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Effects of prenatal DHA supplementation and maternal and offspring *FADS* polymorphisms in relation to cardiometabolic health of Mexican children

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Effects of prenatal DHA supplementation and maternal and offspring *FADS* polymorphisms in relation to cardiometabolic health of Mexican children

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Abstract

Effects of prenatal DHA supplementation and maternal and offspring *FADS* polymorphisms in relation to cardiometabolic health of Mexican children

By Sonia Tandon Wimalasena

Risk factors for cardiometabolic disease are emerging earlier in the life course, including among children and adolescents. Polyunsaturated fatty acids (PUFAs) have a cardioprotective role as precursors to the n-3 and n-6 long-chain PUFAs (LC-PUFAs), which modulate inflammation. However, gaps remain in our understanding of the role of LC-PUFAs for cardiometabolic health (CMH) during critical periods of growth and development, such as gestation and early adolescence. Currently, the long-term effects of prenatal n-3 docosahexaenoic acid (DHA) supplementation are unclear, which may be partially attributable to population heterogeneity in variants of the fatty acid desaturase (*FADS*) genes that regulate LC-PUFA metabolism. The objective of this dissertation was to examine the relationship between prenatal DHA supplementation, maternal and offspring *FADS* polymorphisms, and cardiometabolic health in a population of Mexican children. To accomplish this, we used data from a double-blind randomized controlled trial of prenatal DHA supplementation (POSGRAD) in Mexico, in which the offspring have been followed from birth (n=973) through age 11 years (n = 566).

First, we examined clustering of cardiometabolic markers in 413 children and compared a metabolic syndrome (MetS) score to an exploratory CMH score, which additionally included adipokines and inflammatory markers. We found that measures of adiposity and lipids explained the most variation for both scores, and the MetS score captured nearly as much variation as the exploratory CMH score. Next, we evaluated the effect of prenatal DHA supplementation on offspring MetS scores and examined the role of variations in maternal *FADS* single nucleotide polymorphisms (SNPs) in 314 children. There was no main effect of prenatal DHA supplementation; however, we observed effect modification by variants of maternal SNP rs174602. Offspring of maternal TT genotype who received prenatal DHA had lower MetS scores relative to the placebo group, while offspring of maternal CC genotype who received DHA had higher MetS scores relative to offspring whose mothers received placebo. Finally, we examined the role of variants in both maternal and offspring *FADS* genes on MetS scores and assessed interactions with prenatal DHA supplementation and offspring diet quality (n=203). Offspring SNP rs174602 did not modify the association of prenatal DHA supplementation with MetS score. Although associations between examined *FADS* haplotypes and MetS score were null, there was evidence of interaction between SNP rs174602 and current dietary PUFA intake; children with TT or TC genotype and high dietary n-6:n-3 ratios had higher MetS scores relative to those with low n-6:n-3 ratios, while children with CC genotype and high n-6:n-3 ratios had lower MetS scores relative to those with low n-6:n-3 ratios.

The findings from this dissertation provide valuable insights by improving our understanding of cardiometabolic risk early in the life course and demonstrating the importance of examining gene-nutrient interactions, especially for interpreting results from nutrition supplementation trials. While further research is needed to validate our findings in larger, ethnically diverse populations, this work has the potential to guide and inform the development of targeted nutrition recommendations early in the life course to improve CMH.

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List of abbreviations

AA	Arachidonic acid
AI	Adequate intake
BMI	Body mass index
CMH	Cardiometabolic health
CRP	C-reactive protein
CVD	Cardiovascular disease
DHA	Docosahexaenoic acid
DOHaD	Developmental Origins of Health and Disease
EFA	Essential fatty acid
EPA	Eicosapentaenoic acid
FADS	Fatty acid desaturase
FMI	Fat mass index
FFMI	Fat free mass index
HRC	Haplotype Reference Consortium
HDL	High density lipoprotein cholesterol
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
IDF	International Diabetes Federation
IL-6	Interleukin 6
IMSS	Instituto Mexicano del Seguro Social
INSP	Instituto Nacional de Salud Pública
IRS	Insulin Receptor Substrate
JNK	c-Jun N-terminal kinase

LC-PUFA	Long-chain polyunsaturated fatty acid
LD	Linkage Disequilibrium
LDL	Low density lipoprotein cholesterol
MAF	Minor allele frequency
MetS	Metabolic syndrome
PC	Principal component
PCA	Principal component analysis
POSGRAD	Prenatal Omega-3 Fatty Acid Supplementation and Child Growth and Development
PPAR	Peroxisome proliferator activated receptor
PUFA	Polyunsaturated fatty acid
QC	Quality control
RCT	Randomized controlled trial
SES	Socioeconomic status
SNP	Single nucleotide polymorphism
VLDL	Very low density lipoprotein

