, 2016). This finding is suggestive of a more efficient conversion of ALA to DHA (Ford *et al.*, 2016) thus implying an enhanced D5D and D6D efficiency. These are only some of many studies suggesting that genetics play a role on this LCPUFA biosynthesis pathway.

In the study conducted by Ameer *et al.* in 2012, genetic variation association and haplotype analysis of the *FADS* gene cluster resulted in 28 variants that are strongly associated with each other (Ameer *et al.*, 2012). These 28 variants form two common haplotype blocks that differ in their capability to metabolise LCPUFA (Ameer *et al.*, 2012). These 28 variants account for 95% of the chromosome (Ameer *et al.*, 2012). The two haplotypes are known as haplotype *D* and haplotype *A* and both span a 38.9 kb region of chromosome 11 (which includes promoter regions of both *FADS1* and *FADS2* genes). Haplotype *D* spans across the entire *FADS1* gene and includes the first part of *FADS2* while haplotype *A* spans across the rest of *FADS2* (Ameer *et al.*, 2012).

In the population of African ancestry, haplotype *D* was found to be highly prevalent (Ameer *et al.*, 2012). In addition, Ameer *et al.* (2012) reported a low frequency of haplotype *A* amongst individuals of African descent, however, in populations of European and Asian descent the frequency was moderate to high. Forty-nine percent of the African population appear to carry a haplotype of mixed *FADS* which resembles haplotype *D* more than it resembles haplotype *A* (Ameer *et al.*, 2012). The study discovered that haplotype *D* associates significantly with lower levels of ALA and LA, which are essential precursors in the synthesis of LCPUFA. Haplotype *D* was also associated with higher levels of EPA, DHA, GLA, and AA which shows that haplotype *D* plays a remarkable part in the conversion of ALA and LA to LCPUFA (Ameer *et al.*, 2012). Participants carrying the homozygous genotype for haplotype *D* in this study also had increased levels of DHA and AA (24% and 43% higher levels respectively) (Ameer *et al.*, 2012).

The same researchers also discovered that individuals carrying the homozygous genotype for haplotype *D*, had a significantly increased expression of the *FADS1* gene in the liver than those harbouring the homozygous genotype for haplotype *A* (Ameer *et al.*, 2012). In the population of African descent, a high prevalence for the haplotype *D* as well as high linkage disequilibrium (LD) in the region of the *FADS* gene cluster was also observed (Ameer *et al.*, 2012). This indicates that this specific region in the genome has gone through positive selection and has attained fixation in the African population (Ameer *et al.*, 2012; Mathias *et al.*, 2011).

From a wide range of literature, the following genetic variants have been positively associated with LCPUFA concentration of participants. These identified genetic variants (in no particular order) include: rs102275, rs174448, rs174537, rs174545, rs174544, rs174546, rs174553, rs174556, rs99780, rs174561, rs174568, rs66698963, rs174570, rs174583, rs174589, rs174602, rs174611, rs174620, rs482548, rs526126, rs2072114, rs9688567, and rs968567 (Table 2.2).

Table 2. 2: Summary of FADS1 and FADS2 genetic variants

Gene Symbol	rs number	Ancestral Allele	Alternative Allele	Association	Reference
FADS2	rs102275	T	C	Reduced capacity to desaturate <i>n</i> -6 series PUFA.	(Barman <i>et al.</i> , 2015)
FADS2	rs174448	G	A	Reduced capacity to desaturate <i>n</i> -6 series PUFA.	(Barman <i>et al.</i> , 2015)
FADS1	rs174537	T	G	The presence of the G allele at <i>FADS</i> SNP rs174537 was significantly associated with higher levels of both <i>n</i> -6 and <i>n</i> -3 LCPUFA and genotypes had greater effects of the ratio of AA to DGLA than the effects AA alone or precursor-product ratios of <i>FADS2</i> activity, suggesting that <i>FADS1</i> activity, <i>FADS1</i> expression, or both, are related to genotypic variation in the <i>FADS</i> cluster.	(Mathias <i>et al.</i> , 2011; Reynolds <i>et al.</i> , 2018; Yeates <i>et al.</i> , 2015)
FADS1	rs174548	G	C/T	Variant within the <i>FADS</i> gene cluster impact the transcription of <i>FADS1</i> and <i>FADS2</i> in numerous tissues and raise important questions about how the inverse expression of these two genes impact intermediate molecular (such a LCPUFA and LCPUFA-containing glycerolipid levels) and ultimately clinical phenotypes associated with inflammatory diseases and brain health.	(Reynolds <i>et al.</i> , 2018)
FADS1	rs174545	G	C	Enhanced levels of the fatty acids LA, EDA, DGLA and ALA and <i>n</i> -3 DPA; and decreased levels of GLA, AA, adrenic acid, EPA and DPA.	(Bokor <i>et al.</i> , 2010; Rzehak <i>et al.</i> , 2010; Schaeffer <i>et al.</i> , 2006)
FADS1	rs174546	C	T	The minor allele was consistently associated with lower D5D activity, and associated with much higher levels of ALA and, to a lesser extent, with lower levels of EPA. This variant may be in linkage disequilibrium with a functional variant affecting D5D activity. The rs174546 variant is also in complete linkage disequilibrium with rs174547. Given that LA and ALA compete for D6D and D5D and that the dietary intake of LA is much higher than that of <i>n</i> -3, the enzymes are likely to be saturated with <i>n</i> -6 FA, and this situation would facilitate the expression of a genetic descent in the <i>n</i> -6 pathway. Contrarily, the ALA levels distinctly increase when both the competing effect of dietary LA and the genetic defect interact.	(Bokor <i>et al.</i> , 2010; Rzehak <i>et al.</i> , 2010; Schaeffer <i>et al.</i> , 2006)
FADS1	rs174553	G	A	Enhanced levels of the fatty acids LA, EDA, DGLA and ALA and <i>n</i> -3 DPA; and decreased levels of GLA, AA, adrenic acid, EPA and DPA.	(Schaeffer <i>et al.</i> , 2006)

Gene Symbol	rs number	Ancestral Allele	Alternative Allele	Association	Reference
FADS1	rs174556	C	T	Enhanced levels of the fatty acids LA, EDA, DGLA and ALA and n-3 DPA; and decreased levels of GLA, AA, adrenic acid, EPA and DPA.	(Reynolds <i>et al.</i> , 2018; Rzehak <i>et al.</i> , 2010; Schaeffer <i>et al.</i> , 2006)
FADS1	rs174561	C	T	Enhanced levels of the fatty acids LA, EDA, DGLA and ALA and n-3 DPA; and decreased levels of GLA, AA, adrenic acid, EPA and DPA.	(Rzehak <i>et al.</i> , 2010; Yeates <i>et al.</i> , 2015)
Inter-genic	rs174568	C	A/T	Enhanced levels of the fatty acids LA, EDA, DGLA and ALA and n-3 DPA; and decreased levels of GLA, AA, adrenic acid, EPA and DPA.	(Schaeffer <i>et al.</i> , 2006)
FADS2	rs66698963	ACTTCTCCCT GCCTCCCCA GGG	/	Associated with increased AA concentrations and AA to EPA+DHA ratio, reflecting basal risk of inflammatory and related chronic disease phenotypes, and is correlated with the risk of dyslipidaemia.	(Li <i>et al.</i> , 2018)
FADS2, inter-genic	rs3834458	T	/	Enhanced levels of the fatty acids LA, EDA, DGLA and ALA and n-3 DPA; and decreased levels of GLA, AA, adrenic acid, EPA and DPA.	(Schaeffer <i>et al.</i> , 2006)
FADS2	rs99780	T	C	Enhanced levels of the fatty acids LA, EDA, DGLA and ALA and n-3 DPA; and decreased levels of GLA, AA, adrenic acid, EPA and DPA.	(Schaeffer <i>et al.</i> , 2006)
FADS2	rs174570	C	T	Enhanced levels of the fatty acids LA, EDA, DGLA and ALA and n-3 DPA; and decreased levels of GLA, AA, adrenic acid, EPA and DPA.	(Schaeffer <i>et al.</i> , 2006)
FADS2	rs174589	C	G/T	Enhanced levels of the fatty acids LA, EDA, DGLA and ALA and n-3 DPA; and decreased levels of GLA, AA, adrenic acid, EPA and DPA.	(Lattka <i>et al.</i> , 2009; Malerba <i>et al.</i> , 2008; Schaeffer <i>et al.</i> , 2006)
FADS2	rs482548	C	T	Enhanced levels of the fatty acids LA, EDA, DGLA and ALA and n-3 DPA; and decreased levels of GLA, AA, adrenic acid, EPA and DPA.	(Schaeffer <i>et al.</i> , 2006)
FADS2	rs526126	G	C	Enhanced levels of the fatty acids LA, EDA, DGLA and ALA and n-3 DPA; and decreased levels of GLA, AA, adrenic acid, EPA and DPA.	(Bokor <i>et al.</i> , 2010; Schaeffer <i>et al.</i> , 2006)
FADS2	rs2072114	G	A	Enhanced levels of the fatty acids LA, EDA, DGLA and ALA and n-3 DPA; and decreased levels of GLA, AA, adrenic acid, EPA and DPA.	(Schaeffer <i>et al.</i> , 2006)
FADS1	rs174544	C	A	Enhanced levels of the fatty acids LA, EDA, DGLA and ALA and n-3 DPA; and decreased levels of GLA, AA, adrenic acid, EPA and DPA.	(Schaeffer <i>et al.</i> , 2006)
Inter-genic	rs9688567	T	C	Enhanced levels of the fatty acids LA, EDA, DGLA and ALA and n-3 DPA; and decreased levels of GLA, AA, adrenic acid, EPA and DPA.	(Schaeffer <i>et al.</i> , 2006)
FADS2	rs174583	T	C	Enhanced levels of the fatty acids LA, EDA, DGLA and ALA and n-3 DPA; and decreased levels of GLA, AA, adrenic acid, EPA and DPA.	(Schaeffer <i>et al.</i> , 2006)
FADS1	rs968567	C	T	Enhanced levels of the fatty acids LA, EDA, DGLA and ALA and n-3 DPA; and decreased levels of GLA, AA, adrenic acid, EPA and DPA.	(Schaeffer <i>et al.</i> , 2006)

Gene Symbol	rs number	Ancestral Allele	Alternative Allele	Association	Reference
FADS2	rs174602	C	T	The minor allele was associated with higher levels of LA and lower levels of AA. The minor allele was also associated with lowered D5D activity.	(Bokor <i>et al.</i> , 2010; Schaeffer <i>et al.</i> , 2006)
FADS2	rs174620	G	A	Enhanced levels of the fatty acids LA, EDA, DGLA and ALA and n-3 DPA; and decreased levels of GLA, AA, adrenic acid, EPA and DPA.	(Schaeffer <i>et al.</i> , 2006)
FADS2	rs174611	T	C	Enhanced levels of the fatty acids LA, EDA, DGLA and ALA and n-3 DPA; and decreased levels of GLA, AA, adrenic acid, EPA and DPA.	(Lattka <i>et al.</i> , 2009; Malerba <i>et al.</i> , 2008)
FADS1	rs174547	C	T	The minor allele C was associated with higher levels of LA, DGLA, and ALA, and lower levels of GLA, DTA, EPA and DPA.	(Hu <i>et al.</i> , 2016)
FADS1	rs174549	A	G	Associated with lower FADS1 transcript levels in the human liver.	(Mathias <i>et al.</i> , 2011)
FADS1	rs174560	C	T	SNPs within the <i>FADS</i> gene cluster impact the transcription of <i>FADS1</i> and <i>FADS2</i> in numerous tissues and raise important questions about how the inverse expression of these two genes impact intermediate molecular (such as LCPUFA and LCPUFA-containing glycerolipid levels) and ultimately clinical phenotypes associated with inflammatory diseases and brain health.	(Reynolds <i>et al.</i> , 2018)
FADS2	rs174577	A	C	SNPs within the <i>FADS</i> gene cluster impact the transcription of <i>FADS1</i> and <i>FADS2</i> in numerous tissues and raise important questions about how the inverse expression of these two genes impact intermediate molecular (such a LCPUFA and LCPUFA-containing glycerolipid levels) and ultimately clinical phenotypes associated with inflammatory diseases and brain health.	(Reynolds <i>et al.</i> , 2018)
FADS2	rs174583	T	C	SNPs within the <i>FADS</i> gene cluster impact the transcription of <i>FADS1</i> and <i>FADS2</i> in numerous tissues and raise important questions about how the inverse expression of these two genes impact intermediate molecular (such a LCPUFA and LCPUFA-containing glycerolipid levels) and ultimately clinical phenotypes associated with inflammatory diseases and brain health.	(Reynolds <i>et al.</i> , 2018)
FADS2	rs174616	G	A	The minor allele was associated with higher levels of LA and lower levels of AA. The minor allele was also associated with lowered D5D activity.	(Bokor <i>et al.</i> , 2010; Brayner <i>et al.</i> , 2018)
FADS2	rs2072114	A	G	The minor allele was associated with higher levels of LA and lower levels of AA. The minor allele was also associated with lowered D5D activity.	(Bokor <i>et al.</i> , 2010)

Alpha-linolenic acid (ALA); eicosapentaenoic acid (EPA); docosapentaenoic acid (DPA); docosahexaenoic acid (DHA); linoleic acid (LA); arachidonic acid (AA); dihomogamma linoleic acid (DGLA); long chain polyunsaturated fatty acid (LCPUFA).

2.6 Conclusion

In summary, the *FADS1* and *FADS2* genes code for the desaturase enzymes and therefore play a major role in LCPUFA metabolism. The identification of genetic variants and haplotypes present in the South African population, and how these genetic variants or haplotypes influence the LCPUFA metabolism would be of great value. It is important to understand the appropriate metabolic pathways of the South African population to tailor-make strategies and dietary recommendations, to optimise fatty acid status and its associated health benefits in South Africa. We do, however, not know what the n-3 status of South African women is at entry of pregnancy, and this needs to be confirmed before supplementation programs can be designed.

The information gained from this study gives an opportunity to be familiar with the PUFA status at entry of pregnancy in black South African women, to ensure an adequate n-3 status before pregnancy by means of dietary recommendations and interventions, and to design n-3 supplementation programs for pregnant women (when to start supplementation, amount, frequency, etc.). Further, we can use the information gained from this study to design dietary interventions to not only optimise n-3 status before pregnancy, but also to optimise n-3 status of all South Africans. This would mean that men, women, and children of all ages would be able to benefit from these results.

CHAPTER 3: MANUSCRIPT

Genetic variants in the *FADS1/2* gene cluster of pregnant women of African descent is associated with altered estimated delta-5 and -6 fatty acid desaturase activities.

Anika Prinsloo¹, Linda Malan¹, Tsitsi Chimashu^{1,2}, Lizelle Zandberg^{1*}

¹ Centre of Excellence for Nutrition, North-West University, Potchefstroom, South Africa

² Division of Human Nutrition, Wageningen University, P.O. Box 8129, 6700 EV Wageningen, Netherlands

**Corresponding author: Lizelle Zandberg*
Lizelle.Zandberg@nwu.ac.za
+27 18 299 4018
Centre of Excellence for Nutrition
North-West University
Potchefstroom, 2520
South Africa

Keywords: Long chain polyunsaturated fatty acids, LCPUFA, Fatty acid desaturases; D5D, D6D, *FADS1*; *FADS2*; *FADS1/2*; Phospholipid fatty acids status, Pregnancy, African women

Key findings / Highlights: Irrespective of dietary intake, genetic variation in this African population-based study largely relates to higher D5D and D6D activities and therefore an improved n-3 LCPUFA status.

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Abstract

Background

Apart from dietary intake, red blood cell (RBC) total phospholipid fatty acid (FA) composition depends on the FA desaturase enzyme activities which are coded for by the *FADS1* and *FADS2* genes.

Methods

In this cross-sectional study of 243 pregnant women of African descent at <18 weeks of gestation, the relationship of D5D and D6D activity-estimating polyunsaturated FA (PUFA) ratios and the n-6:n-3 long-chain (LC) PUFA ratio, with genetic variants of the *FADS1* and *FADS2* genes were determined.

Results

We found that the alternative allele carriers of *FADS1*-rs174546_(C/T) and *FADS2*-rs112425493_(G/A) associated with lower AA:DGLA ($P<0.001$) and higher DGLA:LA ratios ($P<0.001$), indicative of lower D5D and higher D6D activities, respectively. The alternative allele carriers for *FADS*-rs174547_(C/T), *FADS1*-rs174549_(A/G), *FADS1*-rs174555_(C/T) and *FADS2*-rs73489307_(T/C), associated with higher DGLA:LA or estimated D6D activity ($P=0.038$, $P=0.04$, $P=0.05$, and $P=0.035$, respectively) compared to the homozygous ancestral genotypes. *FADS2*-rs78678033_(G/A) associated with higher AA:DGLA ratio or estimated D5D activity ($P=0.018$) and lower DGLA:LA ratio or D6D activity ($P=0.038$). Alternative allele carriers of *FADS2*-rs174606_(T/G) and *FADS2*-rs174616_(G/A) associated with lower AA:LA ($P=0.061$; $P=0.053$) and lower DGLA:LA ratios ($P=0.03$; $P=0.014$), or lower combined D5D and D6D activity, and lower individual D6D activity. The associations held when correcting for dietary intake of alpha-linolenic acid (ALA), linoleic acid (LA), docosahexaenoic acid (DHA), arachidonic acid (AA), and total energy.

Conclusion

This data demonstrates that LCPUFA status and estimated D5D and D6D activity are genetically controlled by variants in the *FADS1/2* gene cluster. As desaturase enzymes have a higher affinity for n-3 PUFA, the variants in this sample which favoured increased D5D and D6D activities also favoured higher n-3 LCPUFA status. An important finding is that the relationship between *FADS1/2* gene cluster variants and LCPUFA status seems to be independent of dietary n-6 and n-3 essential and LCPUFA intake in this population.

Keywords: Long chain polyunsaturated fatty acids, LCPUFA, Fatty acid desaturases; D5D, D6D, *FADS1*; *FADS2*; *FADS1/2*; Phospholipid fatty acids status, Pregnancy, African women

1. Introduction

Globally, the dietary shift over the years to a Western diet has caused a drastic change in the intake ratio of omega-6: omega-3 (n-6:n-3) polyunsaturated fatty acids (PUFA) from 1:1 to 20-30:1 [1]. A recent study reported that total fat intake increased from 21% to 30% of energy in urban South African women, and from 15.5% to 21% of energy in rural South African women from 1996 to 2005 [2]. The intake of saturated fatty acids (SFA) varied at 3.9% – 9.1%, monounsaturated fatty acids (MUFA) at 4.2% – 10.4%, and PUFA at 5.5% – 8.3% of total energy intake. Mean PUFA intakes were <10% of energy, with some studies showing <6% of energy [2]; the lower end of the range recommended for PUFA intake [3, 4]. The low PUFA intakes were especially applicable to those with a lower total fat intake (17.6% – 23.8% of energy from fat), and thus compromise the PUFA intake as a result of too low total fat [2].

Many South Africans do not have adequate access to ocean sources of long-chain polyunsaturated fatty acid (LCPUFA) and would benefit from less expensive, plant-based LCPUFA [5]. Plant based LCPUFA are relatively inexpensive, sustainable, easily accessible to the population, and can be obtained from sources such as canola oil [2, 6], but still need to be metabolised from essential n-6 (linoleic acid; LA, 18:2n-6) and n-3 (alpha-linolenic acid; ALA, 18:3n-3) PUFA to LCPUFA [7]. The LCPUFA can be obtained from the diet or can be synthesized endogenously from essential n-3 and n-6 LCPUFA precursors. Through a series of elongation and desaturation steps, delta 5 desaturases (D5D) and delta 6 desaturases (D6D) facilitate the synthesis process [8, 9], see **Figure 1**. Studies done using fish [10, 11] and rodent models [12, 13], suggested that desaturase enzymes have a higher affinity for n-3 PUFA than for n-6 PUFA. The estimated D5D and D6D activities are studied by the use of product-to-precursor ratios [14].

The conversion of ALA to docosahexaenoic acid (DHA) tends to be more effective in children as well as in young women of childbearing age when compared to young men [15]. Young women of childbearing age convert roughly 21% of dietary ALA to eicosapentaenoic acid (EPA) and 9% to DHA, respectively [16] in comparison to young men converting only 8% of the dietary ALA to EPA and 0% – 4% to DHA [17]. Oestrogen has been indicated as a possible mediator for the higher capacity to metabolise ALA into LCPUFA in young women of childbearing age compared to men [18, 19]. The higher conversion of precursor to functional LCPUFA in women of childbearing age is speculated to be an adaptation important to maintain the higher requirements of LCPUFA needed during pregnancy and lactation [20, 21]. Evidently, during pregnancy, the maternal metabolism is modified and altered in order to support the nutritional requirements of the foetus. Early pregnancy is a vulnerable period for the development of the neuro- and immune systems of the foetus [22]. Essential fatty acids

(FA) and LCPUFA are crucial for normal foetal growth and development and are selectively transferred from the mother to the foetus. The predominant FA in the brain tissue is DHA, particularly in the synaptic membranes and retina [22, 23]. The maternal FA status at entry of pregnancy is therefore exceptionally important since it gives the mother a better capacity for the biomagnification of DHA to the foetus during the third trimester [24, 25].

Genome-wide association studies (GWASs) have identified multiple genetic loci that contribute to inter-individual variations in SFAs, trans fatty acids (TFA), MUFA, and PUFA concentrations in whole plasma, plasma phospholipids, and erythrocyte membrane phospholipids [26-28]. Biosynthesis of LCPUFA is associated with genetic variation within the fatty acid desaturase (*FADS*) gene cluster (chromosome 11 bp61567097 – 61634826) containing *FADS1* and *FADS2* genes that encode for D5D and D6D in the LCPUFA biosynthesis pathway [29].