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Chapter 1 : Introduction

Cardiometabolic diseases, which include cardiovascular disease, stroke, and type II diabetes mellitus, are the leading causes of mortality worldwide (1). By 2030, global costs associated with cardiovascular disease alone will exceed $1 trillion (2). Risk factors, including high blood pressure, insulin resistance, obesity, and adverse lipid profiles track from childhood to adulthood and are becoming increasingly prevalent earlier in the life course (3, 4). While it is well known that cardiometabolic risk factors cluster, there is no consensus on how to define cardiometabolic risk in pediatric populations (5). Nutrition is a key modifiable determinant of cardiometabolic health (CMH); therefore, understanding the optimal timing of nutrition interventions to prevent disease development is a critical area of investigation (6-8).

Polyunsaturated fatty acids (PUFAs) are important for CMH because they are precursors to the long-chain PUFAs (LC-PUFAs) n-6 arachidonic acid (AA), n-3 eicosapentaenoic acid (EPA) and n-3 docosahexaenoic acid (DHA), which modulate inflammation (9, 10). Western diets characterized by a high n-6:n-3 intake ratio (~16:1) have been previously associated with multiple chronic diseases in adults, and supplementation with n-3 LC-PUFAs reduces inflammation, insulin resistance, blood pressure, and lipid profiles (11, 12). Despite substantial evidence that nutritional exposures as early as gestation can have permanent metabolic programming effects (13, 14), the role of exposure to n-3 and n-6 LC-PUFAs during critical periods of growth and development, such as gestation and early adolescence, for later CMH remains unclear. In animal models, prenatal n-3 LC-PUFA supplementation reduces adiposity (15-17) and improves adult metabolic profiles among the offspring (15, 17, 18). However, randomized controlled trials (RCTs) of prenatal DHA supplementation report inconsistent findings for offspring CMH outcomes, which may be attributable to heterogeneity across studies with respect to types of omega-3s (e.g. fish oil

vs. DHA only), dose, timing of supplementation, characteristics of mothers, variable length of follow-up, and genetic variation (19-23). Systematic reviews call for additional follow-up of completed trials to account for metabolic changes that may emerge later in childhood (20, 24).

Genetic variants of fatty acid desaturase (*FADS*) genes that regulate the conversion of both n-3 and n-6 precursors into their LC-PUFA forms may explain the inconsistent results observed across trials (25). Most studies incorporating genetic information have been conducted in European populations; however, there is ethnic variation in the genotype distribution of *FADS* variants (26, 27). While European populations predominantly have *FADS* alleles associated with more rapid conversion of precursor PUFAs (25), Native American and Mexican populations have a higher frequency of alleles associated with slower conversion and may have increased n-3 LC-PUFA dietary requirements (28). Additional research is needed across populations that vary in *FADS* genotype and PUFA intake to guide development of targeted recommendations.

1.1 Objective & Specific Aims

The overall objective of this dissertation is to examine the relationship between prenatal DHA supplementation, maternal and offspring *FADS* polymorphisms, and cardiometabolic health in a population of Mexican children (**Figure 1-1**). To meet this objective, we used data from the Prenatal Omega-3 Fatty Acid Supplementation and Child Growth and Development (POSGRAD) randomized controlled trial conducted in Cuernavaca, Mexico from 2005-2007 to address the following three research aims.

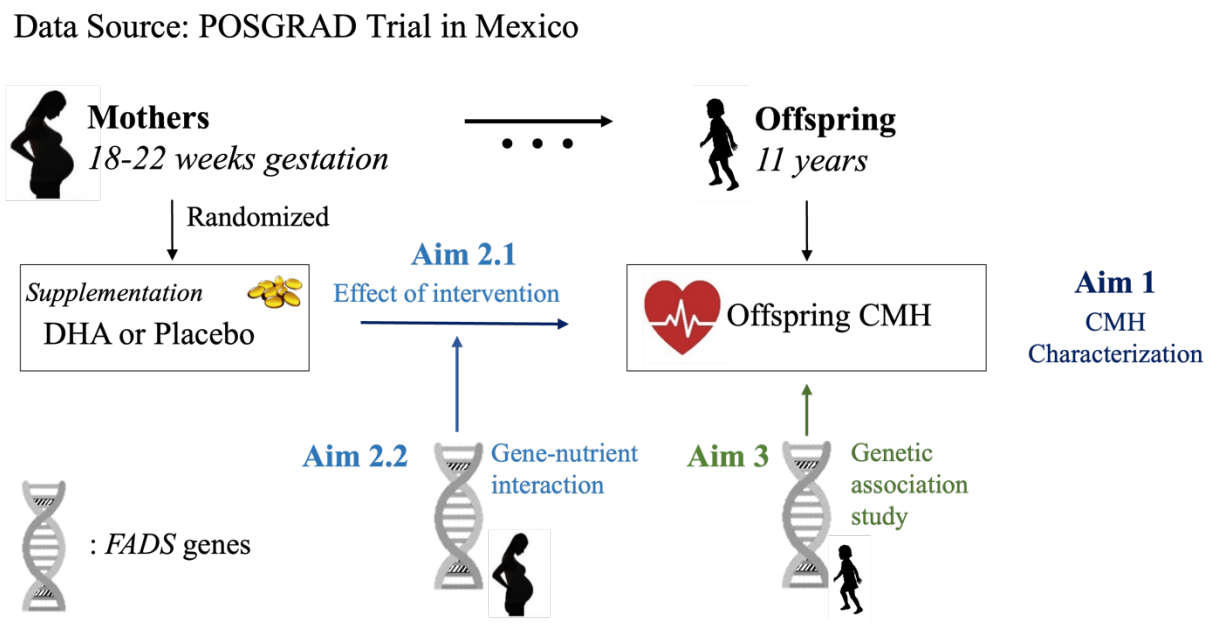

Figure 1-1. Summary of Research Aims.

Aim 1 examines clustering of cardiometabolic markers at 11 years of age. **Aim 2.1** tests the effect of prenatal DHA supplementation on offspring CMH; **Aim 2.2** tests effect modification by variants in maternal *FADS* genes. **Aim 3** assesses the role of selected variants in both maternal and offspring *FADS* genes on CMH.

Abbreviations: POSGRAD, Prenatal Omega-3 Fatty Acid Supplementation and Child Growth and Development; DHA, docosahexaenoic acid; CMH, cardiometabolic health; FADS, fatty acid desaturase.

Specific Aim 1: To examine clustering of cardiometabolic markers in Mexican children at age 11 years and compare a metabolic syndrome (MetS) score to an exploratory CMH score.

We hypothesized that the exploratory CMH score would explain more variation in children's cardiometabolic health at age 11 years compared to the MetS score.

Specific Aim 2: Evaluate the effect of prenatal DHA supplementation on offspring CMH and investigate effect modification by variants in maternal *FADS* genes.

We hypothesized that offspring born to women who are carriers of FADS alleles associated with slower conversion of precursors and exposed to prenatal DHA supplementation would have improved CMH profiles at age 11 y relative to the placebo group, with no differences by treatment group among those born to non-carriers.

Specific Aim 3: Evaluate the role of selected variants in both maternal and offspring *FADS* genes on CMH at age 11y and assess interactions of genotype with offspring diet quality and prenatal DHA supplementation.

a. *We hypothesized that children who carry FADS alleles associated with more rapid conversion of precursors would have poorer CMH profiles relative to those with slower conversion.*
b. *Secondarily, we hypothesized that maternal genotype would be more relevant than offspring genotype for modifying the effect of the prenatal DHA intervention whereas offspring genotype would be more relevant for modifying the effects of dietary intake.*

Relevant literature on the pathophysiology of cardiometabolic diseases in pediatric populations, possible cardioprotective benefits of LC-PUFAs, and interactions between LC-PUFA intake and/or supplementation with *FADS* genetic polymorphisms is reviewed in Chapter 2. Chapter 3 provides additional detail on the data source for the studies, including study setting, participants, design of the original trial and follow-up study, and an in-depth discussion of statistical methods used. Details of the three original research studies based on each of the specific

aims (1-3) are provided in Chapters 4-6. Chapter 7 discusses key findings from this research, strengths and limitations, clinical and public health implications, and recommendations for future research. Through integration of genetic, clinical, and longitudinal data, this work seeks to address important research gaps to ultimately guide the development of targeted dietary and supplementation recommendations early in the life course to improve CMH.