Recent studies on the association between plasma LCPUFA concentration (AA, EPA, DGLA) and *FADS1/2* gene cluster variants (rs174537, rs102274, rs174546, rs1535, rs174576, rs174597) suggest that there is a dramatic difference in LCPUFA levels and product-precursor ratios in total plasma lipids between African Americans and European Americans in the United States, indicating a better pathway efficiency in African Americans. The evidence points to enhanced *FADS1* efficiency in African Americans [30, 31]. An investigation of FA intake in relation to red blood cell (RBC) FA composition revealed that even though black African rural children from the Limpopo Province had the lowest essential FA intake (n-3 and n-6), they had the highest RBC phospholipid DHA composition. This suggests a more efficient conversion from ALA to DHA [32] and could be related to the genetic makeup of the black African population.

The aim of this study was to characterise the genetic variations of the *FADS1/2* gene cluster of pregnant women of African descent living in Johannesburg, South Africa. The genetic variations were to be investigated for associations with LCPUFA status and estimated desaturase activity using precursor-to-product ratios of RBC phospholipid FA at early pregnancy (<18 weeks gestation). The study further investigated whether the relationships are dietary FA intake dependent or endogenously controlled.

2. Methods

Study design and participants

This cross-sectional study was nested within the Nutrition during Pregnancy and Early Development (NuPED) cohort conducted in Johannesburg, South Africa. The protocol of the NuPED study has previously been published [33]. In short, the recruitment was conducted at primary healthcare clinics in Johannesburg from March 2016 to November 2017. Generally healthy pregnant women were recruited and were eligible for inclusion if they were 18 – 39 years of age, had singleton pregnancies of <18 weeks gestation, were proficient in local languages, were South African or born in neighbouring countries, and have been residing in Johannesburg for >12 months. Exclusion criteria included illicit drug usage, smoking, having a non-communicable disease (hypertension, high blood cholesterol, diabetes, and renal disease), - or infectious disease (tuberculosis and hepatitis), - or a serious illness (cancer, lupus or psychosis). Women who were HIV positive were included in the study to assure a better representation of the general population, due to South Africa's high prevalence of HIV (36%) in women 30 – 34 years of age [34]. A total of 250 women volunteered and agreed to participate in the study and were followed up at the antenatal clinic up until June 2018; 243 of these women gave informed consent to take part in genetic related studies such as this one. Ethical approval for this study was obtained from the Health Research Ethics Committee (HREC) of the Faculty of Health Sciences of the North-West University (NWU), Potchefstroom (NWU-00186-15-A1-08) and the study was conducted according to the Declaration of Helsinki. Written informed consent was obtained from the participant allowing for the collection of all the data, the blood sample, and genetic analysis. Only those who consented for genetic testing were included in the genetic analysis

Dietary intake

For this study, maternal dietary intake data was obtained by means of an interviewer administered quantified food frequency questionnaire (QFFQ) that used a set of standardised probing questions [35]. The QFFQ was validated for the population in the Transition and Health during Urbanisation of South Africans (THUSA) study [36] and its reproducibility has been proven [37-39]. According to the 140 food items listed in the QFFQ, the type/brand, cooking methods, frequency and the amount of all food and drink consumed in the past four weeks, was asked from the participating women. The QFFQ data and portion sizes were converted to grams per week per food item, by two registered dietitians using the Condensed Food Composition Tables for South Africa [40] and the South African Medical Research Council (SAMRC) Food Quantities Manual [41]. Dietary data was captured in Microsoft Excel (Microsoft Corporation, Washington, USA); all electronic entries were double checked to

ensure the correct food code was used and that a realistic amount was captured. Raw data was captured in Microsoft Access (Microsoft Corporation, Washington, USA) and 20% of all captured data was randomly checked for correctness.

Biological sample collection

Venous blood was drawn from the participants into labelled ethylenediamine tetra-acetic acid (EDTA)-coated, serum and trace element free evacuated tubes (Becton Dickinson) *via* antecubital venepuncture. Venous blood was processed within one hour after the blood was drawn. Plasma/serum was separated, and red blood cells (RBC) were washed twice with normal saline and stored for phospholipid fatty acid analysis. Buffy coats were stored in 1:1 volume:volume RNeasy (Qiagen) for DNA and RNA isolation.

Phospholipid fatty acid analysis using GS-MS

Lipids were extracted from RBCs with chloroform:methanol by a modification of the method of Folch *et al.* [42]. The lipid extracts were concentrated, and the neutral lipids separated from the phospholipids by thin layer chromatography (TLC) and eluted with diethyl ether: petroleum ether:acetic acid. The lipid band containing phospholipids was removed from the TLC plate and transmethylated with methanol:sulphuric acid to yield FA methyl esters (FAME). The resulting FAME were extracted with water and hexane. The organic layer was evaporated and re-dissolved in hexane. FAMES were analysed with an Agilent Technologies 7890A gas chromatograph system equipped with an Agilent Technologies 7000B triple quad mass selective detector (Agilent Technologies). Mass spectrometry was carried out in positive impact multiple reaction monitoring mode, with at least two transitions per compound. Quantification of FAMES was performed with MassHunter (B.06.00). FAME peaks were identified and calibrated against a standard reference mixture of 33 FAMES (Nu-Check-Prep) and two single FAME standards (Larodan Fine Chemicals AB). Relative percentages of FA were calculated by taking the concentration of a given FA derivative as a percentage of the total concentration of all FAs identified in the sample.

Estimated desaturase activities: Precursor-to-product ratios

The arachidonic acid (AA; C20:4 n-6): LA (C18:2 n-6) ratio was used to determine the overall desaturase activity without distinguishing between D5D- and D6D activities [43-45]. The estimated D5D and D6D activities were determined by the dihomo-gamma-linolenic acid (DGLA; C20:3n-6): LA (C18:2n-6) and AA (C20:4n-6): DGLA (C20:3n-6) ratios, respectively [14, 43]. AA:LA ratio was used to determine the combined D5D-, D6D- and elongase enzymatic activity in the n-6 LC-PUFA pathway, and the eicosapentaenoic acid (EPA; C22:5n-3): ALA (C18:3n-3) ratio was used to determine the same enzymatic activities of the n-3 LC-PUFA pathway. The ratio of docosahexaenoic acid (DHA; C22:6n-3): ALA was used to

determine D5D, D6D, elongase and an additional D6D enzymatic activity whereas DHA:EPA was used to determine D6D activity and β -oxidation.

DNA isolation, targeted gene sequencing and genotyping

Genomic DNA (gDNA) were isolated from buffy coats using the Maxwell[®] 16 instrument and Maxwell[®] 16 DNA Purification Kit (AS1010) was used for DNA isolation (Promega Corporation, USA). DNA quantification was performed using the NanoDrop[®] ND-1000 UV-Vis Spectrophotometer by Thermo Fischer Scientific (Waltham, Massachusetts, USA). Ion Torrent amplicon panel sequencing (Ion AmpliSeq[™]) was performed on a subset of 32 samples, of whom 16 had an RBC composition ratio of n-6:n-3 LCPUFA below the mean and 16 above the mean. A custom Ion AmpliSeq[™] panel was designed using the online AmpliSeq designer (<https://ampliseq.com/>, last accessed July 2020). This customised panel consisted of multiple genes, including a 100% coverage for the *FADS1/2* gene cluster. Library preparation was performed using the Ion Chef[®] as per manufacturer's specifications. A total of 10 ng gDNA (0.67 ng/ μ L) was used as input volume. The Ion AmpliSeq[™] Kit was used for library and template preparation for Chef DL8 for 32 reactions (CAT# A29024) and the Ion 510[™] & Ion 520[™] & Ion 530[™] Kit – Chef (CAT# A34019) respectively. The entire coding region of each selected gene, including flanking regions of introns-exons, was sequenced with the 200bp chemistry, using an Ion 530[™] Chip Kit-4 Reactions (CAT# A27763) and the Ion S5[™] System (CAT# A27212, ThermoFisher, MA, USA).

Bioinformatics: Variant calling, quality assessment and variant selection

Primary data analysis of raw reads obtained from the Ion S5[™] System were analysed with the use of the Torrent Suite (v.5.8). Primary analysis included signalling, base calling and quality control of the bases and were filtered according to a Phred quality score of 20. The sequence files were aligned against Genome Reference Consortium Human Build 37 (hg19), followed by coverage analysis and variant calling by means of the coverage analysis and variant caller plugins from the Torrent Suite. Secondary data analysis of the variant caller files was annotated, filtered and mined according to the process followed by Schoonen, Seyffert [46]. Variants of interest were validated and genotyped for all the included participants.

Variant selection and genotyping using iPLEX[®] MassARRAY

Variants identified with targeted gene sequencing were assessed and considered for validation if a differential $P < 0.001$ between lower and higher n-3 LCPUFA status groups and missingness (5%) of variants were achieved. Genetic variants that passed QC were further assessed for known literature and genomic position and predicted functionality using ensemble genome browser [47]. Genotyping of selected variants was done using the iPLEX[®] MassARRAY system from Agena Bioscience[™]. Analyses were performed by Inqaba

Biotechnology (Pretoria, South Africa). Assays were designed in-house using the Assay Design Suite (ADS) software. The gDNA was amplified in 96 microliter plates using reagents supplied in the iPLEX reagent kits. A nano dispenser RS1000 was used to transfer samples from microtiter plates to a SpectroCHIP® array. Data was obtained from the SpectroCHIP® array using the MassARRAY® analyser. Reports were automatically generated by Typer. Genotype calls were made in real-time during MALDI-TOF analysis and data was automatically saved to the MassARRAY® database.

Statistical analysis

This genetic association study was an exploratory study nested within the NuPED study. Sample size calculation was based on a small effect size F_2 of 0.05; a probability of error (α) of 5%; a power of 80%. The result of the calculation indicated that a total of 196 participants were required in the main study. A dropout rate of 25% was taken into account, and a minimum of 245 participants were considered to be recruited for the NuPED study. The NuPED study had a sample size of 250 pregnant women of African descent. Three women did not agree to genetic testing and were therefore not included in genotyping analysis. Statistical analysis and data processing were performed using SPSS version 26 (SPSS Inc, Chicago, IL, USA). Data was tested for outliers and normality by means of Q-Q plots, histograms, and Shapiro-Wilk tests. Normally distributed data was expressed as mean $\pm$ standard deviation (SD) and non-normally distributed data as median (IQR). Type-1 error (α) was set at 0.05 (5%) and P values of <0.05 were considered significant.

The participants were divided into two groups depending on their n-6:n-3 LCPUFA ratio. The group with an above the mean n-6:n-3 LCPUFA ratio also referred to as the lower n-3 LCPUFA status group, and the group with a below the mean n-6:n-3 LCPUFA ratio also referred to as the higher n-3 LCPUFA status group. The higher n-3 LCPUFA status group is considered the group of women with a more favourable status, whereas the women in the group with lower n-3 LCPUFA ratios are considered to have a less favourable LCPUFA status [22, 23] [48-51]. To test for significant differences of characteristics between n-3 LCPUFA status groups, independent T-tests were used. Univariate analyses per outcome were performed using analysis of covariance (ANCOVA) with Bonferroni post hoc tests to assess differences between genotypes, controlling for gestational age, age of mother, as well as dietary intakes (total energy-, ALA-, LA-, DHA- and AA intake) according to which the data was structured in six different statistical models. Model 1 adjusted for the mother's age and the gestational age. Model 2 adjusted for mother's age, gestational age and total energy intake; model 3 adjusted for mother's age, gestational age and ALA intake; model 4 adjusted for mother's age, gestational age and LA intake; model 5 adjusted for mother's age, gestational age, and DHA intake; and finally model 6 adjusted for mother's age, gestational age and AA intake. The six

models were applied to three genetic models 1) additive, 2) recessive and 3) dominant models. In the additive model the three genotypes, homozygous ancestral (MM) heterozygous (Mm) and homozygous alternative alleles (mm) were assessed as three separate groups. The recessive model considers the homozygous ancestral (MM) + heterozygous (Mm) groups as one versus the homozygous alternative (mm), whereas the dominant model considers the homozygous ancestral (MM) *versus* the combination of the heterozygous (Mm) + homozygous alternative (mm) groups.

3. **Results**

In total, 250 eligible pregnant women living in Johannesburg, South Africa, were enrolled for the NuPED study, and completed data collection at <18 weeks of gestation. Women of African descent who met the inclusion and exclusion criteria and agreed to genetic testing were included in this study (n=243). The participants were divided into two groups depending on their total phospholipid n-6:n-3 LCPUFA ratio. With the mean n-6:n-3 LCPUFA ratio being 3.5, the above the mean (>3.5) group is referred to as the lower n-3 LCPUFA status group, and below mean (≤ 3.5) group is referred to as the higher n-3 LCPUFA status group. The demographics and characteristics of the participants were done for the total group (n=250) as well as a comparison between higher and lower n-3 LCPUFA status groups reported in **Error! Reference source not found.**

The predominantly black African (88%; 220/250) cohort was comprised of mainly South Africans (72%; 180/250), Zimbabweans (25%; 62/250) and the remaining 3% (8/250) were from neighbouring South African countries. HIV positive women made up 26 % (65/250) of the sample population. The median age of the participants (n=243) was 27 (25-32), the height 159 $\pm$ 6.2 cm and middle upper arm circumference (MUAC) was 30.3 $\pm$ 4.68 cm, and compared well between the two groups. The body mass index (BMI) was 26.3 (7.62) kg/m² with 33% (80/243) of the participants being overweight and 28% (68/243) obese. The BMI showed a trend towards significance when comparing between the higher and lower n-3 LCPUFA status groups (P=0.059). The median of the gestational age was 14 weeks at enrolment (12-16 weeks) and differed between the higher and lower n-3 LCPUFA status groups (P=0.021) with women in the lower LCPUFA status group enrolled at earlier gestation comparing to their higher LCPUFA status counterparts.

The dietary intakes were also assessed and summarised in **Table 3.1**. The mean total energy intake (kJ) for the n=243 women was 11063 $\pm$ 3709 and comparable between the higher and lower n-3 LCPUFA status groups. Total PUFA, total n-6 PUFA, LA, EPA, DPA and DHA intakes differed between the two groups with a higher dietary intake of total PUFA (P=0.042),

total n-6 LCPUFA ($P=0.019$), LA ($P=0.019$), EPA ($P=0.006$), DPA ($P=0.024$) and DHA ($P=0.003$) for the higher n-3 LCPUFA status group when compared to the lower n-3 LCPUFA status group. The ratio of n-6:n-3 LCPUFA intake for $n=243$ was 26:1, for the higher n-3 LCPUFA status group the ratio was 25:1, and for the lower n-3 LCPUFA group the ratio was 26:1. Seemingly, these n-6:n-3 LCPUFA ratios are driven by the higher n-6 LCPUFA together with a lower EPA and DHA intake.

The total RBC phospholipid FA composition (%) of the 243 women at <18 weeks gestation was measured and differed between the higher and low n-3 LCPUFA status groups (**Table 3.1**). The higher n-3 LCPUFA status group had higher compositions of ALA ($P<0.001$), EPA ($P<0.001$), DPA ($P<0.001$), DHA ($P<0.001$) and lower AA ($P=0.048$) and DGLA ($P=0.02$), but comparable LA ($P=0.759$) when compared to the lower n-3 LCPUFA status group. The n-3 index and total n-6:n-3 LCPUFA ratios differed between groups ($P<0.001$, $P<0.001$ respectively), with higher n-3 index and lower n6:n3 ratios for the higher n-3 LCPUFA status group comparing the lower n-3 LCPUFA status group. Furthermore, the estimated desaturase activities were also assessed by means of precursor-to-product ratios. The higher n-3 LCPUFA status group had higher EPA:ALA ($P=0.024$) and DHA:ALA ($P=0.018$) ratios, lower DGLA:LA ($P=0.024$) and DHA:EPA ($P<0.001$) ratios than the lower n-3 LCPUFA status group.

3.1 Characterisation of genetic variants in the *FADS1* and *FADS2* gene cluster

The *FADS1/2* gene cluster ranging from 61 567 097bp to 61 634 826bp of 32 samples were sequenced with 100 % coverage. The sequenced samples had 7813 variants in total, of which 36 variants had a significant differential P-value when comparing the lower and higher n-3 LCPUFA status groups. All variants were assessed for quality, biological relevance, genomic position and possible predictive functionality. A total of nine variants were selected for further study. The nine variants (*FADS1*-rs174546, *FADS1*-rs174547, *FADS1*-rs174549, *FADS1/2*-rs174555, *FADS2*-rs174606, *FADS2*-rs174616, *FADS2*-rs78678033, *FADS2*-rs73489307, and *FADS2*-rs112425493) were genotyped in all 243 samples using iPLEX® MassARRAY technology. **Figure 3.2** depicts the position of the selected variants in context of genomic position on chromosome 11.

The nine genetic variants all adhered to the Hardy Weinberg equilibrium (HWE) and the minor allele frequencies are given in **Table 3.3**. The nine selected genetic variants from the *FADS1/2* gene cluster had minor allele frequencies ranging from 0.007 (rare in the sample population) to 0.384 (more common in the population). These variants were all found in non-coding regions of the *FADS1/2* gene cluster. In this study, ancestral and alternative alleles are used

to report the genotypes of the population. The distribution of ancestral (M) and alternative (m) alleles are reported as homozygous for the ancestral allele (MM), heterozygous (Mm) or homozygous for the alternative allele (mm). The genotype distribution (MM/Mm/mm) for the nine selected variants are as follows: *FADS1*-rs174546 (221/16/0), *FADS1*-rs174547 (12/18/213), *FADS1*-rs174549 (9/18/216), *FADS1/2*-rs174555 (111/19/113), *FADS2*-rs174606 (92/98/40), *FADS2*-rs174616 (38/92/100), *FADS2*-rs78678033 (171/51/4), *FADS2*-rs73489307 (226/9/1), and *FADS2*-rs112425493 (211/4/1).

3.2 Associations of genetic variants with LCPUFA ratios and desaturase activity

The variants were assessed for possible associations with the estimated desaturase activities given in **Table 3.2**, using ANCOVA. The six different statistical models were applied to three genetic models.

The *FADS1*-rs174546_(C/T) and *FADS2*-rs112425493_(G/A) both associated with AA:DGLA and DGLA:LA ratios. For *FADS1*-rs174546_(C/T), the T allele carriers_(CT+TT) had lower AA:DGLA ($P<0.001$) and higher DGLA:LA ($P<0.001$) ratios compared to the homozygotes_(CC). For the *FADS2*-rs112425493_(G/A) variant, the A allele_(GA+AA) carriers had a similar pattern with lower AA:DGLA ($P=0.053$) and higher DGLA:LA ($P=0.016$) ratios compared to the homozygotes_(GG). The associations were retained when adjusting for age, gestational age, and total energy intake, or individual intakes of ALA, LA, DHA and AA, respectively. Thus, the T and A alleles of the respective variants *FADS1*-rs174546_(C/T) and *FADS2*-rs112425493_(G/A) associated with lower estimated D5D and higher estimated D6D activities independent of dietary intakes (**Table 4** and **Table 5**).

Four variants, *FADS1*-rs174547_(C/T), *FADS1*-rs174549_(A/G), *FADS1/2*-rs174555_(C/T) and *FADS2*-rs73489307_(T/C) associated with DGLA:LA ratio, indicative of the estimated D6D activity. The alternative allele carriers *FADS1*-rs174547_(CT+TT), *FADS1*-rs174549_(AG+GG), *FADS1/2*-rs174555_(CT+TT) and *FADS2*-rs73489307_(TC+CC), associated with higher DGLA:LA ratios ($P=0.038$, $P=0.04$, $P=0.05$, and $P=0.035$ respectively) compared to the homozygotes. The associations were retained when adjusting for total energy intake, and ALA, LA, DHA and AA dietary intakes, respectively. Thus, the alternative alleles of these variants associated with higher estimated D6D activity and independent of dietary intake (**Table 6-9**).

FADS2-rs78678033_(G/A), a novel variant that is reported here for the first time, associates with estimated desaturase activity. In this study sample, the allele distribution of this variant adhered to HWE ($P=1.00$) and a minor allele frequency of 0.127 (MM/Mm/mm; 171/51/4). *FADS2*-rs78678033_(G/A) associated with AA:DGLA ($P=0.018$) and DGLA:LA ($P=0.038$) ratios.

The A allele carriers_(GA+AA) had higher AA:DGLA and lower DGLA:LA ratios when compared to the homozygotes_(GG), meaning that the A allele carriers have higher estimated D5D and lower estimated D6D activity. The associations were retained when adjusting for total energy intake, and ALA, LA, DHA and AA dietary intake, respectively (**Table 10**), suggesting the association to be independent of dietary intake.

FADS2-rs174606_(T/G) associated with AA:LA ($P=0.07$) and *FADS2*-rs174616_(G/A) had a trend towards association with AA:LA ($P=0.083$). *FADS2*-rs174606_(T/G) had a trend for associating with DGLA:LA ratio ($P=0.058$) whereas *FADS2*-rs174616_(G/A) associated with DGLA:LA ratio ($P=0.034$). For *FADS2*-rs174606_(T/G), the G allele carriers_(TG+GG) had lower DGLA:LA ratio ($P=0.03$) and trend towards a lower AA:LA ratio ($P=0.061$) compared to the homozygotes_(TT). The *FADS2*-rs174616_(G/A) variant had a similar pattern with lower DGLA:LA ($P=0.014$) and trend towards lower AA:LA ($P=0.053$) ratios for the A allele carriers_(AG+AA) comparing to the homozygotes_(GG). The associations were retained when adjusting for total energy intake, and ALA, LA, DHA and AA dietary intakes, respectively. Therefore, the G alleles for both *FADS2*-rs174606_(T/G) and *FADS2*-rs174616_(G/A) variants associated with lower combined estimated D5D and D6D activity, as well as lower individual estimated D6D activity (**Table 11** and **Table 12**).

The carriers for the G allele for *FADS2*-rs112425493_(G/A), T allele for *FADS1*-rs174546_(C/T), T allele for *FADS1*-rs174547_(C/T), and the G allele for *FADS1*-rs174549_(A/G) all associated or tended to associate with a lower n-6:n-3 LCPUFA ratio and therefore with a higher n-3 LCPUFA status, as seen in the model 1 analysis of the recessive models each in Table 3.4 ($P=0.033$), Table 3.5 ($P=0.059$), Table 3.6 ($P=0.062$) and Table 3.8 ($P=0.071$), respectively. To assess the number of participants that were either carriers or homozygotes for the genetic variants that improved n-3 LCPUFA status, we selected the participants who were carriers or homozygotes simultaneously of the G allele for *FADS2*-rs112425493_(G/A), T allele for *FADS1*-rs174546_(C/T), T allele for *FADS1*-rs174547_(C/T), and the G allele for *FADS1*-rs174549_(A/G). It was found that 207/243 (85%) participants had the GTTG combination, of which 188/243 (77%) were homozygous for the four mentioned variants. There was no difference in n-3 LCPUFA status between the homozygotes and non-carriers of these variants.

4. Discussion

The most profound finding of this study is that the genetic variation in this sub sample of an African population-based study largely related to higher D5D and D6D activities and thus improved n-3 LCPUFA status, irrespective of individual n-3 or n-6 essential PUFA or LCPUFA dietary intakes. This finding supports the notion that genetic drivers in the *FADS1/2* gene cluster enhance the endogenous synthesis of functional LCPUFA in people from African descent. It was remarkable that the associations between the studied genetic variants and estimate desaturase activities were all independent of dietary intake which suggest a predominantly endogenously controlled pathway dictated by the underpinning genetic makeup.

Nine genetic variants associated with estimated D5D and D6D activities, regardless of the dietary intake of individual n-3 or n-6 essential PUFA (ALA and LA) or LCPUFA (DHA and AA). *FADS2*-rs78678033_(G/A) associated with higher D5D activity, and *FADS1*-rs174547_(C/T), *FADS1*-rs174549_(A/G), *FADS1/2*-rs174555_(C/T), *FADS2*-rs73489307_(T/C), *FADS1*-rs174546_(C/T) and *FADS2*-rs112425493_(G/A) associated with higher D6D activity. Furthermore, A allele carriers of *FADS2*-rs112425493_(G/A), T allele carriers of *FADS1*-rs174546_(C/T), T allele carriers of *FADS1*-rs174547_(C/T), and G allele carriers of *FADS1*-rs174549_(A/G) had lower n-6:n-3 LCPUFA ratios or tendencies to be lower, and thus better n-3 LCPUFA status, which were also independent from dietary intake of the same individual n-3 and n-6 essential PUFA and LCPUFA. Since D5D and D6D have a higher affinity for n-3 PUFA, the variants which increased their activities generally also enhanced the n-3 LCPUFA status, as was apparent from the lower n-6:n-3 LCPUFA ratios being associated with these variants in our study. Moreover, most of our study population were carriers for these genetic variants that were associated with a better n-3 LCPUFA status, and the majority were homozygous for the variant combination as well.

One of the nine genetic variants, *FADS2*-rs78678033_(G/A), a non-coding transcript exon variant, is a novel variant for the first time reported here to associate with estimated desaturase activity. The A allele carriers for *FADS2*-rs78678033_(GA+AA) had higher estimated D5D and lower estimated D6D activities which were both independent of dietary intake.

Nine genetic variants from non-coding regions in the *FADS1/2* gene cluster were characterised. As previously suggested, variations in the *FADS1/2* gene cluster are responsible for altered product-to-precursor ratios indicative of altered estimated activities of the D5D and D6D enzymes in the LCPUFA pathways [29]. Studies conducted in European countries and the United States of America have demonstrated associations between

LCPUFA status and genetic variants in the *FADS1/2* gene cluster, whereas studies in the African context are scarce.

When RBC PUFA ratios were compared between the higher and the lower n-3 LCPUFA status groups, it was evident that the higher n-3 LCPUFA status group had a lower DGLA:LA ratio, indicating lower D6D activity, in the n-6 LCPUFA pathway. In contrast, the EPA:ALA ratio representing the overall n-3 PUFA pathway was higher in the higher n-3 LCPUFA status group, whereas the n-6 PUFA AA:LA ratio did not differ between the higher and lower n-3 LCPUFA status groups. Given that LA and ALA compete for D5D and D6D, our results confirmed that the desaturases have a higher affinity for n-3 PUFA than n-6 PUFA. The higher n-3 LCPUFA status group also had a higher DHA:ALA ratio, indicating a higher combined D5D and D6D activity, but a lower DHA:EPA ratio, indicative of lower combined D6D activity and β -oxidation.

In this study *FADS2*-rs174606_(T/G) and *FADS2*-rs174616_(G/A) (both located in the same intron region) were associated with lowered AA:LA ratios as well as lowered DGLA:LA ratios, independent of dietary intake. This association suggests that G and A allele carriers of the respective variants have lowered D6D activity. This finding is in agreement with Brayner *et al.* and Bokor *et al.* who reported lowered D5D activity for the A allele of *FADS2*-rs174616_(G/A) [43, 52].

We found that *FADS1*-rs174546_(C/T) and *FADS2*-rs112425493_(G/A) (located in the 3'UTR of *FADS1* and *FADS2* respectively) both associated with lower AA:DGLA and higher DGLA:LA ratios indicative of lower D5D activity, and higher D6D activity for the T and A allele carriers. The T allele of *FADS1*-rs174546_(C/T) has been associated with lower D5D activity before [9, 43, 53], which is in agreement with what we found in our study. Furthermore, *FADS*-rs174547_(C/T), *FADS1*-rs174549_(A/G), *FADS1*-rs174555_(C/T) and *FADS2*-rs73489307_(T/C) associated with higher DGLA:LA ratio or D6D activity in this study. In contrast, a study done by Mathias *et al.*, 2011 found that the G allele of *FADS1*-rs174579_(A/G) associated with lower *FADS1* transcript levels, thus resulting in lowered D5D levels [30].

The current study agrees with results from other researchers that were conducted in individuals of African ancestry. In previous studies conducted in the United States, ancestry-related differences were observed for the genetic variant *FADS1*-rs174537_(T/G) that were associated with higher AA levels, and it was found that 79% – 82% African Americans carried two copies of the alternative G allele in comparison to only 42% – 45% of European Americans. The allelic effect of the G allele (enhanced conversion of DGLA to AA) on D5D enzymatic efficiency was similar in both the European American- and African American groups

[30]. Sergeant and colleagues (2012) had similar results where seven genetic variants (including *FADS1*-rs174537_(T/G)) in the *FADS1/2* gene cluster had a strong association with AA, EPA, DGLA and AA:DGLA ratio [31]. For the genetic variant *FADS1*-rs174537_(T/G), European Americans homozygous for the ancestral T allele had significantly lower AA levels and AA:DGLA ratios but higher DGLA levels compared to African Americans homozygous for the alternative G allele, suggesting lower D5D activity in the European American group. Allele frequency patterns suggested that the GG genotype at rs174537 (which associated with higher AA:DGLA ratio and higher D5D activity) was much higher in African Americans in comparison to European Americans [31]. In our sample, this particular genetic variant did not have a significant differential P-value when the lower and higher n-3 LCPUFA status groups were compared.

These results are of importance to the South African population since this study offers insight into the South African population's genetic makeup and how it affects RBC LCPUFA status, which may result in inflammatory diseases and suboptimal birth outcomes if not managed well.

It is evident from this study that the quantity and quality of FA intake is skewed in this population. This data is the first step to determining the LCPUFA status of the South African population and gives us a possible opportunity to improve current diet recommendations (FA amounts, types and sources) in the future. These results can then be used to optimise n-3 PUFA status of pregnant women via supplementation programs, to decrease inflammatory diseases and increase optimal birth outcomes.

5. **Conclusion**

This study demonstrates that genetic variation in these pregnant African women is largely related to higher D5D and D6D activities, irrespective of essential n-3 or n-6 PUFA or LCPUFA dietary intake. Four genetic variants were associated with both increased desaturase activities and an improved n-3 LCPUFA status, and may therefore be beneficial to optimise n-3 LCPUFA status in pregnant women of African descent.

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Figures and Tables

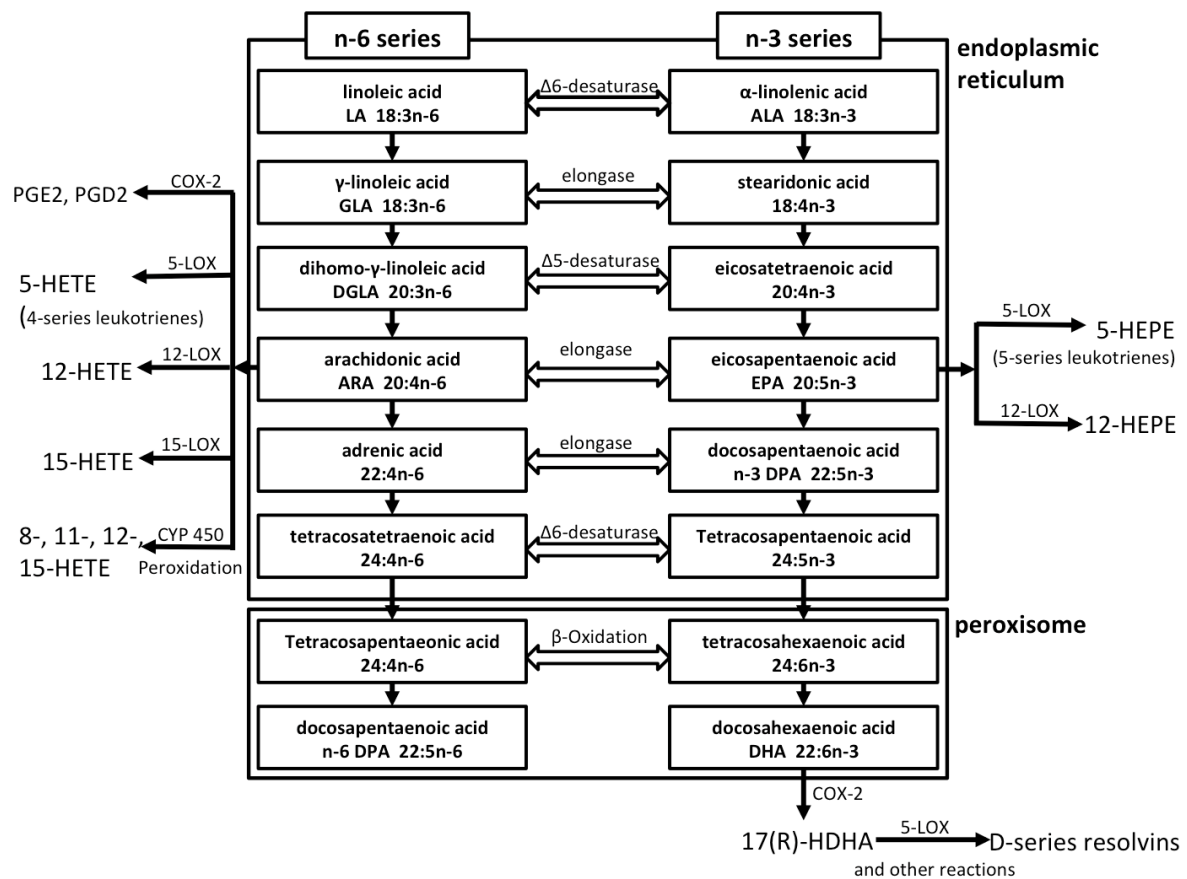


Figure 3.1: Metabolic pathway of n-6 and n-3 LCPUFA in humans

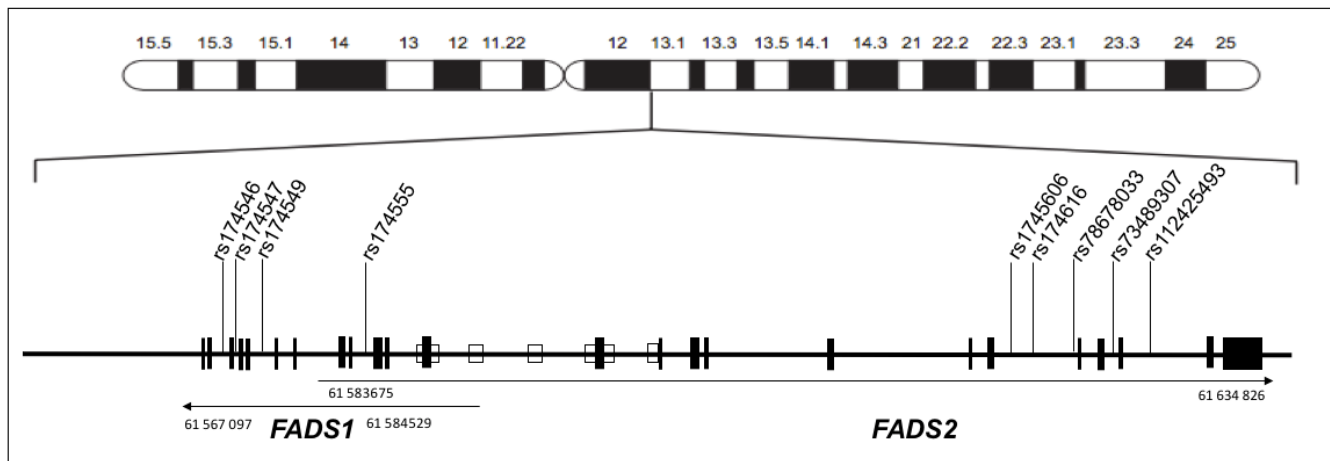


Figure 3. 2: Schematic presentation of *FADS1* and *FADS2* genes located on chromosome 11 and location of genetic variants selected for this study

FADS = Fatty acid desaturase: ■ = exon coding region; □ = Promoter area

Table 3.1: Participant characteristics

	Total group		n-3 LCPUFA status				P-value
	n		n	Higher n-3 LCPUFA status	n	Lower n-3 LCPUFA status	
General characteristics							
Age (years)	243	27.7 ± 5.0 [#]	130	27.6 ± 5.07	113	28.2 ± 4.93	0.283
Gestational age (days)	243	100 (28) [@]	130	104 (29)	113	95 (31)	0.021*
Height (cm)	243	159 ± 6.2 [#]	130	159 ± 6.6	113	159 ± 8.0	0.834
BMI (kg/m ²)	243	26.3 (7.62) [@]	130	28.2 (6.23)	113	26.8 (5.66)	0.059
MUAC (cm)	243	30.3 ± 4.68 [#]	130	30.8 ± 4.9	113	30 ± 4.42	0.17
Dietary intake							
Total energy intake (kJ)	243	11063 ± 3709 [#]	130	10809 ± 5365	113	10777 ± 4836	0.864
Total protein as % of energy (%)	243	13.3 ± 2.6 [#]	130	13.1 ± 2.49	113	13.7 ± 2.7	0.113
Total carbohydrates as % of energy (%)	243	56.3 ± 8.26 [#]	130	56.5 ± 8.3	113	56.0 ± 8.26	0.654
Total fat % of energy (%)	243	31.1 ± 6.47 [#]	130	31.5 ± 6.47	113	30.7 ± 6.67	0.363
Total Saturated Fatty Acids (g)	243	27.3 (18.5) [@]	130	27.0 (19.3)	113	27.9 (17.2)	0.461
Total Trans Fatty Acids (g)	243	1.25 (1.58) [@]	130	1.15 (1.5)	113	1.35 (1.75)	0.409
Total Monounsaturated Fatty acids (g)	243	29.2 (21.0) [@]	130	29.1 (23.3)	113	29.8 (18.3)	0.988
Total Polyunsaturated Fatty acids (g)	243	21.5 (15.6) [@]	130	25.6 (12.2)	113	21.1 (14.6)	0.042*
Total n-6 LCPUFA (g)	243	21.8 (16.4) [@]	130	23.1 (16.4)	113	20.9 (16.9)	0.019*
Linoleic acid (LA) (18:2 n-6) (g)	243	21.5 (16.4) [@]	130	22.9 (16.4)	113	20.7 (16.9)	0.019*
Arachidonic acid (AA) (20:4 n-6) (mg)	243	142 (124) [@]	130	149 (138)	113	134(102)	0.145
Total n-3 LCPUFA (g)	243	0.85 (0.69) [@]	130	0.93 (0.82)	113	0.82 (0.51)	0.156
Dihomo-gamma-linolenic acid (DGLA) (20:3 n-6) (mg)	243	8.27 (13) [@]	130	8.17 (12.4)	113	8.93 (14.2)	0.199
Alpha-linolenic acid (ALA) (18:3 n-3) (mg)	243	584 (445) [@]	130	560 (461)	113	630 (395)	0.436
Eicosapentaenoic acid (EPA) (20:5 n-3) (mg)	243	61.8 (87.5) [@]	130	73.5 (113)	113	51.8 (63.1)	0.006*
Docosapentaenoic acid (DPA) (22:5 n-3) (mg)	243	25.1 (30.5) [@]	130	25.2 (39.6)	113	23.6 (21.9)	0.024*
Docosahexaenoic acid (DHA) (22:6 n-3) (mg)	243	128 (174) [@]	130	170 (215)	113	106 (127)	0.003*

[#]Normally distributed data presented as mean ± SD, all such values; [@]non-normally distributed data presented as median (IQR), all such values; *statistically significant; interquartile range (IQR); body mass index (BMI); mid upper arm circumference (MUAC); kilojoule (kJ); monounsaturated fatty acid (MUFA); polyunsaturated fatty acid (PUFA); saturated fatty acid (SFA).

Table 3.2: Red blood cell total phospholipid fatty acid composition

	Total group	n-3 LCPUFA status		P-value
		Higher n-3 LCPUFA status (n=130)	Lower n-3 LCPUFA status (n=113)	
Red blood cell total phospholipid fatty acid composition (% of total)				
n-3 polyunsaturated fatty acids				
C18:3n-3 ALA	0.075 (0.0) [@]	0.078 (0.0)	0.069 (0.0)	<0.001*
C20:5n-3 EPA	0.253 (0.2) [@]	0.325 (0.2)	0.202 (0.1)	<0.001*
C22:5n-3 DPA	1.85 ± 0.39 [#]	2.05 ± 0.38	1.63 ± 0.28	<0.001*
C22:6n-3 DHA	5.28 ± 1.16 [#]	5.96 ± 1.04	4.49 ± 0.69	<0.001*
n-3 index (DHA + EPA)	5.57 ± 1.23 [#]	6.33 ± 1.08	4.7 ± 0.71	<0.001*
n-6 polyunsaturated fatty acids				
C18:2n-6 LA	11.4 ± 1.7 [#]	11.4 ± 1.68	11.4 ± 1.72	0.759
C20:4n-6 AA	14.4 ± 1.75 [#]	14 ± 2.1	14.6 ± 1.64	0.048*
C20:3n-6 DGLA	1.18 (0.4) [@]	1.14 (0.5)	1.23 (0.5)	0.020*
n-6:n-3 fatty acid ratios and estimated desaturase activities of n-6 and n-3 pathways				
Total n-6:n-3 LCPUFA ratio	4.53 ± 0.94 [#]	3.88 ± 0.51	5.28 ± 0.75	<0.001*
Delta 5 desaturase (AA:DGLA)	12.2 (5.47) [@]	12.6 (5.21)	12.0 (5.64)	0.278
Delta 6 desaturase (DGLA:LA)	0.108 (0.05) [@]	0.102 (0.04)	0.112 (0.06)	0.024*
Delta 5+6 desaturase (AA:LA)	1.29 ± 0.232 [#]	1.27 ± 0.231	1.29 ± 0.3	0.095
Delta 5+6 desaturase + elongase (EPA:ALA)	3.44 (2.63) [@]	4.34 (2.73)	2.8 (1.69)	<0.001*
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	73.1 (45.0) [@]	76.4 (50.1)	67.2 (36.5)	0.018*
Delta 6 desaturase + B-Oxidation (DHA: EPA)	20.4 (11.3) [@]	18.7 (11.4)	22.9 (9.85)	<0.001*

[#]Normally distributed data presented as mean ± SD, all such values; [@]non-normally distributed data presented as median (IQR);*statistically significant P<0.05; alpha linolenic acid (ALA); eicosapentaenoic acid (EPA); docosapentaenoic acid (DPA); docosahexaenoic acid (DHA); linoleic acid (LA); arachidonic acid (AA); dihomo-gamma linoleic acid (DGLA); long chain polyunsaturated fatty acid LCPUFA.

Table 3.3: Characteristics of the nine selected genetic variants in the *FADS1/2* gene cluster of the NuPED study sample

rs Number	Gene	Position (bp)	Region	Alleles (ancestral/ alternative)	Minor allele frequency	HWE P- value
rs174546*	<i>FADS 1</i>	61569829	3'UTR	C/T	0.033	1.00
rs174547#	<i>FADS 1</i>	61570782	intron	C/T	0.038	1.00
rs174549#	<i>FADS 1</i>	61571382	5'UTR	A/G	0.037	1.00
rs174555#	<i>FADS 1/2</i>	61579760	intron	C/T	0.069	1.00
rs174606\$	<i>FADS 2</i>	61626973	intron	T/G	0.384	0.13
rs174616\$	<i>FADS 2</i>	61629122	intron	G/A	0.363	0.04
rs78678033@	<i>FADS 2</i>	61630383	non-coding transcript exon	G/A	0.127	1.00
rs73489307#	<i>FADS 2</i>	61630969	intron	T/C	0.017	1.00
rs112425493*	<i>FADS 2</i>	61631376	3'UTR	G/A	0.007	1.00

Fatty acid desaturase 1 (*FADS1*); Fatty acid desaturase 2 (*FADS2*); Hardy Weinberg equilibrium (HWE); *Group genetic variants with alternative allele associated with lowered D5D and higher D6D activity; #group genetic variants with alternative allele associated of higher D6D activity; \$group genetic variants with alternative allele associated with lowered D5D and D6D activity; @Genetic variant with alternative allele associated with higher D5D and lower D6D activity.