1.2 References

1. Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, et al. Global, Regional, and National Burden of Cardiovascular Diseases for 10 Causes, 1990 to 2015. J Am Coll Cardiol 2017;70(1):1-25. doi: 10.1016/j.jacc.2017.04.052.
2. Bloom DE, Cafiero, E.T., Jané-Llopis, E., Abrahams-Gessel, S., Bloom, L.R., Fathima, S., Feigl,, A.B. G, T., Mowafi, M., Pandya, A., Prettner, K., Rosenberg, L., Seligman, B., Stein, A.Z., & Weinstein, C. The Global Economic Burden of Noncommunicable Diseases. Geneva: World Economic Forum., 2011.
3. Saklayen MG. The Global Epidemic of the Metabolic Syndrome. Curr Hypertens Rep 2018;20(2):12. doi: 10.1007/s11906-018-0812-z.
4. Weiss R, Bremer AA, Lustig RH. What is metabolic syndrome, and why are children getting it? Ann N Y Acad Sci 2013;1281(1):123-40. doi: 10.1111/nyas.12030.
5. Magge SN, Goodman E, Armstrong SC. The Metabolic Syndrome in Children and Adolescents: Shifting the Focus to Cardiometabolic Risk Factor Clustering. Pediatrics 2017;140(2):e20171603. doi: 10.1542/peds.2017-1603.
6. DeBoer MD. Assessing and Managing the Metabolic Syndrome in Children and Adolescents. Nutrients 2019;11(8). doi: 10.3390/nu11081788.
7. Nicklas TA, von Duvillard SP, Berenson GS. Tracking of serum lipids and lipoproteins from childhood to dyslipidemia in adults: the Bogalusa Heart Study. Int J Sports Med 2002;23 Suppl 1:S39-43. doi: 10.1055/s-2002-28460.
8. Francesco C, Maria Loredana M. Insulin resistance and obesity in childhood. European Journal of Endocrinology 2008;159(suppl_1):S67-S74. doi: 10.1530/EJE-08-0245.

9. Saravanan P, Davidson NC, Schmidt EB, Calder PC. Cardiovascular effects of marine omega-3 fatty acids. Lancet 2010;376(9740):540-50. doi: 10.1016/s0140-6736(10)60445-x.

10. Lorente-Cebrián S, Costa AG, Navas-Carretero S, Zabala M, Martínez JA, Moreno-Aliaga MJ. Role of omega-3 fatty acids in obesity, metabolic syndrome, and cardiovascular diseases: a review of the evidence. J Physiol Biochem 2013;69(3):633-51. doi: 10.1007/s13105-013-0265-4.

11. Eslick GD, Howe PR, Smith C, Priest R, Bensoussan A. Benefits of fish oil supplementation in hyperlipidemia: a systematic review and meta-analysis. Int J Cardiol 2009;136(1):4-16. doi: 10.1016/j.ijcard.2008.03.092.

12. Simopoulos AP. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp Biol Med (Maywood) 2008;233(6):674-88. doi: 10.3181/0711-mr-311.

13. Rinaudo P, Wang E. Fetal programming and metabolic syndrome. Annu Rev Physiol 2012;74:107-30. doi: 10.1146/annurev-physiol-020911-153245.

14. Koletzko B, Brands B, Poston L, Godfrey K, Demmelmair H. Early nutrition programming of long-term health. Proc Nutr Soc 2012;71(3):371-8. doi: 10.1017/s0029665112000596.

15. Sardinha FL, Fernandes FS, Tavares do Carmo MG, Herrera E. Sex-dependent nutritional programming: fish oil intake during early pregnancy in rats reduces age-dependent insulin resistance in male, but not female, offspring. Am J Physiol Regul Integr Comp Physiol 2013;304(4):R313-20. doi: 10.1152/ajpregu.00392.2012.

16. D'Asti E, Long H, Tremblay-Mercier J, Grajzer M, Cunnane SC, Di Marzo V, et al. Maternal dietary fat determines metabolic profile and the magnitude of endocannabinoid

inhibition of the stress response in neonatal rat offspring. Endocrinology 2010;151(4):1685-94. doi: 10.1210/en.2009-1092.

17. Hussain A, Nookaew I, Khoomrung S, Andersson L, Larsson I, Hulthén L, et al. A maternal diet of fatty fish reduces body fat of offspring compared with a maternal diet of beef and a post-weaning diet of fish improves insulin sensitivity and lipid profile in adult C57BL/6 male mice. Acta Physiol (Oxf) 2013;209(3):220-34. doi: 10.1111/apha.12130.