Table 3.4: Distribution of plasma phospholipid LCPUFA ratios as classified by *FADS2*-112425493_(G/A)

	Additive				Recessive		
	GG	GA	AA	P-value	GG	GA + AA	P-value
n	211	4	1		211	5	
Model 1 (adjusted for mother age and gestational age)							
Delta 5 desaturase (AA:DGLA)	12.81 ± 4.02 ^a	8.22 ± 3.1 ^b	9.1 ± 0 ^{ab}	0.053	12.81 ± 4.02	8.39 ± 2.71	0.016*
Delta 6 desaturase (DGLA:LA)	0.11 ± 0.37 ^a	0.167 ± 0.046 ^b	0.115 ± 0 ^{ab}	0.016*	0.11 ± 0.037	0.156 ± 0.046	0.011*
Delta 5+6 desaturase (AA:LA)	1.29 ± 0.227	1.27 ± 0.17	1.04 ± 0	0.311	1.29 ± 0.23	1.22 ± 0.18	0.296
Delta 5+6 desaturase + elongase (EPA:ALA)	3.95 ± 2.28	4.01 ± 2.85	4.18 ± 0	0.997	3.95 ± 2.28	4.043 ± 2.47	0.949
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	76.17 ± 34.33	56.55 ± 22.03	54.0 ± 0	0.391	76.17 ± 34.33	56.035 ± 19.11	0.171
Delta 6 desaturase + B-Oxidation (DHA:EPA)	21.76 ± 9.03	17.08 ± 5.88	12.91 ± 0	0.451	21.76 ± 9.03	16.24 ± 5.37	0.220
n-6:n-3 LCPUFA ratio	4.53 ± 0.904	5.53 ± 1.79	5.05 ± 0	0.090	4.53 ± 0.90	5.43 ± 1.56	0.033*
Model 2 (adjusted for mother age, gestational age and total energy intake)							
Delta 5 desaturase (AA:DGLA)delta 5 (AA:DGLA)	12.81 ± 4.02 ^a	8.22 ± 3.1 ^b	0.1031 ± 0 ^{ab}	0.042*	12.81 ± 4.02	8.38 ± 2.71	0.012*
Delta 6 desaturase (DGLA:LA)	0.11 ± 0.037 ^a	0.167 ± 0.046 ^b	0.1146 ± 0 ^{ab}	0.014*	0.11 ± 0.037	0.16 ± 0.046	0.009*
Delta 5+6 desaturase (AA:LA)	1.29 ± 0.227	1.27 ± 0.169	1.0436 ± 0	0.326	1.29 ± 0.23	1.22 ± 0.177	0.311
Delta 5+6 desaturase + elongase (EPA:ALA)	3.95 ± 2.28	4.01 ± 2.85	4.183 ± 0	1.00	3.95 ± 2.28	4.04 ± 2.47	0.988
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	76.17 ± 34.33	56.55 ± 22.03	54.0 ± 0	0.313	76.17 ± 34.33	56.04 ± 19.11	0.129
Delta 6 desaturase + B-Oxidation (DHA:EPA)	21.76 ± 9.03	17.08 ± 5.81	12.9085 ± 0	0.352	21.76 ± 9.03	16.24 ± 5.37	0.163
n-6:n-3 LCPUFA ratio	4.53 ± 0.90	5.53 ± 1.79	5.05 ± 0	0.074	4.53 ± 0.90	5.43 ± 1.56	0.025*
Model 3 (mother age, gestational age, ALA intake)							
Delta 5 desaturase (AA:DGLA)	12.81 ± 4.03 ^a	8.22 ± 3.1 ^b	9.1 ± 0 ^{ab}	0.044*	12.81 ± 4.03	8.39 ± 2.71	0.013*
Delta 6 desaturase (DGLA:LA)	0.11 ± 0.038 ^a	0.117 ± 0.046 ^b	0.115 ± 0 ^{ab}	0.013*	0.11 ± 0.038	0.156 ± 0.046	0.008*
Delta 5+6 desaturase (AA:LA)	1.29 ± 0.228	1.27 ± 0.169	1.04 ± 0	0.342	1.29 ± 0.228	1.22 ± 0.177	0.329
Delta 5+6 desaturase + elongase (EPA:ALA)	3.96 ± 2.28	4.01 ± 2.85	4.18 ± 0	0.999	3.96 ± 2.28	4.04 ± 2.47	0.966
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	76.1 ± 34.32	56.55 ± 22.03	53.99 ± 0	0.328	76.1 ± 34.32	56.04 ± 19.11	0.137
Delta 6 desaturase + B-Oxidation (DHA:EPA)	21.57 ± 8.76	17.08 ± 5.81	12.91 ± 0	0.356	21.57 ± 8.76	16.24 ± 5.37	0.165
n-6:n-3 LCPUFA ratio	4.51 ± 0.90	5.53 ± 1.79	5.05 ± 0	0.074	4.51 ± 0.90	5.43 ± 1.56	0.026*
Model 4 (mother age, gestational age, LA intake)							
Delta 5 desaturase (AA:DGLA)	12.81 ± 4.03 ^a	8.22 ± 3.1 ^a	9.1 ± 0 ^a	0.063	12.81 ± 4.03	8.39 ± 2.71	0.019*
Delta 6 desaturase (DGLA:LA)	0.11 ± 0.038 ^a	0.167 ± 0.046 ^b	0.115 ± 0 ^{ab}	0.025*	0.11 ± 0.038	0.156 ± 0.046	0.018*
Delta 5+6 desaturase (AA:LA)	1.29 ± 0.228	1.27 ± 0.169	1.04 ± 0	0.259	1.29 ± 0.228	1.22 ± 0.177	0.235
Delta 5+6 desaturase + elongase (EPA:ALA)	3.96 ± 2.285	4 ± 2.85	4.18 ± 0	0.978	3.96 ± 2.285	4.04 ± 2.47	0.834
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	76.1 ± 34.32	56.55 ± 22.03	54.0 ± 0	0.43	76.1 ± 34.32	56.04 ± 19.11	0.194
Delta 6 desaturase + B-Oxidation (DHA:EPA)	21.57 ± 8.76	17.08 ± 5.81	12.91 ± 0	0.366	21.57 ± 8.76	16.24 ± 5.37	0.169

	Additive				Recessive		
	GG	GA	AA	P-value	GG	GA + AA	P-value
n	211	4	1		211	5	
n-6:n-3 LCPUFA ratio	4.51 ± 0.90	5.53 ± 1.79	5.05 ± 0	0.099	4.51 ± 0.90	5.43 ± 1.56	0.037*
Model 5 (mother age, gestational age, DHA intake)							
Delta 5 desaturase (AA:DGLA)	12.81 ± 4.03	8.22 ± 3.1	9.1 ± 0	0.066	12.81 ± 4.03	8.39 ± 2.71	0.020*
Delta 6 desaturase (DGLA:LA)	0.11 ± 0.038 ^a	0.167 ± 0.046 ^b	0.115 ± 0 ^{ab}	0.021*	0.11 ± 0.038	0.156 ± 0.046	0.014*
Delta 5+6 desaturase (AA:LA)	1.29 ± 0.228	1.27 ± 0.169	1.04 ± 0	0.311	1.29 ± 0.228	1.22 ± 0.177	0.303
Delta 5+6 desaturase + elongase (EPA:ALA)	3.96 ± 2.285	4 ± 2.85	4.18 ± 0	0.951	3.96 ± 2.285	4.04 ± 2.47	0.831
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	76.1 ± 34.32	56.55 ± 22.03	54.0 ± 0	0.435	76.1 ± 34.32	56.04 ± 19.11	0.2
Delta 6 desaturase + B-Oxidation (DHA:EPA)	21.57 ± 8.76	17.08 ± 5.81	12.91 ± 0	0.349	21.57 ± 8.76	16.24 ± 5.37	0.152
n-6:n-3 LCPUFA ratio	4.51 ± 0.9	5.53 ± 1.79	5.05 ± 0	0.114	4.51 ± 0.9	5.43 ± 1.56	0.042*
Model 6 (mother age, gestational age, AA intake)							
Delta 5 desaturase (AA:DGLA)	12.81 ± 4.03 ^a	8.22 ± 3.1 ^b	9.1 ± 0 ^{ab}	0.039*	12.81 ± 4.03	8.39 ± 2.71	0.011*
Delta 6 desaturase (DGLA:LA)	0.11 ± 0.038 ^a	0.167 ± 0.046 ^b	0.115 ± 0 ^{ab}	0.012*	0.11 ± 0.038	0.156 ± 0.046	0.009*
Delta 5+6 desaturase (AA:LA)	1.29 ± 0.228	1.27 ± 0.169	1.04 ± 0	0.315	1.29 ± 0.228	1.22 ± 0.177	0.277
Delta 5+6 desaturase + elongase (EPA:ALA)	3.96 ± 2.285	4 ± 2.85	4.18 ± 0	0.995	3.96 ± 2.285	4.04 ± 2.47	0.919
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	76.1 ± 34.32	56.55 ± 22.03	54.0 ± 0	0.377	76.1 ± 34.32	56.04 ± 19.11	0.162
Delta 6 desaturase + B-Oxidation (DHA:EPA)	21.57 ± 8.76	17.08 ± 5.81	12.91 ± 0	0.422	21.57 ± 8.76	16.24 ± 5.37	0.204
n-6:n-3 LCPUFA ratio	4.51 ± 0.9	5.53 ± 1.79	5.05 ± 0	0.08	4.51 ± 0.9	5.43 ± 1.56	0.03*

Data presented as mean ± SD; alphabetic letters indicate between group differences with Bonferroni adjust P<0.05, *significance P<0.05

Table 3.5: Distribution of plasma phospholipid LCPUFA ratios as classified by *FADS1*-rs174546_(CT)

	Additive				Recessive		
	CC	CT	TT	P-value	CC	CT + TT	P-value
n	221	16	0		221	16	
Model 1 (adjusted for mother age and gestational age)							
Delta 5 desaturase (AA:DGLA)	12.96 ± 4.05 ^a	8.58 ± 1.75 ^b		0.000*	12.96 ± 4.05	8.58 ± 1.75	0.000
Delta 6 desaturase (DGLA:LA)	0.108 ± 0.036 ^a	0.153 ± 0.044 ^b		0.000*	0.11 ± 0.036	0.153 ± 0.044	0.000
Delta 5+6 desaturase (AA:LA)	1.29 ± 0.229	1.25 ± 0.132		0.146	1.29 ± 0.229	1.25 ± 0.132	0.146
Delta 5+6 desaturase + elongase (EPA:ALA)	4.06 ± 2.35	3.63 ± 2.67		0.268	4.06 ± 2.35	3.63 ± 2.67	0.268
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	76.57 ± 33.75	65.36 ± 28.45		0.165	76.57 ± 33.75	65.36 ± 28.45	0.165
Delta 6 desaturase + B-Oxidation (DHA:EPA)	21.56 ± 9.16	20.78 ± 7.47		0.939	21.56 ± 9.16	20.78 ± 7.47	0.939
n-6:n-3 LCPUFA ratio	4.51 ± 0.934	5.01 ± 0.998		0.059	4.51 ± 0.934	5.01 ± 0.998	0.059
Model 2 (adjusted for mother age, gestational age and total energy intake)							
Delta 5 desaturase (AA:DGLA)	12.96 ± 4.05 ^a	8.58 ± 1.75 ^b		0.000	12.96 ± 4.05	8.58 ± 1.75	0.000
Delta 6 desaturase (DGLA:LA)	0.108 ± 0.036 ^a	0.153 ± 0.044 ^b		0.000	0.11 ± 0.036	0.153 ± 0.044	0.000
Delta 5+6 desaturase (AA:LA)	1.29 ± 0.229	1.25 ± 0.132		0.152	1.29 ± 0.229	1.25 ± 0.132	0.152
Delta 5+6 desaturase + elongase (EPA:ALA)	4.06 ± 2.35	3.63 ± 2.67		0.278	4.06 ± 2.35	3.63 ± 2.67	0.278
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	76.57 ± 33.75	65.36 ± 28.45		0.144	76.57 ± 33.75	65.36 ± 28.45	0.144
Delta 6 desaturase + B-Oxidation (DHA:EPA)	21.56 ± 9.16	20.78 ± 7.47		0.976	21.56 ± 9.16	20.78 ± 7.47	0.976
n-6:n-3 LCPUFA ratio	4.51 ± 0.934	5.01 ± 0.998		0.053	4.51 ± 0.934	5.01 ± 0.998	0.053
Model 3 (mother age, gestational age, ALA intake)							
Delta 5 desaturase (AA:DGLA)	12.96 ± 4.06 ^a	8.58 ± 1.75 ^b		0.000*	12.96 ± 4.06	8.58 ± 1.75	0.000*
Delta 6 desaturase (DGLA:LA)	0.109 ± 0.036 ^a	0.153 ± 0.044 ^b		0.000*	0.109 ± 0.036	0.153 ± 0.044	0.000*
Delta 5+6 desaturase (AA:LA)	1.29 ± 0.23	1.25 ± 0.132		0.137	1.29 ± 0.23	1.25 ± 0.132	0.137
Delta 5+6 desaturase + elongase (EPA:ALA)	4.07 ± 2.35	3.63 ± 2.67		0.266	4.07 ± 2.35	3.63 ± 2.67	0.266
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	76.5 ± 33.74	65.36 ± 28.45		0.183	76.5 ± 33.74	65.36 ± 28.45	0.183
Delta 6 desaturase + B-Oxidation (DHA:EPA)DHA:EPA	21.38 ± 8.9	20.78 ± 7.47		0.888	21.38 ± 8.9	20.78 ± 7.47	0.888
n-6:n-3 LCPUFA ratio	4.5 ± 0.931	5.01 ± 0.998		0.062	4.5 ± 0.931	5.01 ± 0.998	0.062
Model 4 (mother age, gestational age, LA intake)							
Delta 5 desaturase (AA:DGLA)	12.96 ± 4.06 ^a	8.58 ± 1.75 ^b		0.000*	12.96 ± 4.06	8.58 ± 1.75	0.000*
Delta 6 desaturase (DGLA:LA)	0.109 ± 0.036 ^a	0.153 ± 0.044 ^b		0.000*	0.109 ± 0.036	0.153 ± 0.044	0.000*
Delta 5+6 desaturase (AA:LA)	1.29 ± 0.23	1.25 ± 0.132		0.14	1.29 ± 0.23	1.25 ± 0.132	0.14

	Additive				Recessive		
	CC	CT	TT	P-value	CC	CT + TT	P-value
n	221	16	0		221	16	
Delta 5+6 desaturase + elongase (EPA:ALA)	4.07 ± 2.35	3.63 ± 2.67		0.294	4.07 ± 2.35	3.63 ± 2.67	0.294
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	76.5 ± 33.74	65.36 ± 28.45		0.172	76.5 ± 33.74	65.36 ± 28.45	0.172
Delta 6 desaturase + B-Oxidation (DHA:EPA)	21.38 ± 8.9	20.78 ± 7.47		0.929	21.38 ± 8.9	20.78 ± 7.47	0.929
n-6:n-3 LCPUFA ratio	4.5 ± 0.931	5.01 ± 0.998		0.058	4.5 ± 0.931	5.01 ± 0.998	0.058
Model 5 (mother age, gestational age, DHA intake)							
Delta 5 desaturase (AA:DGLA)	12.96 ± 4.06 ^a	8.58 ± 1.75 ^b		0.000	12.96 ± 4.06	8.58 ± 1.75	0.000
Delta 6 desaturase (DGLA:LA)	0.109 ± 0.036 ^a	0.153 ± 0.044 ^b		0.000	0.109 ± 0.036	0.153 ± 0.044	0.000
Delta 5+6 desaturase (AA:LA)	1.29 ± 0.23	1.25 ± 0.132		0.16	1.29 ± 0.23	1.25 ± 0.132	0.16
Delta 5+6 desaturase + elongase (EPA:ALA)	4.07 ± 2.35	3.63 ± 2.67		0.379	4.07 ± 2.35	3.63 ± 2.67	0.379
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	76.5 ± 33.74	65.36 ± 28.45		0.203	76.5 ± 33.74	65.36 ± 28.45	0.203
Delta 6 desaturase + B-Oxidation (DHA:EPA)	21.38 ± 8.9	20.78 ± 7.47		0.964	21.38 ± 8.9	20.78 ± 7.47	0.964
n-6:n-3 LCPUFA ratio	4.5 ± 0.931	5.01 ± 0.998		0.076	4.5 ± 0.931	5.01 ± 0.998	0.076
Model 6 (mother age, gestational age, AA intake)							
Delta 5 desaturase (AA:DGLA)	12.96 ± 4.06 ^a	8.58 ± 1.75 ^b		0.000	12.96 ± 4.06	8.58 ± 1.75	0.000
Delta 6 desaturase (DGLA:LA)	0.109 ± 0.036 ^a	0.153 ± 0.044 ^b		0.000	0.109 ± 0.036	0.153 ± 0.044	0.000
Delta 5+6 desaturase (AA:LA)	1.29 ± 0.23	1.25 ± 0.132		0.168	1.29 ± 0.23	1.25 ± 0.132	0.168
Delta 5+6 desaturase + elongase (EPA:ALA)	4.07 ± 2.35	3.63 ± 2.67		0.289	4.07 ± 2.35	3.63 ± 2.67	0.289
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	76.5 ± 33.74	65.36 ± 28.45		0.167	76.5 ± 33.74	65.36 ± 28.45	0.167
Delta 6 desaturase + B-Oxidation (DHA:EPA)	21.38 ± 8.9	20.78 ± 7.47		0.931	21.38 ± 8.9	20.78 ± 7.47	0.931
n-6:n-3 LCPUFA ratio	4.5 ± 0.931	5.01 ± 0.998		0.061	4.5 ± 0.931	5.01 ± 0.998	0.061

Data presented as mean ± SD; alphabetic letters indicate between group differences with Bonferroni adjust P<0.05, *significance P<0.05

Table 3.6: Distribution of plasma phospholipid LCPUFA ratios as classified by *FADS2*-rs73489307_(T/C)

	Additive				Recessive		
	TT	TC	CC	P-value	TT	CT +CC	P-value
n	226	9	1		226	10	
Model 1 (adjusted for mother age and gestational age)							
Delta 5 desaturase (AA:DGLA)	12.78 ± 4.07	10.73 ± 4.18	9.1	0.227	12.78 ± 4.07	10.57 ± 3.97	0.095
Delta 6 desaturase (DGLA:LA)	0.11 ± 0.038	0.138 ± 0.051	0.115	0.082	0.11 ± 0.038	0.136 ± 0.048	0.035*
Delta 5+6 desaturase (AA:LA)	1.29 ± 0.225	1.3 ± 0.206	1.04	0.319	1.29 ± 0.225	1.28 ± 0.21	0.896
Delta 5+6 desaturase + elongase (EPA:ALA)	4.04 ± 2.39	3.73 ± 2.1	4.18	0.95	4.04 ± 2.39	3.77 ± 1.99	0.749
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	76.62 ± 33.75	57.93 ± 24.42	53.996	0.211	76.62 ± 33.75	57.53 ± 23.06	0.079
Delta 6 desaturase + B-Oxidation (DHA:EPA)	21.7 ± 9.13	18.09 ± 6.51	12.91	0.315	21.7 ± 9.13	17.57 ± 6.35	0.141
n-6:n-3 LCPUFA ratio	4.53 ± 0.923	4.94 ± 1.44	5.05 ± 0	0.383	4.53 ± 0.923	4.95 ± 1.36	0.166
Model 2 (adjusted for mother age, gestational age and total energy intake)							
Delta 5 desaturase (AA:DGLA)	12.78 ± 4.07	10.73 ± 4.18	9.10	0.216	12.78 ± 4.07	10.57 ± 3.97	0.092
Delta 6 desaturase (DGLA:LA)	0.11 ± 0.038	0.138 ± 0.051	0.115	0.083	0.11 ± 0.038	0.136 ± 0.048	0.034*
Delta 5+6 desaturase (AA:LA)	1.29 ± 0.225	1.3 ± 0.206	1.04	0.342	1.29 ± 0.225	1.28 ± 0.21	0.908
Delta 5+6 desaturase + elongase (EPA:ALA)	4.04 ± 2.39	3.73 ± 2.1	4.18	0.954	4.04 ± 2.39	3.77 ± 1.99	0.761
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	76.62 ± 33.75	57.93 ± 24.42	53.996	0.185	76.62 ± 33.75	57.53 ± 23.06	0.07
Delta 6 desaturase + B-Oxidation (DHA:EPA)	21.7 ± 9.13	18.09 ± 6.51	12.91	0.287	21.7 ± 9.13	17.57 ± 6.35	0.132
n-6:n-3 LCPUFA ratio	4.53 ± 0.923	4.94 ± 1.44	5.05	0.363	4.53 ± 0.923	4.95 ± 1.36	0.156
Model 3 (mother age, gestational age, ALA intake)							
Delta 5 desaturase (AA:DGLA)	12.78 ± 4.08	10.73 ± 4.18	9.10	0.217	12.78 ± 4.08	10.57 ± 3.97	0.091
Delta 6 desaturase (DGLA:LA)	0.11 ± 0.038	0.138 ± 0.051	0.115	0.08	0.11 ± 0.038	0.136 ± 0.048	0.032*
Delta 5+6 desaturase (AA:LA)	1.29 ± 0.226	1.3 ± 0.206	1.04	0.343	1.29 ± 0.226	1.28 ± 0.21	0.943
Delta 5+6 desaturase + elongase (EPA:ALA)	4.05 ± 2.39	3.73 ± 2.1	4.18	0.945	4.05 ± 2.39	3.77 ± 1.99	0.735
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	76.56 ± 33.74	57.93 ± 24.42	53.99	0.181	76.56 ± 33.74	57.53 ± 23.06	0.067
Delta 6 desaturase + B-Oxidation (DHA:EPA)	21.53 ± 8.88	18.09 ± 6.51	12.91	0.288	21.53 ± 8.88	17.57 ± 6.35	0.133
n-6:n-3 LCPUFA ratio	4.52 ± 0.92	4.94 ± 1.44	5.05	0.325	4.52 ± 0.92	4.95 ± 1.36	0.134
Model 4 (mother age, gestational age, LA intake)							
Delta 5 desaturase (AA:DGLA)	12.78 ± 4.08	10.73 ± 4.18	9.10	0.262	12.78 ± 4.08	10.57 ± 3.97	0.112
Delta 6 desaturase (DGLA:LA)	0.11 ± 0.038	0.138 ± 0.051	0.115	0.115	0.11 ± 0.038	0.136 ± 0.048	0.054
Delta 5+6 desaturase (AA:LA)	1.29 ± 0.226	1.3 ± 0.206	1.04	0.298	1.29 ± 0.226	1.28 ± 0.21	0.828

	Additive				Recessive		
	TT	TC	CC	P-value	TT	CT +CC	P-value
n	226	9	1		226	10	
Delta 5+6 desaturase + elongase (EPA:ALA)	4.05 ± 2.39	3.73 ± 2.1	4.18	0.99	4.05 ± 2.39	3.77 ± 1.99	0.903
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	76.56 ± 33.74	57.93 ± 24.42	54.0	0.234	76.56 ± 33.74	57.53 ± 23.06	0.089
Delta 6 desaturase + B-Oxidation (DHA:EPA)	21.53 ± 8.88	18.09 ± 6.51	12.91	0.288	21.53 ± 8.88	17.57 ± 6.35	0.130
n-6:n-3 LCPUFA ratio	4.52 ± 0.92	4.94 ± 1.44	5.05	0.388	4.52 ± 0.92	4.95 ± 1.36	0.168
Model 5 (mother age, gestational age, DHA intake)							
Delta 5 desaturase (AA:DGLA)	12.78 ± 4.08	10.73 ± 4.18	9.1	0.273	12.78 ± 4.08	10.57 ± 3.97	0.123
Delta 6 desaturase (DGLA:LA)	0.11 ± 0.038	0.138 ± 0.051	0.115	0.104	0.11 ± 0.038	0.136 ± 0.048	0.045*
Delta 5+6 desaturase (AA:LA)	1.29 ± 0.226	1.3 ± 0.206	1.04	0.307	1.29 ± 0.226	1.28 ± 0.21	0.946
Delta 5+6 desaturase + elongase (EPA:ALA)	4.05 ± 2.39	3.73 ± 2.1	4.18	0.991	4.05 ± 2.39	3.77 ± 1.99	0.991
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	76.56 ± 33.74	57.93 ± 24.42	54.0	0.265	76.56 ± 33.74	57.53 ± 23.06	0.107
Delta 6 desaturase + B-Oxidation (DHA:EPA)	21.53 ± 8.88	18.09 ± 6.51	12.91	0.235	21.53 ± 8.88	17.57 ± 6.35	0.096
n-6:n-3 LCPUFA ratio	4.52 ± 0.92	4.94 ± 1.44	5.05	0.464	4.52 ± 0.92	4.95 ± 1.36	0.217
Model 6 (mother age, gestational age, AA intake)							
Delta 5 desaturase (AA:DGLA)	12.78 ± 4.08	10.73 ± 4.18	9.10	0.198	12.78 ± 4.08	10.57 ± 3.97	0.076
Delta 6 desaturase (DGLA:LA)	0.11 ± 0.038	0.138 ± 0.051	0.115	0.067	0.11 ± 0.038	0.136 ± 0.048	0.030*
Delta 5+6 desaturase (AA:LA)	1.29 ± 0.226	1.3 ± 0.206	1.04	0.336	1.29 ± 0.226	1.28 ± 0.21	0.875
Delta 5+6 desaturase + elongase (EPA:ALA)	4.05 ± 2.39	3.73 ± 2.1	4.18	0.934	4.05 ± 2.39	3.77 ± 1.99	0.713
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	76.56 ± 33.74	57.93 ± 24.42	54.0	0.211	76.56 ± 33.74	57.53 ± 23.06	0.079
Delta 6 desaturase + B-Oxidation (DHA:EPA)	21.53 ± 8.88	18.09 ± 6.51	12.91	0.319	21.53 ± 8.88	17.57 ± 6.35	0.148
n-6:n-3 LCPUFA ratio	4.52 ± 0.92	4.94 ± 1.44	5.05	0.346	4.52 ± 0.92	4.95 ± 1.36	0.145

Data presented as mean ± SD; alphabetic letters indicate between group differences with Bonferroni adjust P<0.05, *significance P<0.05

Table 3.7: Distribution of plasma phospholipid LCPUFA ratios as classified by *FADS1*-rs174547_(CT)

	Additive				Recessive			Dominant		
	CC	CT	TT	P-value	CC	CT + TT	P-value	CC + CT	TT	P-value
n	12	18	213		12	231		30	213	
Model 1 (adjusted for mother age and gestational age)										
Delta 5 desaturase (AA:DGLA)	11.76 ± 4.88	10.96 ± 5.1	12.82 ± 3.91	0.119	11.76 ± 4.88	12.67 ± 4.03	0.436	10.96 ± 5.1	12.82 ± 3.91	0.121
Delta 6 desaturase (DGLA:LA)	0.116 ± 0.043 ^{ab}	0.136 ± 0.055 ^a	0.11 ± 0.036 ^b	0.036*	0.116 ± 0.043	0.112 ± 0.038	0.781	0.136 ± 0.055	0.11 ± 0.036	0.038*
Delta 5+6 desaturase (AA:LA)	1.2 ± 0.273	1.26 ± 0.2	1.3 ± 0.23	0.158	1.2 ± 0.273	1.29 ± 0.229	0.110	1.26 ± 0.2	1.3 ± 0.23	0.162
Delta 5+6 desaturase + elongase (EPA:ALA)	4.46 ± 2.16	3.74 ± 2.73	4.03 ± 2.32	0.647	4.46 ± 2.16	4.01 ± 2.35	0.613	3.74 ± 2.73	4.03 ± 2.32	0.650
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	74.34 ± 29.08	69 ± 30.99	76.5 ± 33.69	0.611	74.34 ± 29.08	75.91 ± 33.48	0.850	69 ± 30.99	76.5 ± 33.69	0.616
Delta 6 desaturase + B-Oxidation (DHA:EPA)	18.79 ± 9.48	21.29 ± 7.95	21.62 ± 9.08	0.677	18.79 ± 9.48	21.6 ± 8.98	0.382	21.29 ± 7.95	21.62 ± 9.08	0.681
n-6:n-3 LCPUFA ratio	5.05 ± 1.7	4.7 ± 1.07	4.49 ± 0.865	0.127	5.05 ± 1.7	4.6 ± 0.882	0.062	4.7 ± 1.07	4.49 ± 0.865	0.128
Model 2 (adjusted for mother age, gestational age and total energy intake)										
Delta 5 desaturase (AA:DGLA)	11.76 ± 4.88	10.96 ± 5.1	12.82 ± 3.91	0.120	11.76 ± 4.88	12.67 ± 4.03	0.443	10.96 ± 5.1	12.82 ± 3.91	0.121
Delta 6 desaturase (DGLA:LA)	0.116 ± 0.043 ^{ab}	0.136 ± 0.055 ^a	0.11 ± 0.036 ^b	0.036*	0.116 ± 0.043	0.112 ± 0.038	0.787	0.136 ± 0.055	0.11 ± 0.036	0.039*
Delta 5+6 desaturase (AA:LA)	1.2 ± 0.273	1.26 ± 0.205	1.3 ± 0.232	0.158	1.2 ± 0.273	1.29 ± 0.229	0.109	1.26 ± 0.2	1.3 ± 0.23	0.161
Delta 5+6 desaturase + elongase (EPA:ALA)	4.46 ± 2.16	3.74 ± 2.73	4.03 ± 2.32	0.651	4.46 ± 2.16	4.01 ± 2.35	0.621	3.74 ± 2.73	4.03 ± 2.32	0.652
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	74.34 ± 29.08	69 ± 30.99	76.5 ± 33.69	0.610	74.34 ± 29.08	75.91 ± 33.48	0.868	69 ± 30.99	76.5 ± 33.69	0.660
Delta 6 desaturase + B-Oxidation (DHA:EPA)	18.79 ± 9.48	21.29 ± 7.95	21.62 ± 9.08	0.688	18.79 ± 9.48	21.6 ± 8.98	0.392	21.29 ± 7.95	21.62 ± 9.08	0.693
n-6:n-3 LCPUFA ratio	5.05 ± 1.7	4.7 ± 1.07	4.49 ± 0.865	0.130	5.05 ± 1.7	4.6 ± 0.882	0.064	4.7 ± 1.07	4.49 ± 0.865	0.133
Model 3 (mother age, gestational age, ALA intake)										
Delta 5 desaturase (AA:DGLA)	11.76 ± 4.88	10.96 ± 5.1	12.82 ± 3.92	0.126	11.76 ± 4.88	12.67 ± 4.04	0.444	10.96 ± 5.1	12.82 ± 3.92	0.132
Delta 6 desaturase (DGLA:LA)	0.116 ± 0.043 ^{ab}	0.136 ± 0.05 ^a	0.11 ± 0.036 ^b	0.039*	0.116 ± 0.043	0.112 ± 0.038	0.789	0.136 ± 0.055	0.11 ± 0.036	0.043*
Delta 5+6 desaturase (AA:LA)	1.2 ± 0.273	1.26 ± 0.2	1.3 ± 0.232	0.158	1.2 ± 0.273	1.29 ± 0.23	0.113	1.26 ± 0.205	1.3 ± 0.232	0.162
Delta 5+6 desaturase + elongase (EPA:ALA)	4.46 ± 2.16	3.74 ± 2.73	4.05 ± 2.32	0.645	4.46 ± 2.16	4.02 ± 2.35	0.626	3.74 ± 2.73	4.05 ± 2.32	0.654
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	74.34 ± 29.08	69 ± 30.99	76.43 ± 33.68	0.640	74.34 ± 29.08	75.84 ± 33.47	0.867	69 ± 30.99	76.43 ± 33.68	0.696
Delta 6 desaturase + B-Oxidation (DHA:EPA)	18.79 ± 9.48	21.29 ± 7.95	21.44 ± 8.81	0.694	18.79 ± 9.48	21.43 ± 8.73	0.407	21.29 ± 7.95	21.44 ± 8.81	0.721
n-6:n-3 LCPUFA ratio	5.05 ± 1.7	4.7 ± 1.07	4.47 ± 0.861	0.119	5.05 ± 1.7	4.49 ± 0.878	0.057	4.7 ± 1.07	4.47 ± 0.861	0.120
Model 4 (mother age, gestational age, LA intake)										

	Additive				Recessive			Dominant		
	CC	CT	TT	P-value	CC	CT + TT	P-value	CC + CT	TT	P-value
n	12	18	213		12	231		30	213	
Delta 5 desaturase (AA:DGLA)	11.76 ± 4.88	10.96 ± 5.1	12.82 ± 3.92	0.129	11.76 ± 4.88	12.67 ± 4.04	0.460	10.96 ± 5.1	12.82 ± 3.92	0.130
Delta 6 desaturase (DGLA:LA)	0.116 ± 0.043 ^{ab}	0.136 ± 0.055 ^a	0.11 ± 0.036 ^b	0.041*	0.116 ± 0.043	0.112 ± 0.038	0.840	0.136 ± 0.055	0.11 ± 0.036	0.045*
Delta 5+6 desaturase (AA:LA)	1.2 ± 0.273	1.26 ± 0.2	1.3 ± 0.232	0.150	1.2 ± 0.273	1.29 ± 0.23	0.105	1.26 ± 0.205	1.3 ± 0.232	0.220
Delta 5+6 desaturase + elongase (EPA:ALA)	4.46 ± 2.16	3.74 ± 2.73	4.05 ± 2.32	0.639	4.46 ± 2.16	4.02 ± 2.35	0.569	3.74 ± 2.73	4.05 ± 2.32	0.644
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	74.34 ± 29.08	69 ± 30.99	76.43 ± 33.68	0.628	74.34 ± 29.08	75.84 ± 33.47	0.882	69 ± 30.99	76.43 ± 33.68	0.630
Delta 6 desaturase + B-Oxidation (DHA:EPA)	18.79 ± 9.48	21.29 ± 7.95	21.44 ± 8.81	0.689	18.79 ± 9.48	21.43 ± 8.73	0.397	21.29 ± 7.95	21.44 ± 8.81	0.690
n-6:n-3 LCPUFA ratio	5.05 ± 1.7	4.7 ± 1.07	4.47 ± 0.861	0.119	5.05 ± 1.7	4.49 ± 0.878	0.059	4.7 ± 1.07	4.47 ± 0.861	0.120
Model 5 (mother age, gestational age, DHA intake)										
Delta 5 desaturase (AA:DGLA)	11.76 ± 4.88	10.96 ± 5.1	12.82 ± 3.92	0.131	11.76 ± 4.88	12.67 ± 4.04	0.495	10.96 ± 5.1	12.82 ± 3.92	0.132
Delta 6 desaturase (DGLA:LA)	0.116 ± 0.043 ^{ab}	0.136 ± 0.055 ^a	0.11 ± 0.036 ^b	0.038*	0.116 ± 0.043	0.112 ± 0.038	0.835	0.136 ± 0.055	0.11 ± 0.036	0.040*
Delta 5+6 desaturase (AA:LA)	1.2 ± 0.273	1.26 ± 0.2	1.3 ± 0.232	0.172	1.2 ± 0.273	1.29 ± 0.23	0.12	1.26 ± 0.205	1.3 ± 0.232	0.175
Delta 5+6 desaturase + elongase (EPA:ALA)	4.46 ± 2.16	3.74 ± 2.73	4.05 ± 2.32	0.541	4.46 ± 2.16	4.02 ± 2.35	0.433	3.74 ± 2.73	4.05 ± 2.32	0.550
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	74.34 ± 29.08	69 ± 30.99	76.43 ± 33.68	0.633	74.34 ± 29.08	75.84 ± 33.47	0.955	69 ± 30.99	76.43 ± 33.68	0.645
Delta 6 desaturase + B-Oxidation (DHA:EPA)	18.79 ± 9.48	21.29 ± 7.95	21.44 ± 8.81	0.616	18.79 ± 9.48	21.43 ± 8.73	0.331	21.29 ± 7.95	21.44 ± 8.81	0.621
n-6:n-3 LCPUFA ratio	5.05 ± 1.7	4.7 ± 1.07	4.47 ± 0.861	0.146	5.05 ± 1.7	4.49 ± 0.878	0.074	4.7 ± 1.07	4.47 ± 0.861	0.152
Model 6 (mother age, gestational age, AA intake)										
Delta 5 desaturase (AA:DGLA)	11.76 ± 4.88	10.96 ± 5.1	12.82 ± 3.92	0.149	11.76 ± 4.88	12.67 ± 4.04	0.445	10.96 ± 5.1	12.82 ± 3.92	0.153
Delta 6 desaturase (DGLA:LA)	0.116 ± 0.043 ^{ab}	0.136 ± 0.055 ^a	0.11 ± 0.036 ^b	0.044*	0.116 ± 0.043	0.112 ± 0.038	0.791	0.136 ± 0.055	0.11 ± 0.036	0.050*
Delta 5+6 desaturase (AA:LA)	1.2 ± 0.273	1.26 ± 0.2	1.3 ± 0.232	0.173	1.2 ± 0.273	1.29 ± 0.23	0.115	1.26 ± 0.205	1.3 ± 0.232	0.182
Delta 5+6 desaturase + elongase (EPA:ALA)	4.46 ± 2.16	3.74 ± 2.73	4.05 ± 2.32	0.665	4.46 ± 2.16	4.02 ± 2.35	0.621	3.74 ± 2.73	4.05 ± 2.32	0.667
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	74.34 ± 29.08	69 ± 30.99	76.43 ± 33.68	0.623	74.34 ± 29.08	75.84 ± 33.47	0.860	69 ± 30.99	76.43 ± 33.68	0.632
Delta 6 desaturase + B-Oxidation (DHA:EPA)	18.79 ± 9.48	21.29 ± 7.95	21.44 ± 8.81	0.694	18.79 ± 9.48	21.43 ± 8.73	0.402	21.29 ± 7.95	21.44 ± 8.81	0.700
n-6:n-3 LCPUFA ratio	5.05 ± 1.7	4.7 ± 1.07	4.47 ± 0.861	0.118	5.05 ± 1.7	4.49 ± 0.878	0.057	4.7 ± 1.07	4.47 ± 0.861	0.120

Data presented as mean ± SD; alphabetic letters indicate between group differences with Bonferroni adjust P<0.05, *significance P<0.05

Table 3.8: Distribution of plasma phospholipid LCPUFA ratios as classified by *FADS1*-rs174549_(A/G)

	Additive				Recessive			Dominant		
	AA	AG	GG	P-value	AA	AG + GG	P-value	AA + AG	GG	P-value
n	9	18	216		9	234		27	216	
Model 1 (adjusted for mother age and gestational age)										
Delta 5 desaturase (AA:DGLA)	12.14 ± 5.48	10.96 ± 5.1	12.78 ± 3.9	0.162	12.14 ± 5.48	12.64 ± 4.02	0.711	10.96 ± 5.1	12.78 ± 3.9	0.163
Delta 6 desaturase (DGLA:LA)	0.115 ± 0.05 ^{ab}	0.136 ± 0.055 ^a	0.11 ± 0.036 ^b	0.038*	0.115 ± 0.05	0.112 ± 0.038	0.851	0.136 ± 0.055	0.11 ± 0.036	0.040*
Delta 5+6 desaturase (AA:LA)	1.19 ± 0.285	1.26 ± 0.205	1.3 ± 0.232	0.198	1.19 ± 0.285	1.29 ± 0.229	0.141	1.26 ± 0.205	1.3 ± 0.232	0.197
Delta 5+6 desaturase + elongase (EPA:ALA)	4.71 ± 2.29	3.74 ± 2.73	4.02 ± 2.31	0.528	4.71 ± 2.29	4 ± 2.34	0.409	3.74 ± 2.73	4.02 ± 2.31	0.528
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	77.89 ± 10.82	69 ± 30.99	76.32 ± 33.56	0.628	77.89 ± 10.82	75.76 ± 33.38	0.862	69 ± 30.99	76.32 ± 33.57	0.623
Delta 6 desaturase + B-Oxidation (DHA:EPA)	18.42 ± 9.06	21.29 ± 7.95	21.6 ± 9.11	0.633	18.42 ± 9.06	21.58 ± 9.01	0.343	21.29 ± 7.95	21.6 ± 9.11	0.638
n-6:n-3 LCPUFA ratio	5.1 ± 1.89	4.7 ± 1.07	4.49 ± 0.87	0.145	5.1 ± 1.89	4.51 ± 0.885	0.071	4.7 ± 1.07	4.49 ± 8.69	0.146
Model 2 (adjusted for mother age, gestational age and total energy intake)										
Delta 5 desaturase (AA:DGLA)	12.14 ± 5.48	10.96 ± 5.1	12.78 ± 3.9	0.164	12.14 ± 5.48	12.64 ± 4.02	0.733	10.96 ± 5.1	12.78 ± 3.9	0.167
Delta 6 desaturase (DGLA:LA)	0.115 ± 0.05 ^{ab}	0.136 ± 0.055 ^a	0.11 ± 0.036 ^b	0.038*	0.115 ± 0.05	0.112 ± 0.038	0.864	0.136 ± 0.055	0.11 ± 0.036	0.039*
Delta 5+6 desaturase (AA:LA)	1.19 ± 0.285	1.26 ± 0.205	1.3 ± 0.233	0.194	1.19 ± 0.285	1.29 ± 0.229	0.138	1.26 ± 0.205	1.3 ± 0.233	0.191
Delta 5+6 desaturase + elongase (EPA:ALA)	4.71 ± 2.29	3.74 ± 2.73	4.02 ± 2.31	0.540	4.71 ± 2.29	4 ± 2.34	0.424	3.74 ± 2.73	4.04 ± 2.31	0.543
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	77.89 ± 30.82	69 ± 30.99	76.32 ± 33.56	0.617	77.89 ± 30.82	75.76 ± 33.38	0.813	69 ± 30.99	76.32 ± 33.56	0.618
Delta 6 desaturase + B-Oxidation (DHA:EPA)	18.42 ± 9.06	21.29 ± 7.95	21.6 ± 9.11	0.660	18.42 ± 9.06	21.58 ± 9.01	0.367	21.29 ± 7.95	21.6 ± 9.11	0.677
n-6:n-3 LCPUFA ratio	5.1 ± 1.89	4.7 ± 1.07	4.49 ± 0.87	0.156	5.1 ± 1.89	4.51 ± 0.885	0.077	4.7 ± 1.07	4.49 ± 8.69	0.158
Model 3 (mother age, gestational age, ALA intake)										
Delta 5 desaturase (AA:DGLA)	12.14 ± 5.48	10.96 ± 5.1	12.78 ± 3.91	0.171	12.14 ± 5.48	12.64 ± 4.03	0.725	10.96 ± 5.1	12.78 ± 3.91	0.176
Delta 6 desaturase (DGLA:LA)	0.115 ± 0.05 ^{ab}	0.136 ± 0.055 ^a	0.11 ± 0.036 ^b	0.042*	0.115 ± 0.05	0.112 ± 0.038	0.866	0.136 ± 0.055	0.11 ± 0.036	0.045*
Delta 5+6 desaturase (AA:LA)	1.19 ± 0.28	1.26 ± 0.205	1.3 ± 0.233	0.195	1.19 ± 0.28	1.29 ± 0.23	0.142	1.26 ± 0.205	1.3 ± 0.233	0.210
Delta 5+6 desaturase + elongase (EPA:ALA)	4.72 ± 2.29	3.74 ± 2.73	4.04 ± 2.31	0.531	4.72 ± 2.29	4.02 ± 2.34	0.420	3.74 ± 2.73	4.04 ± 2.31	0.567
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	77.89 ± 30.82	69 ± 30.99	76.25 ± 33.56	0.649	77.89 ± 30.82	75.69 ± 33.36	0.834	69 ± 30.99	76.25 ± 33.56	0.653
Delta 6 desaturase + B-Oxidation (DHA:EPA)	18.42 ± 9.06	21.29 ± 7.95	21.42 ± 8.84	0.653	18.42 ± 9.06	21.41 ± 8.76	0.369	21.29 ± 7.95	21.42 ± 8.84	0.664
n-6:n-3 LCPUFA ratio	5.1 ± 1.89	4.7 ± 1.07	4.48 ± 0.87	0.140	5.1 ± 1.89	4.51 ± 0.881	0.068	4.70 ± 1.07	4.48 ± 0.87	0.141
Model 4 (mother age, gestational age, LA intake)										
Delta 5 desaturase (AA:DGLA)	12.14 ± 5.48	10.96 ± 5.1	12.78 ± 3.9	0.170	12.14 ± 5.48	12.64 ± 4.03	0.723	10.96 ± 5.1	12.78 ± 3.91	0.182
Delta 6 desaturase (DGLA:LA)	0.115 ± 0.05 ^{ab}	0.136 ± 0.055 ^a	0.11 ± 0.036 ^b	0.042*	0.115 ± 0.05	0.112 ± 0.038	0.870	0.136 ± 0.055	0.11 ± 0.036	0.043*
Delta 5+6 desaturase (AA:LA)	1.19 ± 0.28	1.26 ± 0.205	1.3 ± 0.233	0.197	1.19 ± 0.28	1.29 ± 0.23	0.142	1.26 ± 0.205	1.3 ± 0.233	0.230

	Additive				Recessive			Dominant		
	AA	AG	GG	P-value	AA	AG + GG	P-value	AA + AG	GG	P-value
n	9	18	216		9	234		27	216	
Delta 5+6 desaturase + elongase (EPA:ALA)	4.72 ± 2.29	3.74 ± 2.73	4.04 ± 2.31	0.534	4.72 ± 2.29	4.02 ± 2.34	0.403	3.74 ± 2.73	4.04 ± 2.31	0.551
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	77.89 ± 30.82	69 ± 30.99	76.25 ± 33.56	0.637	77.89 ± 30.82	75.69 ± 33.36	0.847	69 ± 30.99	76.25 ± 33.56	0.641
Delta 6 desaturase + B-Oxidation (DHA:EPA)	18.42 ± 9.06	21.29 ± 7.95	21.42 ± 8.84	0.646	18.42 ± 9.06	21.41 ± 8.76	0.359	21.29 ± 7.95	21.42 ± 8.84	0.662
n-6:n-3 LCPUFA ratio	5.1 ± 1.89	4.7 ± 1.07	4.48 ± 0.87	0.134	5.1 ± 1.89	4.51 ± 0.881	0.066	4.7 ± 1.07	4.48 ± 0.87	0.135
Model 5 (mother age, gestational age, DHA intake)										
Delta 5 desaturase (AA:DGLA)	12.14 ± 5.48	10.96 ± 5.1	12.78 ± 3.9	0.169	12.14 ± 5.48	12.64 ± 4.03	0.764	10.96 ± 5.1	12.78 ± 3.91	0.173
Delta 6 desaturase (DGLA:LA)	0.115 ± 0.05 ^{ab}	0.136 ± 0.055 ^a	0.11 ± 0.036 ^b	0.040*	0.115 ± 0.05	0.112 ± 0.038	0.890	0.136 ± 0.055	0.11 ± 0.036	0.041*
Delta 5+6 desaturase (AA:LA)	1.19 ± 0.28	1.26 ± 0.205	1.3 ± 0.233	0.211	1.19 ± 0.28	1.29 ± 0.23	0.151	1.26 ± 0.205	1.3 ± 0.233	0.220
Delta 5+6 desaturase + elongase (EPA:ALA)	4.72 ± 2.29	3.74 ± 2.73	4.04 ± 2.31	0.43	4.72 ± 2.29	4.02 ± 2.34	0.298	3.74 ± 2.73	4.04 ± 2.31	0.441
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	77.89 ± 30.82	69 ± 30.99	76.25 ± 33.56	0.623	77.89 ± 30.82	75.69 ± 33.36	0.785	69 ± 30.99	76.25 ± 33.56	0.626
Delta 6 desaturase + B-Oxidation (DHA:EPA)	18.42 ± 9.06	21.29 ± 7.95	21.42 ± 8.84	0.590	18.42 ± 9.06	21.41 ± 8.76	0.31	21.29 ± 7.95	21.42 ± 8.84	0.640
n-6:n-3 LCPUFA ratio	5.1 ± 1.89	4.7 ± 1.07	4.48 ± 0.87	0.153	5.1 ± 1.89	4.51 ± 0.881	0.077	4.7 ± 1.07	4.48 ± 0.87	0.157
Model 6 (mother age, gestational age, AA intake)										
Delta 5 desaturase (AA:DGLA)	12.14 ± 5.48	10.96 ± 5.1	12.78 ± 3.9	0.199	12.14 ± 5.48	12.64 ± 4.03	0.719	10.96 ± 5.1	12.78 ± 3.91	0.220
Delta 6 desaturase (DGLA:LA)	0.115 ± 0.05 ^{ab}	0.136 ± 0.055 ^a	0.11 ± 0.036 ^b	0.046*	0.115 ± 0.05	0.112 ± 0.038	0.856	0.136 ± 0.055	0.11 ± 0.036	0.048*
Delta 5+6 desaturase (AA:LA)	1.19 ± 0.28	1.26 ± 0.205	1.3 ± 0.233	0.215	1.19 ± 0.28	1.29 ± 0.23	0.147	1.26 ± 0.205	1.3 ± 0.233	0.222
Delta 5+6 desaturase + elongase (EPA:ALA)	4.72 ± 2.29	3.74 ± 2.73	4.04 ± 2.31	0.546	4.72 ± 2.29	4.02 ± 2.34	0.417	3.74 ± 2.73	4.04 ± 2.31	0.551
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	77.89 ± 30.82	69 ± 30.99	76.25 ± 33.56	0.637	77.89 ± 30.82	75.69 ± 33.36	0.854	69 ± 30.99	76.25 ± 33.56	0.639
Delta 6 desaturase + B-Oxidation (DHA:EPA)	18.42 ± 9.06	21.29 ± 7.95	21.42 ± 8.84	0.648	18.42 ± 9.06	21.41 ± 8.76	0.360	21.29 ± 7.95	21.42 ± 8.84	0.645
n-6:n-3 LCPUFA ratio	5.1 ± 1.89	4.7 ± 1.07	4.48 ± 0.87	0.136	5.1 ± 1.89	4.51 ± 0.881	0.065	4.7 ± 1.07	4.48 ± 0.87	0.149

Data presented as mean ± SD; alphabetic letters indicate between group differences with Bonferroni adjust P<0.05, *significance P<0.05

Table 3.9: Distribution of plasma phospholipid LCPUFA ratios as classified by *FADS1*-rs174555_(CT)

	Additive				Recessive			Dominant		
	CC	CT	TT	P-value	CC	CT + TT	P-value	CC + CT	TT	P-value
n	111	19	113		111	132		130	113	
Model 1 (adjusted for mother age and gestational age)										
Delta 5 desaturase (AA:DGLA)	12.68 ± 4.07	10.87 ± 4.98	12.87 ± 3.87	0.121	12.68 ± 4.07	12.58 ± 4.09	0.847	10.87 ± 4.98	12.87 ± 3.87	0.121
Delta 6 desaturase (DGLA:LA)	0.11 ± 0.035 ^a	0.135 ± 0.054 ^b	0.111 ± 0.038 ^{ab}	0.049*	0.11 ± 0.035	0.114 ± 0.041	0.466	0.135 ± 0.054	0.111 ± 0.038	0.050*
Delta 5+6 desaturase (AA:LA)	1.28 ± 0.233	1.25 ± 0.205	1.3 ± 0.236	0.369	1.28 ± 0.233	1.29 ± 0.232	0.807	1.25 ± 0.205	1.3 ± 0.236	0.369
Delta 5+6 desaturase + elongase (EPA:ALA)	4.08 ± 2.33	3.76 ± 2.66	4.02 ± 2.31	0.703	4.08 ± 2.33	3.98 ± 2.35	0.703	3.76 ± 2.66	4.02 ± 2.31	0.705
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	76.15 ± 36.78	68.21 ± 30.31	76.81 ± 29.97	0.539	76.15 ± 36.78	75.57 ± 30.06	0.884	68.21 ± 30.31	76.81 ± 29.97	0.539
Delta 6 desaturase + B-Oxidation (DHA:EPA)	20.7 ± 8.19	20.85 ± 7.96	22.31 ± 9.89	0.448	20.7 ± 8.19	22.1 ± 9.63	0.222	20.85 ± 7.96	22.31 ± 9.89	0.445
n-6:n-3 LCPUFA ratio	4.55 ± 0.966	4.72 ± 1.04	4.48 ± 0.9	0.655	4.55 ± 0.966	4.51 ± 0.921	0.779	4.72 ± 1.04	4.48 ± 0.9	0.655
Model 2 (adjusted for mother age, gestational age and total energy intake)										
Delta 5 desaturase (AA:DGLA)	12.68 ± 4.07	10.87 ± 4.98	12.87 ± 3.87	0.117	12.68 ± 4.07	12.58 ± 4.09	0.849	10.87 ± 4.98	12.87 ± 3.87	0.119
Delta 6 desaturase (DGLA:LA)	0.11 ± 0.035 ^a	0.135 ± 0.054 ^b	0.111 ± 0.038 ^{ab}	0.048*	0.11 ± 0.035	0.114 ± 0.041	0.468	0.135 ± 0.054	0.111 ± 0.038	0.048*
Delta 5+6 desaturase (AA:LA)	1.28 ± 0.233	1.25 ± 0.205	1.3 ± 0.236	0.374	1.28 ± 0.233	1.29 ± 0.232	0.808	1.25 ± 0.205	1.3 ± 0.236	0.374
Delta 5+6 desaturase + elongase (EPA:ALA)	4.08 ± 2.33	3.76 ± 2.66	4.02 ± 2.31	0.711	4.08 ± 2.33	3.98 ± 2.35	0.702	3.76 ± 2.66	4.02 ± 2.31	0.712
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	76.15 ± 36.78	68.21 ± 30.31	76.81 ± 29.97	0.514	76.15 ± 36.78	75.57 ± 30.06	0.888	68.21 ± 30.31	76.81 ± 29.97	0.514
Delta 6 desaturase + B-Oxidation (DHA:EPA)	20.7 ± 8.19	20.85 ± 7.96	22.31 ± 9.89	0.440	20.7 ± 8.19	22.1 ± 9.63	0.219	20.85 ± 7.96	22.31 ± 9.89	0.450
n-6:n-3 LCPUFA ratio	4.55 ± 0.966	4.72 ± 1.04	4.48 ± 0.9	0.640	4.55 ± 0.966	4.51 ± 0.921	0.776	4.72 ± 1.04	4.48 ± 0.9	0.650
Model 3 (mother age, gestational age, ALA intake)										
Delta 5 desaturase (AA:DGLA)	12.68 ± 4.07	10.87 ± 4.98	12.87 ± 3.89	0.124	12.68 ± 4.07	12.58 ± 4.11	0.859	10.87 ± 4.98	12.87 ± 3.89	0.123
Delta 6 desaturase (DGLA:LA)	0.11 ± 0.035	0.135 ± 0.054	0.111 ± 0.038	0.052	0.11 ± 0.035	0.114 ± 0.041	0.494	0.135 ± 0.054	0.111 ± 0.038	0.053
Delta 5+6 desaturase (AA:LA)	1.28 ± 0.233	1.25 ± 0.205	1.3 ± 0.238	0.391	1.28 ± 0.233	1.29 ± 0.233	0.895	1.25 ± 0.205	1.3 ± 0.238	0.392
Delta 5+6 desaturase + elongase (EPA:ALA)	4.08 ± 2.33	3.76 ± 2.66	4.05 ± 2.31	0.704	4.08 ± 2.33	4.01 ± 2.36	0.761	3.76 ± 2.66	4.05 ± 2.31	0.704
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	76.15 ± 36.78	68.21 ± 30.31	76.68 ± 29.87	0.547	76.15 ± 36.78	75.44 ± 29.97	0.905	68.21 ± 30.31	76.68 ± 29.87	0.549
Delta 6 desaturase + B-Oxidation (DHA:EPA)	20.7 ± 8.19	20.85 ± 7.96	21.97 ± 9.46	0.572	20.7 ± 8.19	21.8 ± 9.23	0.303	20.85 ± 7.96	21.97 ± 9.46	0.573
n-6:n-3 LCPUFA ratio	4.55 ± 0.966	4.72 ± 1.04	4.46 ± 0.892	0.546	4.55 ± 0.966	4.49 ± 0.916	0.594	4.72 ± 1.04	4.46 ± 0.892	0.549
Model 4 (mother age, gestational age, LA intake)										
Delta 5 desaturase (AA:DGLA)	12.68 ± 4.07	10.87 ± 4.98	12.87 ± 3.89	0.131	12.68 ± 4.07	12.58 ± 4.11	0.823	10.87 ± 4.98	12.87 ± 3.89	0.131
Delta 6 desaturase (DGLA:LA)	0.11 ± 0.035	0.135 ± 0.054	0.111 ± 0.038	0.057	0.11 ± 0.035	0.114 ± 0.041	0.451	0.135 ± 0.054	0.111 ± 0.038	0.058
Delta 5+6 desaturase (AA:LA)	1.28 ± 0.233	1.25 ± 0.205	1.3 ± 0.238	0.367	1.28 ± 0.233	1.29 ± 0.233	0.852	1.25 ± 0.205	1.3 ± 0.238	0.369

	Additive				Recessive			Dominant		
	CC	CT	TT	P-value	CC	CT + TT	P-value	CC + CT	TT	P-value
n	111	19	113		111	132		130	113	
Delta 5+6 desaturase + elongase (EPA:ALA)	4.08 ± 2.33	3.76 ± 2.66	4.05 ± 2.31	0.738	4.08 ± 2.33	4.01 ± 2.36	0.751	3.76 ± 2.66	4.05 ± 2.31	0.738
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	76.15 ± 36.78	68.21 ± 30.31	76.68 ± 29.87	0.558	76.15 ± 36.78	75.44 ± 29.97	0.838	68.21 ± 30.31	76.68 ± 29.87	0.555
Delta 6 desaturase + B-Oxidation (DHA:EPA)	20.7 ± 8.19	20.85 ± 7.96	21.97 ± 9.46	0.607	20.7 ± 8.19	21.8 ± 9.23	0.332	20.85 ± 7.96	21.97 ± 9.46	0.609
n-6:n-3 LCPUFA ratio	4.55 ± 0.966	4.72 ± 1.04	4.46 ± 0.892	0.578	4.55 ± 0.966	4.49 ± 0.916	0.645	4.72 ± 1.04	4.46 ± 0.892	0.578
Model 5 (mother age, gestational age, DHA intake)										
Delta 5 desaturase (AA:DGLA)	12.68 ± 4.07	10.87 ± 4.98	12.87 ± 3.89	0.127	12.68 ± 4.07	12.58 ± 4.11	0.744	10.87 ± 4.98	12.87 ± 3.89	0.129
Delta 6 desaturase (DGLA:LA)	0.11 ± 0.035 ^a	0.135 ± 0.054 ^b	0.111 ± 0.038 ^{ab}	0.05*	0.11 ± 0.035	0.114 ± 0.041	0.415	0.135 ± 0.054	0.111 ± 0.038	0.051
Delta 5+6 desaturase (AA:LA)	1.28 ± 0.233	1.25 ± 0.205	1.3 ± 0.238	0.395	1.28 ± 0.233	1.29 ± 0.233	0.881	1.25 ± 0.205	1.3 ± 0.238	0.420
Delta 5+6 desaturase + elongase (EPA:ALA)	4.08 ± 2.33	3.76 ± 2.66	4.05 ± 2.31	0.641	4.08 ± 2.33	4.01 ± 2.36	0.532	3.76 ± 2.66	4.05 ± 2.31	0.647
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	76.15 ± 36.78	68.21 ± 30.31	76.68 ± 29.87	0.546	76.15 ± 36.78	75.44 ± 29.97	0.731	68.21 ± 30.31	76.68 ± 29.87	0.548
Delta 6 desaturase + B-Oxidation (DHA:EPA)	20.7 ± 8.19	20.85 ± 7.96	21.97 ± 9.46	0.509	20.7 ± 8.19	21.8 ± 9.23	0.261	20.85 ± 7.96	21.97 ± 9.46	0.510
n-6:n-3 LCPUFA ratio	4.55 ± 0.966	4.72 ± 1.04	4.46 ± 0.892	0.635	4.55 ± 0.966	4.49 ± 0.916	0.772	4.72 ± 1.04	4.46 ± 0.892	0.637
Model 6 (mother age, gestational age, AA intake)										
Delta 5 desaturase (AA:DGLA)	12.68 ± 4.07	10.87 ± 4.98	12.87 ± 3.89	0.154	12.68 ± 4.07	12.58 ± 4.11	0.882	10.87 ± 4.98	12.87 ± 3.89	0.155
Delta 6 desaturase (DGLA:LA)	0.11 ± 0.035	0.135 ± 0.054	0.111 ± 0.038	0.061	0.11 ± 0.035	0.114 ± 0.041	0.49	0.135 ± 0.054	0.111 ± 0.038	0.069
Delta 5+6 desaturase (AA:LA)	1.28 ± 0.233	1.25 ± 0.205	1.3 ± 0.238	0.409	1.28 ± 0.233	1.29 ± 0.233	0.838	1.25 ± 0.205	1.3 ± 0.238	0.411
Delta 5+6 desaturase + elongase (EPA:ALA)	4.08 ± 2.33	3.76 ± 2.66	4.05 ± 2.31	0.731	4.08 ± 2.33	4.01 ± 2.36	0.782	3.76 ± 2.66	4.05 ± 2.31	0.738
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	76.15 ± 36.78	68.21 ± 30.31	76.68 ± 29.87	0.552	76.15 ± 36.78	75.44 ± 29.97	0.849	68.21 ± 30.31	76.68 ± 29.87	0.555
Delta 6 desaturase + B-Oxidation (DHA:EPA)	20.7 ± 8.19	20.85 ± 7.96	21.97 ± 9.46	0.610	20.7 ± 8.19	21.8 ± 9.23	0.335	20.85 ± 7.96	21.97 ± 9.46	0.611
n-6:n-3 LCPUFA ratio	4.55 ± 0.966	4.72 ± 1.04	4.46 ± 0.892	0.580	4.55 ± 0.966	4.49 ± 0.916	0.628	4.72 ± 1.04	4.46 ± 0.892	0.591

Data presented as mean ± SD; alphabetic letters indicate between group differences with Bonferroni adjust P<0.05, *significance P<0.05

Table 3.10: Distribution of plasma phospholipid LCPUFA ratios as classified by *FADS2*-rs78678033_(G/A)

	Additive				Recessive		
	GG	GA	AA	P-value	GG	GA + AA	P-value
n	171	51	4		171	55	
Model 1 (adjusted for mother age and gestational age)							
Delta 5 desaturase (AA:DGLA)	12.31 ± 4.09	13.9 ± 4.18	13.17 ± 3.24	0.058	12.31 ± 4.09	13.85 ± 4.09	0.018*
Delta 6 desaturase (DGLA:LA)	0.114 ± 0.039	0.104 ± 0.038	0.096 ± 0.016	0.096	0.114 ± 0.039	0.103 ± 0.037	0.038*
Delta 5+6 desaturase (AA:LA)	1.28 ± 0.224	1.32 ± 0.243	1.23 ± 0.124	0.484	1.28 ± 0.224	1.31 ± 0.236	0.642
Delta 5+6 desaturase + elongase (EPA:ALA)	3.91 ± 2.35	4.26 ± 2.46	4.56 ± 3.76	0.530	3.91 ± 2.35	4.37 ± 2.53	0.260
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	75.99 ± 33.56	75.1 ± 33.73	59.68 ± 20.11	0.612	75.99 ± 33.56	73.98 ± 33.04	0.691
Delta 6 desaturase + B-Oxidation (DHA:EPA)	22.09 ± 9.17	19.92 ± 8.89	17.67 ± 10.37	0.273	22.09 ± 9.17	19.76 ± 8.91	0.118
n-6:n-3 LCPUFA ratio	4.56 ± 0.921	4.41 ± 0.882	4.32 ± 0.68	0.468	4.56 ± 0.921	4.4 ± 0.864	0.227
Model 2 (adjusted for mother age, gestational age and total energy intake)							
Delta 5 desaturase (AA:DGLA)	12.31 ± 4.09	13.9 ± 4.18	13.17 ± 3.24	0.054	12.31 ± 4.09	13.85 ± 4.09	0.017*
Delta 6 desaturase (DGLA:LA)	0.114 ± 0.039	0.104 ± 0.038	0.096 ± 0.016	0.094	0.114 ± 0.039	0.103 ± 0.037	0.036*
Delta 5+6 desaturase (AA:LA)	1.28 ± 0.224	1.32 ± 0.243	1.23 ± 0.124	0.503	1.28 ± 0.224	1.31 ± 0.236	0.657
Delta 5+6 desaturase + elongase (EPA:ALA)	3.91 ± 2.35	4.26 ± 2.46	4.56 ± 3.76	0.546	3.91 ± 2.35	4.37 ± 2.53	0.273
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	75.99 ± 33.56	75.1 ± 33.73	59.68 ± 20.11	0.584	75.99 ± 33.56	73.98 ± 33.04	0.728
Delta 6 desaturase + B-Oxidation (DHA:EPA)	22.09 ± 9.17	19.92 ± 8.89	17.67 ± 10.37	0.284	22.09 ± 9.17	19.76 ± 8.91	0.129
n-6:n-3 LCPUFA ratio	4.56 ± 0.921	4.41 ± 0.882	4.32 ± 0.68	0.461	4.56 ± 0.921	4.4 ± 0.864	0.220
Model 3 (mother age, gestational age, ALA intake)							
Delta 5 desaturase (AA:DGLA)	12.31 ± 4.1	13.9 ± 4.18	13.17 ± 3.24	0.056	12.31 ± 4.1	13.85 ± 4.09	0.018*
Delta 6 desaturase (DGLA:LA)	0.115 ± 0.039	0.104 ± 0.038	0.096 ± 0.016	0.096	0.115 ± 0.039	0.103 ± 0.037	0.036*
Delta 5+6 desaturase (AA:LA)	1.28 ± 0.225	1.32 ± 0.243	1.23 ± 0.124	0.507	1.28 ± 0.225	1.31 ± 0.236	0.657
Delta 5+6 desaturase + elongase (EPA:ALA)	3.94 ± 2.36	4.36 ± 2.46	4.56 ± 3.76	0.556	3.94 ± 2.36	4.37 ± 2.53	0.280
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	75.89 ± 33.54	75.1 ± 33.73	59.68 ± 20.11	0.565	75.89 ± 33.56	73.98 ± 33.04	0.716
Delta 6 desaturase + B-Oxidation (DHA:EPA)	21.86 ± 8.84	19.92 ± 8.89	17.67 ± 10.37	0.282	21.86 ± 8.84	19.76 ± 8.91	0.134
n-6:n-3 LCPUFA ratio	4.55 ± 0.918	4.41 ± 0.882	4.32 ± 0.68	0.496	4.55 ± 0.918	4.40 ± 0.864	0.245
Model 4 (mother age, gestational age, LA intake)							
Delta 5 desaturase (AA:DGLA)	12.31 ± 4.1	13.9 ± 4.18	13.17 ± 3.24	0.056	12.31 ± 4.1	13.85 ± 4.09	0.017*
Delta 6 desaturase (DGLA:LA)	0.115 ± 0.039	0.104 ± 0.038	0.096 ± 0.016	0.085	0.115 ± 0.039	0.103 ± 0.037	0.033*
Delta 5+6 desaturase (AA:LA)	1.28 ± 0.225	1.32 ± 0.243	1.23 ± 0.124	0.478	1.28 ± 0.225	1.31 ± 0.236	0.657

	Additive				Recessive		
	GG	GA	AA	P-value	GG	GA + AA	P-value
n	171	51	4		171	55	
Delta 5+6 desaturase + elongase (EPA:ALA)	3.94 ± 2.36	4.36 ± 2.46	4.56 ± 3.76	0.495	3.94 ± 2.36	4.37 ± 2.53	0.237
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	75.89 ± 33.54	75.1 ± 33.73	59.68 ± 20.11	0.607	75.89 ± 33.56	73.98 ± 33.04	0.709
Delta 6 desaturase + B-Oxidation (DHA:EPA)	21.86 ± 8.84	19.92 ± 8.89	17.67 ± 10.37	0.266	21.86 ± 8.84	19.76 ± 8.91	0.120
n-6:n-3 LCPUFA ratio	4.55 ± 0.918	4.41 ± 0.882	4.32 ± 0.68	0.475	4.55 ± 0.918	4.4 ± 0.864	0.235
Model 5 (mother age, gestational age, DHA intake)							
Delta 5 desaturase (AA:DGLA)	12.31 ± 4.1	13.9 ± 4.18	13.17 ± 3.24	0.054	12.31 ± 4.1	13.85 ± 4.09	0.020*
Delta 6 desaturase (DGLA:LA)	0.115 ± 0.039	0.104 ± 0.038	0.096 ± 0.016	0.115	0.115 ± 0.039	0.103 ± 0.037	0.041*
Delta 5+6 desaturase (AA:LA)	1.28 ± 0.225	1.32 ± 0.243	1.23 ± 0.124	0.416	1.28 ± 0.225	1.31 ± 0.236	0.644
Delta 5+6 desaturase + elongase (EPA:ALA)	3.94 ± 2.36	4.36 ± 2.46	4.56 ± 3.76	0.455	3.94 ± 2.36	4.37 ± 2.53	0.294
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	75.89 ± 33.54	75.1 ± 33.73	59.68 ± 20.11	0.384	75.89 ± 33.56	73.98 ± 33.04	0.651
Delta 6 desaturase + B-Oxidation (DHA:EPA)	21.86 ± 8.84	19.92 ± 8.89	17.67 ± 10.37	0.324	21.86 ± 8.84	19.76 ± 8.91	0.134
n-6:n-3 LCPUFA ratio	4.55 ± 0.918	4.41 ± 0.882	4.32 ± 0.68	0.539	4.55 ± 0.918	4.4 ± 0.864	0.271
Model 6 (mother age, gestational age, AA intake)							
Delta 5 desaturase (AA:DGLA)	12.31 ± 4.1	13.9 ± 4.18	13.17 ± 3.24	0.07	12.31 ± 4.1	13.85 ± 4.09	0.022*
Delta 6 desaturase (DGLA:LA)	0.115 ± 0.039	0.104 ± 0.038	0.096 ± 0.016	0.106	0.115 ± 0.039	0.103 ± 0.037	0.044*
Delta 5+6 desaturase (AA:LA)	1.28 ± 0.225	1.32 ± 0.243	1.23 ± 0.124	0.49	1.28 ± 0.225	1.31 ± 0.236	0.653
Delta 5+6 desaturase + elongase (EPA:ALA)	3.94 ± 2.36	4.36 ± 2.46	4.56 ± 3.76	0.567	3.94 ± 2.36	4.37 ± 2.53	0.288
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	75.89 ± 33.54	75.1 ± 33.73	59.68 ± 20.11	0.60	75.89 ± 33.56	73.98 ± 33.04	0.682
Delta 6 desaturase + B-Oxidation (DHA:EPA)	21.86 ± 8.84	19.92 ± 8.89	17.67 ± 10.37	0.281	21.86 ± 8.84	19.76 ± 8.91	0.129
n-6:n-3 LCPUFA ratio	4.55 ± 0.918	4.41 ± 0.882	4.32 ± 0.680	0.511	4.55 ± 0.918	4.4 ± 0.864	0.261

Data presented as mean ± SD; *significance P<0.05

Table 3.11: Distribution of plasma phospholipid LCPUFA ratios as classified by *FADS2*-rs174606_(T/G)

	Additive				Recessive			Dominant		
	TT	TG	GG	P-value	TT	TG + GG	P-value	TT + TG	GG	P-value
n	92	98	40		92	138		190	40	
Model 1 (adjusted for mother age and gestational age)										
Delta 5 desaturase (AA:DGLA)	12.76 ± 3.98	12.99 ± 4.22	11.53 ± 3.57	0.252	12.76 ± 3.98	12.57 ± 4.08	0.868	12.88 ± 4.09	11.53 ± 3.57	0.137
Delta 6 desaturase (DGLA:LA)	0.111 ± 0.038	0.107 ± 0.037	0.127 ± 0.038	0.058	0.111 ± 0.038	0.113 ± 0.039	0.949	0.109 ± 0.037	0.127 ± 0.038	0.030*
Delta 5+6 desaturase (AA:LA)	1.3 ± 0.236	1.26 ± 0.22	1.36 ± 0.232	0.070	1.3 ± 0.236	1.29 ± 0.228	0.187	1.28 ± 0.228	1.36 ± 0.232	0.061
Delta 5+6 desaturase + elongase (EPA:ALA)	4.36 ± 2.38	3.73 ± 2.49	3.96 ± 1.85	0.233	4.36 ± 2.38	3.8 ± 2.32	0.123	4.03 ± 2.45	3.96 ± 1.85	0.899
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	76.8 ± 31.43	77.87 ± 37.13	70.98 ± 27.93	0.552	76.8 ± 31.43	75.87 ± 34.76	0.676	77.35 ± 34.4	70.98 ± 27.93	0.355
Delta 6 desaturase + B-Oxidation (DHA:EPA)	20.39 ± 8.91 ^a	23.54 ± 9.27 ^b	19.98 ± 8 ^{ab}	0.023*	20.39 ± 8.91	22.51 ± 9.04	0.056	22.02 ± 9.21	19.98 ± 8	0.277
n-6:n-3 LCPUFA ratio	4.41 ± 0.875	4.62 ± 0.935	4.47 ± 0.728	0.266	4.41 ± 0.875	4.57 ± 0.88	0.216	4.52 ± 0.91	4.47 ± 0.728	0.393
Model 2 (adjusted for mother age, gestational age and total energy intake)										
Delta 5 desaturase (AA:DGLA)	12.76 ± 3.98	12.99 ± 4.22	11.53 ± 3.57	0.252	12.76 ± 3.98	12.57 ± 4.08	0.867	12.88 ± 4.09	11.53 ± 3.57	0.145
Delta 6 desaturase (DGLA:LA)	0.111 ± 0.038	0.107 ± 0.037	0.127 ± 0.038	0.058	0.111 ± 0.038	0.113 ± 0.039	0.946	0.109 ± 0.037	0.127 ± 0.038	0.032*
Delta 5+6 desaturase (AA:LA)	1.3 ± 0.236	1.26 ± 0.22	1.36 ± 0.232	0.07	1.3 ± 0.236	1.29 ± 0.228	0.187	1.28 ± 0.228	1.36 ± 0.232	0.062
Delta 5+6 desaturase + elongase (EPA:ALA)	4.36 ± 2.38	3.73 ± 2.49	3.96 ± 1.85	0.233	4.36 ± 2.38	3.8 ± 2.32	0.127	4.03 ± 2.45	3.96 ± 1.85	0.889
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	76.8 ± 31.43	77.87 ± 37.13	70.98 ± 27.93	0.552	76.8 ± 31.43	75.87 ± 34.76	0.685	77.35 ± 34.4	70.98 ± 27.93	0.384
Delta 6 desaturase + B-Oxidation (DHA:EPA)	20.39 ± 8.91 ^a	23.54 ± 9.27 ^b	19.98 ± 8 ^{ab}	0.023*	20.39 ± 8.91	22.51 ± 9.04	0.06	22.02 ± 9.21	19.98 ± 8	0.295
n-6:n-3 LCPUFA ratio	4.41 ± 0.875	4.62 ± 0.935	4.47 ± 0.728	0.256	4.41 ± 0.875	4.57 ± 0.88	0.216	4.52 ± 0.91	4.47 ± 0.728	0.397
Model 3 (mother age, gestational age, ALA intake)										
Delta 5 desaturase (AA:DGLA)	12.79 ± 3.99	12.96 ± 4.23	11.53 ± 3.57	0.276	12.79 ± 3.99	12.54 ± 4.09	0.840	12.88 ± 4.11	11.53 ± 3.57	0.148
Delta 6 desaturase (DGLA:LA)	0.11 ± 0.038	0.107 ± 0.037	0.127 ± 0.038	0.066	0.111 ± 0.038	0.113 ± 0.039	0.921	0.109 ± 0.038	0.127 ± 0.038	0.033*
Delta 5+6 desaturase (AA:LA)	1.3 ± 0.237	1.26 ± 0.221	1.36 ± 0.235	0.061	1.3 ± 0.237	1.29 ± 0.229	0.166	1.28 ± 0.229	1.36 ± 0.235	0.056
Delta 5+6 desaturase + elongase (EPA:ALA)	4.38 ± 2.38	3.75 ± 2.5	3.96 ± 1.85	0.224	4.38 ± 2.38	3.81 ± 2.33	0.117	4.06 ± 2.45	3.96 ± 1.85	0.885
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	77.09 ± 31.47	77.45 ± 37.09	70.98 ± 27.93	0.618	77.09 ± 31.47	75.56 ± 34.69	0.693	77.28 ± 34.4	70.98 ± 27.93	0.409
Delta 6 desaturase + B-Oxidation (DHA:EPA)	20.32 ± 8.93 ^a	23.21 ± 8.72 ^b	19.98 ± 8.01 ^{ab}	0.043*	20.32 ± 8.93	22.27 ± 8.62	0.082	21.81 ± 8.92	19.98 ± 8.01	0.338
n-6:n-3 LCPUFA ratio	4.4 ± 0.868	4.61 ± 0.933	4.47 ± 0.728	0.263	4.4 ± 0.868	4.57 ± 0.88	0.211	4.5 ± 0.906	4.47 ± 0.728	0.426
Model 4 (mother age, gestational age, LA intake)										
Delta 5 desaturase (AA:DGLA)	12.79 ± 3.99	12.96 ± 4.23	11.53 ± 3.57	0.285	12.79 ± 3.99	12.54 ± 4.09	0.821	12.88 ± 4.11	11.53 ± 3.57	0.153
Delta 6 desaturase (DGLA:LA)	0.11 ± 0.038	0.107 ± 0.037	0.127 ± 0.038	0.080	0.111 ± 0.038	0.113 ± 0.039	0.906	0.109 ± 0.038	0.127 ± 0.038	0.039*
Delta 5+6 desaturase (AA:LA)	1.3 ± 0.237	1.26 ± 0.221	1.36 ± 0.235	0.071	1.3 ± 0.237	1.29 ± 0.229	0.186	1.28 ± 0.229	1.36 ± 0.235	0.064

	Additive				Recessive			Dominant		
	TT	TG	GG	P-value	TT	TG + GG	P-value	TT + TG	GG	P-value
n	92	98	40		92	138		190	40	
Delta 5+6 desaturase + elongase (EPA:ALA)	4.38 ± 2.38	3.75 ± 2.5	3.96 ± 1.85	0.177	4.38 ± 2.38	3.81 ± 2.33	0.094	4.06 ± 2.45	3.96 ± 1.85	0.928
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	77.09 ± 31.47	77.45 ± 37.09	70.98 ± 27.93	0.588	77.09 ± 31.47	75.56 ± 34.69	0.646	77.28 ± 34.4	70.98 ± 27.93	0.381
Delta 6 desaturase + B-Oxidation (DHA:EPA)	20.32 ± 8.93 ^a	23.21 ± 8.72 ^b	19.98 ± 8.01 ^{ab}	0.033*	20.32 ± 8.93	22.27 ± 8.62	0.070	21.81 ± 8.92	19.98 ± 8.01	0.299
n-6:n-3 LCPUFA ratio	4.4 ± 0.868	4.61 ± 0.933	4.47 ± 0.728	0.245	4.4 ± 0.868	4.57 ± 0.88	0.194	4.5 ± 0.906	4.47 ± 0.728	0.387
Model 5 (mother age, gestational age, DHA intake)										
Delta 5 desaturase (AA:DGLA)	12.79 ± 3.99	12.96 ± 4.23	11.53 ± 3.57	0.258	12.79 ± 3.99	12.54 ± 4.09	0.913	12.88 ± 4.11	11.53 ± 3.57	0.146
Delta 6 desaturase (DGLA:LA)	0.11 ± 0.038	0.107 ± 0.037	0.127 ± 0.038	0.057	0.111 ± 0.038	0.113 ± 0.039	0.966	0.109 ± 0.038	0.127 ± 0.038	0.032*
Delta 5+6 desaturase (AA:LA)	1.3 ± 0.237	1.26 ± 0.221	1.36 ± 0.235	0.067	1.3 ± 0.237	1.29 ± 0.229	0.194	1.28 ± 0.229	1.36 ± 0.235	0.062
Delta 5+6 desaturase + elongase (EPA:ALA)	4.38 ± 2.38	3.75 ± 2.5	3.96 ± 1.85	0.409	4.38 ± 2.38	3.81 ± 2.33	0.239	4.06 ± 2.45	3.96 ± 1.85	0.889
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	77.09 ± 31.47	77.45 ± 37.09	70.98 ± 27.93	0.587	77.09 ± 31.47	75.56 ± 34.69	0.740	77.28 ± 34.4	70.98 ± 27.93	0.394
Delta 6 desaturase + B-Oxidation (DHA:EPA)	20.32 ± 8.93 ^a	23.21 ± 8.72 ^b	19.98 ± 8.01 ^{ab}	0.048*	20.32 ± 8.93	22.27 ± 8.62	0.092	21.81 ± 8.92	19.98 ± 8.01	0.255
n-6:n-3 LCPUFA ratio	4.4 ± 0.868	4.61 ± 0.933	4.47 ± 0.728	0.378	4.4 ± 0.868	4.57 ± 0.88	0.299	4.5 ± 0.906	4.47 ± 0.728	0.432
Model 6 (mother age, gestational age, AA intake)										
Delta 5 desaturase (AA:DGLA)	12.79 ± 3.99	12.96 ± 4.23	11.53 ± 3.57	0.223	12.79 ± 3.99	12.54 ± 4.09	0.799	12.88 ± 4.11	11.53 ± 3.57	0.123
Delta 6 desaturase (DGLA:LA)	0.11 ± 0.038	0.107 ± 0.037	0.127 ± 0.038	0.056	0.111 ± 0.038	0.113 ± 0.039	0.968	0.109 ± 0.038	0.127 ± 0.038	0.031*
Delta 5+6 desaturase (AA:LA)	1.3 ± 0.237	1.26 ± 0.221	1.36 ± 0.235	0.058	1.3 ± 0.237	1.29 ± 0.229	0.162	1.28 ± 0.229	1.36 ± 0.235	0.05*
Delta 5+6 desaturase + elongase (EPA:ALA)	4.38 ± 2.38	3.75 ± 2.5	3.96 ± 1.85	0.242	4.38 ± 2.38	3.81 ± 2.33	0.128	4.06 ± 2.45	3.96 ± 1.85	0.881
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	77.09 ± 31.47	77.45 ± 37.09	70.98 ± 27.93	0.557	77.09 ± 31.47	75.56 ± 34.69	0.640	77.28 ± 34.4	70.98 ± 27.93	0.355
Delta 6 desaturase + B-Oxidation (DHA:EPA)	20.32 ± 8.93 ^a	23.21 ± 8.72 ^b	19.98 ± 8.01 ^{ab}	0.035*	20.32 ± 8.93	22.27 ± 8.62	0.071	21.81 ± 8.92	19.98 ± 8.01	0.305
n-6:n-3 LCPUFA ratio	4.4 ± 0.868	4.61 ± 0.933	4.47 ± 0.728	0.255	4.4 ± 0.868	4.57 ± 0.88	0.192	4.5 ± 0.906	4.47 ± 0.728	0.352

Data presented as mean ± SD; alphabetic letters indicate between group differences with Bonferroni adjust P<0.05, *significance P<0.05

Table 3.12: Distribution of plasma phospholipid LCPUFA ratios as classified by *FADS2*-rs174616_(G/A)

	Additive				Recessive			Dominant		
	GG	GA	AA	p-value	GG	GA + AA	p-value	GG + GA	AA	P-value
n	38	92	100		38	192		130	100	
Model 1 (adjusted for mother age and gestational age)										
Delta 5 desaturase (AA:DGLA)	11.45 ± 3.64	12.89 ± 4.18	12.8 ± 3.92	0.268	11.45 ± 3.64	12.84 ± 4.04	0.141	12.47 ± 4.07	12.8 ± 3.92	0.814
Delta 6 desaturase (DGLA:LA)	0.13 ± 0.038 ^a	0.108 ± 0.037 ^b	0.11 ± 0.037 ^{ab}	0.034*	0.13 ± 0.038	0.109 ± 0.037	0.014*	0.114 ± 0.039	0.110 ± 0.037	0.715
Delta 5+6 desaturase (AA:LA)	1.37 ± 0.228	1.26 ± 0.222	1.29 ± 0.233	0.083	1.37 ± 0.228	1.28 ± 0.227	0.053	1.29 ± 0.228	1.29 ± 0.233	0.338
Delta 5+6 desaturase + elongase (EPA:ALA)	3.91 ± 1.88	3.7 ± 2.53	4.34 ± 2.32	0.218	3.91 ± 1.88	4.03 ± 2.44	0.842	3.76 ± 2.36	4.34 ± 2.32	0.112
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	70.66 ± 27.59	77.57 ± 38.42	76.44 ± 30.78	0.705	70.66 ± 27.59	76.98 ± 34.57	0.507	75.55 ± 35.64	76.44 ± 30.78	0.936
Delta 6 desaturase + B-Oxidation (DHA:EPA)	20.1 ± 7.86 ^a	23.57 ± 9.06 ^b	20.34 ± 9.13 ^{ab}	0.047*	20.1 ± 7.86	21.89 ± 9.22	0.610	22.56 ± 8.84	20.34 ± 9.13	0.095
n-6:n-3 LCPUFA ratio	4.5 ± 0.727	4.59 ± 0.946	4.41 ± 0.88	0.103	4.5 ± 0.727	4.5 ± 0.914	0.103	4.56 ± 0.886	4.41 ± 0.879	0.053
Model 2 (adjusted for mother age, gestational age and Total energy intake)										
Delta 5 desaturase (AA:DGLA)	11.45 ± 3.64	12.89 ± 4.18	12.8 ± 3.92	0.287	11.45 ± 3.64	12.84 ± 4.04	0.152	12.47 ± 4.07	12.8 ± 3.92	0.801
Delta 6 desaturase (DGLA:LA)	0.13 ± 0.038 ^a	0.108 ± 0.037 ^b	0.11 ± 0.037 ^{ab}	0.036*	0.13 ± 0.038	0.109 ± 0.037	0.015*	0.114 ± 0.039	0.11 ± 0.037	0.707
Delta 5+6 desaturase (AA:LA)	1.37 ± 0.228	1.26 ± 0.222	1.29 ± 0.233	0.087	1.37 ± 0.228	1.28 ± 0.227	0.054	1.29 ± 0.228	1.29 ± 0.233	0.33
Delta 5+6 desaturase + elongase (EPA:ALA)	3.91 ± 1.88	3.7 ± 2.53	4.34 ± 2.32	0.228	3.91 ± 1.88	4.03 ± 2.44	0.824	3.76 ± 2.36	4.34 ± 2.32	0.116
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	70.66 ± 27.59	77.57 ± 38.42	76.44 ± 30.78	0.764	70.66 ± 27.59	76.98 ± 34.57	0.566	75.55 ± 35.64	76.44 ± 30.78	0.941
Delta 6 desaturase + B-Oxidation (DHA:EPA)	20.1 ± 7.86	23.57 ± 9.06	20.34 ± 9.13	0.057	20.1 ± 7.86	21.89 ± 9.22	0.660	22.56 ± 8.84	20.34 ± 9.13	0.103
n-6:n-3 LCPUFA ratio	4.5 ± 0.727	4.59 ± 0.946	4.41 ± 0.88	0.105	4.5 ± 0.727	4.5 ± 0.914	0.113	4.56 ± 0.886	4.41 ± 0.879	0.057
Model 3 (mother age, gestational age, ALA intake)										
Delta 5 desaturase (AA:DGLA)	11.45 ± 3.64	12.86 ± 4.19	12.83 ± 3.93	0.288	11.45 ± 3.64	12.84 ± 4.05	0.152	12.45 ± 4.08	12.83 ± 3.93	0.731
Delta 6 desaturase (DGLA:LA)	0.13 ± 0.038 ^a	0.108 ± 0.038 ^b	0.11 ± 0.038 ^{ab}	0.040*	0.13 ± 0.038	0.109 ± 0.037	0.016*	0.114 ± 0.039	0.11 ± 0.038	0.645
Delta 5+6 desaturase (AA:LA)	1.37 ± 0.228	1.26 ± 0.222	1.29 ± 0.234	0.082	1.37 ± 0.228	1.28 ± 0.228	0.052	1.29 ± 0.229	1.29 ± 0.234	0.318
Delta 5+6 desaturase + elongase (EPA:ALA)	3.91 ± 1.88	3.71 ± 2.54	4.37 ± 2.32	0.210	3.91 ± 1.88	4.05 ± 2.44	0.827	3.77 ± 2.36	4.37 ± 2.32	0.106
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	70.66 ± 27.59	77.13 ± 38.4	76.71 ± 30.82	0.767	70.66 ± 27.59	76.91 ± 34.56	0.565	75.22 ± 35.57	76.71 ± 30.82	0.882
Delta 6 desaturase + B-Oxidation (DHA:EPA)	20.1 ± 7.86	23.22 ± 8.46	20.27 ± 9.15	0.092	20.1 ± 7.86	21.68 ± 8.93	0.702	22.3 ± 8.38	20.27 ± 9.15	0.146
n-6:n-3 LCPUFA ratio	4.5 ± 0.727	4.58 ± 0.944	4.4 ± 0.873	0.097	4.5 ± 0.727	4.48 ± 0.91	0.111	4.55 ± 0.883	4.4 ± 0.873	0.051

	Additive				Recessive			Dominant		
	GG	GA	AA	p-value	GG	GA + AA	p-value	GG + GA	AA	P-value
n	38	92	100		38	192		130	100	
Model 4 (mother age, gestational age, LA intake)										
Delta 5 desaturase (AA:DGLA)	11.45 ± 3.64	12.86 ± 4.19	12.83 ± 3.93	0.290	11.45 ± 3.64	12.84 ± 4.05	0.153	12.45 ± 4.08	12.83 ± 3.93	0.743
Delta 6 desaturase (DGLA:LA)	0.13 ± 0.038 ^a	0.108 ± 0.038 ^b	0.11 ± 0.038 ^{ab}	0.043*	0.13 ± 0.038	0.109 ± 0.037	0.017*	0.114 ± 0.039	0.11 ± 0.038	0.643
Delta 5+6 desaturase (AA:LA)	1.37 ± 0.228	1.26 ± 0.222	1.29 ± 0.234	0.087	1.37 ± 0.228	1.28 ± 0.228	0.057	1.29 ± 0.229	1.29 ± 0.234	0.350
Delta 5+6 desaturase + elongase (EPA:ALA)	3.91 ± 1.88	3.71 ± 2.54	4.37 ± 2.32	0.166	3.91 ± 1.88	4.05 ± 2.44	0.868	3.77 ± 2.36	4.37 ± 2.32	0.084
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	70.66 ± 27.59	77.13 ± 38.4	76.71 ± 30.82	0.739	70.66 ± 27.59	76.91 ± 34.56	0.532	75.22 ± 35.57	76.71 ± 30.82	0.887
Delta 6 desaturase + B-Oxidation (DHA:EPA)	20.1 ± 7.86	23.22 ± 8.46	20.27 ± 9.15	0.069	20.1 ± 7.86	21.68 ± 8.93	0.654	22.3 ± 8.38	20.27 ± 9.15	0.123
n-6:n-3 LCPUFA ratio	4.5 ± 0.727	4.58 ± 0.944	4.4 ± 0.873	0.092	4.5 ± 0.727	4.48 ± 0.91	0.096	4.55 ± 0.883	4.40 ± 0.873	0.046*
Model 5 (mother age, gestational age, DHA intake)										
Delta 5 desaturase (AA:DGLA)	11.45 ± 3.64	12.86 ± 4.19	12.83 ± 3.93	0.261	11.45 ± 3.64	12.84 ± 4.05	0.138	12.45 ± 4.08	12.83 ± 3.93	0.854
Delta 6 desaturase (DGLA:LA)	0.13 ± 0.038 ^a	0.108 ± 0.038 ^b	0.11 ± 0.038 ^{ab}	0.033*	0.13 ± 0.038	0.109 ± 0.037	0.014*	0.114 ± 0.039	0.11 ± 0.038	0.763
Delta 5+6 desaturase (AA:LA)	1.37 ± 0.228	1.26 ± 0.222	1.29 ± 0.234	0.080	1.37 ± 0.228	1.28 ± 0.228	0.052	1.29 ± 0.229	1.29 ± 0.234	0.342
Delta 5+6 desaturase + elongase (EPA:ALA)	3.91 ± 1.88	3.71 ± 2.54	4.37 ± 2.32	0.393	3.91 ± 1.88	4.05 ± 2.44	0.803	3.77 ± 2.36	4.37 ± 2.32	0.224
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	70.66 ± 27.59	77.13 ± 38.4	76.71 ± 30.82	0.683	70.66 ± 27.59	76.91 ± 34.56	0.499	75.22 ± 35.57	76.71 ± 30.82	0.945
Delta 6 desaturase + B-Oxidation (DHA:EPA)	20.1 ± 7.86	23.22 ± 8.46	20.27 ± 9.15	0.118	20.1 ± 7.86	21.68 ± 8.93	0.669	22.3 ± 8.38	20.27 ± 9.15	0.186
n-6:n-3 LCPUFA ratio	4.5 ± 0.727	4.58 ± 0.944	4.4 ± 0.873	0.112	4.5 ± 0.727	4.48 ± 0.91	0.080	4.55 ± 0.883	4.40 ± 0.873	0.053
Model 6 (mother age, gestational age, AA intake)										
Delta 5 desaturase (AA:DGLA)	11.45 ± 3.64	12.86 ± 4.19	12.83 ± 3.93	0.251	11.45 ± 3.64	12.84 ± 4.05	0.135	12.45 ± 4.08	12.83 ± 3.93	0.890
Delta 6 desaturase (DGLA:LA)	0.13 ± 0.038 ^a	0.108 ± 0.038 ^b	0.11 ± 0.038 ^{ab}	0.032*	0.13 ± 0.038	0.109 ± 0.037	0.014*	0.114 ± 0.039	0.11 ± 0.038	0.787
Delta 5+6 desaturase (AA:LA)	1.37 ± 0.228	1.26 ± 0.222	1.29 ± 0.234	0.081	1.37 ± 0.228	1.28 ± 0.228	0.051	1.29 ± 0.229	1.29 ± 0.234	0.333
Delta 5+6 desaturase + elongase (EPA:ALA)	3.91 ± 1.88	3.71 ± 2.54	4.37 ± 2.32	0.237	3.91 ± 1.88	4.05 ± 2.44	0.828	3.77 ± 2.36	4.37 ± 2.32	0.122
Delta 5+6 desaturase + elongase + delta 6 desaturase (DHA:ALA)	70.66 ± 27.59	77.13 ± 38.4	76.71 ± 30.82	0.721	70.66 ± 27.59	76.91 ± 34.56	0.515	75.22 ± 35.57	76.71 ± 30.82	0.909
Delta 6 desaturase + B-Oxidation (DHA:EPA)	20.1 ± 7.86	23.22 ± 8.46	20.27 ± 9.15	0.072	20.1 ± 7.86	21.68 ± 8.93	0.659	22.3 ± 8.38	20.27 ± 9.15	0.125
n-6:n-3 LCPUFA ratio	4.5 ± 0.727	4.58 ± 0.944	4.4 ± 0.873	0.102	4.5 ± 0.727	4.48 ± 0.91	0.093	4.55 ± 0.883	4.40 ± 0.873	0.050*

Data presented as mean ± SD; alphabetic letters indicate between group differences with Bonferroni adjust P<0.05, *significance P<0.05

CHAPTER 4: Conclusion, recommendations and future prospects

This chapter concludes the results from the present study in terms of the larger body of scientific research.

4.1 Summary

The aim of this study was to determine and characterise the genetic variations of the delta-5 and -6 *FADS* gene cluster and to study associations with the LCPUFA status in pregnant women of African descent.

More specifically, this study's objectives were:

- A.) To characterise the genetic variations of the *FADS* gene cluster, which encodes for D5D and D6D
- B.) To study the associations of selected *FADS* gene cluster variations with the ratios of the respective fatty acid desaturase estimated enzyme activities
- C.) To assess whether the associations in B are dependent on ALA, LA, DHA, EPA and AA intake, respectively

Each objective and how it was reached will be discussed in context of this mini-dissertation.

4.2 Objective A: The characterisation of the genetic variations of the *FADS* gene cluster, which encodes for delta-5 and -6 fatty acid desaturases

We aimed to characterise genetic variations of the *FADS* gene cluster in order to identify whether pregnant women of African descent have genetic variations that associate with D5D and D6D enzymatic activities. This objective was achieved when the *FADS1/2* gene cluster of 32 samples was sequenced and a total of 7813 variants were identified. Only 36 had a significant differential P-value ($P < 0.05$) when the lower and higher n-3 LCPUFA status groups were compared. The nine genetic variants *FADS1*-rs174546_(C/T), *FADS*-rs174547_(C/T), *FADS1*-rs174549_(A/G), *FADS1*-rs174555_(C/T), *FADS2*-rs174606_(T/G), *FADS2*-rs174616_(G/A), *FADS2*-rs78678033_(G/A), *FADS2*-rs73489307_(T/C), and *FADS2*-rs112425493_(G/A) were selected for further study.

4.3 Objective B: The associations of *FADS1/2* genetic variants with LCPUFA ratios and desaturase activity

After the genetic variants were identified and all participants were genotyped, association analyses were done to identify and study the impact of these nine identified variants on the PUFA ratios (which indicate the estimated D5D- as well as D6D activities), and the n-6:n-3 LCPUFA ratio (indicating a low or high n-3 LCPUFA status). As hypothesised, the results from the association of the *FADS1/2* gene cluster variations with RBC total phospholipid LCPUFA composition showed that *FADS1/2* gene variations contribute to the variability of several n-6 and n-3 PUFA pathways, thus indicating changes in estimated D5D and D6D enzymes. Since desaturase enzymes have a higher affinity to the n-3 PUFA pathway, enzymatic activity will be increased, leading to an advantaged n-3 LCPUFA status. The research provided results that support the growing body of evidence pointing to enhanced *FADS1* efficiency in people of African descent (particularly pregnant women in this study), increasing D5D activity.

The genetic variant *FADS2*-rs78678033_(G/A) is a novel variant and characterised for the first time. The associations showed promising results as the alternative allele of *FADS2*-rs78678033_(G/A) associated with higher AA:DGLA and lower DGLA:LA ratios, indicative of higher D5D activity and lowered D6D activity. This genetic variant is therefore important since it can improve n-3 LCPUFA status, and even though this is a newly researched variant, it is highly prevalent within this population.

From the nine genetic variants, our top findings were that A allele carriers of *FADS2*-rs112425493_(G/A), T allele carriers of *FADS1*-rs174546_(C/T), T allele carriers of *FADS1*-rs174547_(C/T), and G allele carriers of *FADS1*-rs174549_(A/G) had lower n-6:n-3 LCPUFA ratios or tendencies to be lower, and thus better n-3 LCPUFA status. These associations were also independent from dietary intake of the same individual n-3 and n-6 essential PUFA and LCPUFA. It was found that most participants were carriers for these alleles, but that no dramatic difference in n-3 LCPUFA was found between the homozygotes and carriers of these alleles.

It can be assumed that most of the South African population are therefore also carriers of these four genetic variants, and have the potential to have increased desaturase activity and lower n-6:n-3 LCPUFA status. This means that if South Africans increase n-3 PUFA and decrease n-6 PUFA intake even just a little (by means of using balanced n-6:n-3 PUFA oils and margarines such as canola oil), there is an opportunity to lower the n-6:n-3 LCPUFA status of the South African population.

Current results also indicated that alternative alleles of the genetic variants *FADS1*-rs174546_(C/T) and *FADS2*-rs112425493_(G/A) seemed to be associated with the exact opposite namely lower AA:DGLA and higher DGLA:LA ratios, and therefore lowered D5D activity and heightened D6D activity. Alternative alleles of *FADS*-rs174547_(C/T), *FADS1*-rs174549_(A/G), *FADS1*-rs174555_(C/T) and *FADS2*-rs73489307_(T/C) were associated with higher D6D activity in this specific population, with no effect on D5D activity, since all of these were associated with higher DGLA:LA ratios. Alternative allele carriers of *FADS2*-rs174606_(T/G) and *FADS2*-rs174616_(G/A) were associated with both lowered DGLA:LA ratio as well as lowered AA:LA ratio, indicating lower D5D and -D6D activity. Alternative allele carriers of *FADS2*-rs112425493_(G/A), *FADS1*-rs174546_(C/T), *FADS1*-rs174547_(C/T), and *FADS1*-rs174549_(A/G) had lower n-6:n-3 LCPUFA status or tendencies to be lower, and therefore a better n-3 LCPUFA status.

4.4 Objective C: The impact of dietary intake on the associations of *FADS1/2* genetic variants with LCPUFA ratios and desaturase activity

To assess the association between genotypes and LCPUFA status, univariate analyses were performed, adjusting for gestational age, age of mother, and dietary intakes (total energy-, ALA-, LA-, DHA- and AA intake), structured in six different statistical models. All of the associations were retained when adjusting for dietary intake. This finding indicates that precursors (such as ALA) are used very effectively and therefore if n-3 PUFA intake increases even just slightly, it could lead to a noteworthy improvement of the n-3 LCPUFA status of the population.

4.5 Strengths

The strengths of this study include that we used RBCs to measure FAs instead of plasma, since RBC concentrations reflect longer-term dietary FA intake. By means of whole genome sequencing, it was also possible for us to sequence and identify all existing genetic variants in our population, instead of only common genetic variants.

4.6 Limitations

As it is not possible to control for all factors that may influence study variables, residual confounding could have been possible as with all studies, although adjustments were made for known covariates. Another limitation of the study was the low prevalence of the alternative alleles in this study population that might have resulted in the study to be underpowered.

4.7 Future research

This MSc study was nested within a larger study focusing on nutrition during pregnancy and early development of children. In future research, we would recommend that the current results found in this study be replicated. If possible, we recommend that future studies be done on other ethnic groups or black African populations (including men and women) in other regions of South Africa, to more accurately represent our black South African population and to contribute to literature on populations of African descent. We would recommend a larger population group for the genetic association studies such as the present study. Furthermore, a study that will include *FADS3* and *ELOVL* genes should also be considered to investigate all genetic variants that influence the n-3 and n-6 PUFA metabolic pathways.

4.8 Conclusion

To conclude, the most profound finding of this study was that the genetic variation in this sub-sample of an African population-based study, largely related to higher D5D and D6D activities. These genetic variants therefore improved the n-3 LCPUFA status, irrespective of individual n-3 or n-6 essential PUFA or LCPUFA dietary intake. It is evident that the associations between the studied genetic variants and estimate desaturase activities were independent of dietary intake of the relevant PUFA, which suggests a predominantly endogenously controlled pathway dictated by the genetic makeup.

As hypothesised, this study demonstrated that LCPUFA status and estimated D5D and D6D activities are genetically controlled by the *FADS1/2* gene cluster, particularly benefitting this sample and maybe also the population with a higher n-3 LCPUFA status. It was also found that desaturase enzymes have an affinity to the n-3 LCPUFA pathway, meaning that increased activity of these enzymes directly result in higher n-3 LCPUFA status individuals, thus benefitting participants with these genetic variants

We conclude that it is important to understand the genetics of LCPUFA metabolism since it appears in this study, as well as previous studies, that people from different ethnicities may exhibit differences in the LCPUFA metabolic pathway, which may result in inflammatory diseases and suboptimal birth outcomes. This study offers insight in how the South African population's genetic makeup may affect RBC LCPUFA status, and thus gives us an opportunity to improve current diet recommendations (FA amounts, types and sources) and supplementation programs to optimise n-3 LCPUFA status of pregnant women, to decrease inflammatory diseases and increase optimal birth outcomes.

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APPENDIXES

Appendix A: Ethics approval of the North West University for the affiliated study



Private Bag X1290, Potchefstroom South Africa
2520

Tel: 086 016 9698
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**North-West University Health Research Ethics
Committee (NWU-HREC)**

Tel: 018 299-1206
Email: Ethics-HRECAppl@nwu.ac.za (for human
studies)

4 November 2020

ETHICS APPROVAL LETTER OF STUDY

Based on approval by the North-West University Health Research Ethics Committee (NWU-HREC) on

04/11/2020, the NWU-HREC hereby approves your study as indicated below. This implies that the NWU-HREC grants its permission that, provided the general conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: Genetic characterisation of delta-5 and -6 fatty acid desaturases and its associations with fatty acid status in pregnant women of African descent
Principal Investigator/Study Supervisor/Researcher: Dr L Zandberg **Student:** A Prinsloo - 25976648

Ethics number:

N	W	U	-	0	0	1	8	6	-	1	5	-	A	1	-	0	8
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Institution Study Number Year Status Sub-study

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation;
 A = Authorisation

Application Type: Sub-study

Commencement date: 04/11/2020

Risk:

Minimal

Expiry date: 30/11/2021

Approval of the study is provided for a year, after which continuation of the study is dependent on receipt and review of an annual monitoring report and the concomitant issuing of a letter of continuation. A monitoring report is due at the end of November annually until completion.

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, the following general terms and conditions will apply:

- *The principal investigator/study supervisor/researcher must report in the prescribed format to the NWU-HREC:*
 - *annually on the monitoring of the study, whereby a letter of continuation will be provided annually, and upon completion of the study; and*
 - *without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.*
- *The approval applies strictly to the proposal as stipulated in the application form. Should any amendments to the proposal be deemed necessary during the course of the study, the principal investigator/study supervisor/researcher must apply for approval of these amendments at the NWUHREC, prior to implementation. Should there be any*

deviations from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.

- *Annually a number of studies may be randomly selected for active monitoring.*
- *The date of approval indicates the first date that the study may be started.*
- *In the interest of ethical responsibility, the NWU-HREC reserves the right to:*
 - *request access to any information or data at any time during the course or after completion of the study;*
 - *to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process;*

– *withdraw or postpone approval if:*

- *any unethical principles or practices of the study are revealed or suspected;*
 - *it becomes apparent that any relevant information was withheld from the NWU-HREC or that information has been false or misrepresented;*
 - *submission of the annual monitoring report, the required amendments, or reporting of adverse events or incidents was not done in a timely manner and accurately; and/or*
 - *new institutional rules, national legislation or international conventions deem it necessary.*
- *NWU-HREC can be contacted for further information via Ethics-HRECAppl@nwu.ac.za or 018 299 1206*

Special conditions of the research approval due to the COVID-19 pandemic:

Please note: Due to the nature of the study i.e. laboratory work using previously collected biological samples from the NUPED study, this study will be able to proceed during the current alert level, following receipt of the approval letter. No additional COVID-19 restrictions have been placed on the study except that the researcher must ensure that before proceeding with the study that all research team members have

reviewed the North-West University COVID-19 Occupational Health and Safety Standard Operating Procedure.

The NWU-HREC would like to remain at your service and wishes you well with your study. Please do not hesitate to contact the NWU-HREC for any further enquiries or requests for assistance.

Yours sincerely,

Chairperson NWU-HREC

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Appendix B: Authors guidelines



PROSTAGLANDINS, LEUKOTRIENES & ESSENTIAL FATTY ACIDS

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PACK **AUTHOR INFORMATION**

CONTENTS

TABLE OF

- Description p.1
- Impact Factor p.1
- Abstracting and Indexing p.1
- Editorial Board p.2
- Guide for Authors p.3



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DESCRIPTION

The role of **lipids**, including **essential fatty acids** and their **prostaglandin, leukotriene** and other derivatives, is now evident in almost all areas of biomedical science. **Cell membrane** behaviour and **cell signalling** in all tissues are highly dependent on the lipid constituents of cells. *Prostaglandins, Leukotrienes & Essential Fatty Acids* aims to cover all aspects of the roles of lipids in cellular, organ and whole organism function, and places a particular emphasis on human studies. Papers concerning all medical specialties are published. Much of the material is particularly relevant to the development of novel treatments for disease.

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Anika Prinsloo

Genetic characterisation of delta-5
and -6 fatty acid desaturases and
associations with fatty acid status in
pregnant women of African descent

2 December 2020

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