18. Siemelink M, Verhoef A, Dormans JA, Span PN, Piersma AH. Dietary fatty acid composition during pregnancy and lactation in the rat programs growth and glucose metabolism in the offspring. Diabetologia 2002;45(10):1397-403. doi: 10.1007/s00125-002-0918-2.

19. Stratakis N, Gielen M, Chatzi L, Zeegers MP. Effect of maternal n-3 long-chain polyunsaturated fatty acid supplementation during pregnancy and/or lactation on adiposity in childhood: a systematic review and meta-analysis of randomized controlled trials. Eur J Clin Nutr 2014;68(12):1277-87. doi: 10.1038/ejcn.2014.158.

20. Middleton P, Gomersall JC, Gould JF, Shepherd E, Olsen SF, Makrides M. Omega-3 fatty acid addition during pregnancy. Cochrane Database Syst Rev 2018;11(11):Cd003402. doi: 10.1002/14651858.CD003402.pub3.

21. Voortman T, Tielemans MJ, Stroobant W, Schoufour JD, Kiefte-de Jong JC, Steenweg-de Graaff J, et al. Plasma fatty acid patterns during pregnancy and child's growth, body composition, and cardiometabolic health: The Generation R Study. Clin Nutr 2018;37(3):984-92. doi: 10.1016/j.clnu.2017.04.006.

22. He S, Stein AD. Early-Life Nutrition Interventions and Associated Long-Term Cardiometabolic Outcomes: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Adv Nutr 2021;12(2):461-89. doi: 10.1093/advances/nmaa107.

23. Vahdaninia M, Mackenzie H, Dean T, Helps S. The effectiveness of ω-3 polyunsaturated fatty acid interventions during pregnancy on obesity measures in the offspring: an up-to-date systematic review and meta-analysis. Eur J Nutr 2019;58(7):2597-613. doi: 10.1007/s00394-018-1824-9.

24. Voortman T, van den Hooven EH, Braun KV, van den Broek M, Bramer WM, Chowdhurry R, et al. Effects of polyunsaturated fatty acid intake and status during pregnancy, lactation, and early childhood on cardiometabolic health: A systematic review. Prog Lipid Res 2015;59:67-87. doi: 10.1016/j.plipres.2015.05.001.

25. Koletzko B, Reischl E, Tanjung C, Gonzalez-Casanova I, Ramakrishnan U, Meldrum S, et al. FADS1 and FADS2 Polymorphisms Modulate Fatty Acid Metabolism and Dietary Impact on Health. Annu Rev Nutr 2019;39:21-44. doi: 10.1146/annurev-nutr-082018-124250.

26. Ameur A, Enroth S, Johansson A, Zaboli G, Igl W, Johansson AC, et al. Genetic adaptation of fatty-acid metabolism: a human-specific haplotype increasing the biosynthesis of long-chain omega-3 and omega-6 fatty acids. American journal of human genetics 2012;90(5):809-20. doi: 10.1016/j.ajhg.2012.03.014.

27. Mathias RA, Fu W, Akey JM, Ainsworth HC, Torgerson DG, Ruczinski I, et al. Adaptive evolution of the FADS gene cluster within Africa. PLoS One 2012;7(9):e44926. doi: 10.1371/journal.pone.0044926.

28. Gibbs RA, Belmont JW, Hardenbol P, Willis TD, Yu F, Yang H, et al. The International HapMap Project. Nature 2003;426(6968):789-96. doi: 10.1038/nature02168.

Chapter 2 : Literature Review

This chapter provides relevant background information on how and why cardiometabolic diseases develop, how certain nutrients, such as LC-PUFAs, may improve cardiometabolic risk factors, and how nutrients interact with genetic polymorphisms to influence cardiometabolic disease risk. First, I discuss the epidemiology and etiology of cardiometabolic disease, with an emphasis on Mexican children and adolescents. Next, I overview the role of LC-PUFAs in human health and discuss their potential cardioprotective benefits. Third, I review the evidence supporting the role of prenatal DHA supplementation in offspring cardiometabolic health, drawing on evidence from animal models, human observational studies, and human RCTs. Finally, I discuss the role of gene-nutrient interactions in CMH, focusing on the *FADS* genetic polymorphisms involved in LC-PUFA metabolism.

2.1 Cardiometabolic disease in pediatric populations

2.1.1 Health of school-age children, obesity epidemic, and tracking of risk factors across life course

It is now well understood that early nutrition and lifestyle factors can have long-lasting impacts on the lifelong risk of obesity and associated non-communicable diseases, including cardiovascular disease (CVD), hypertension, and type II diabetes (1). Exposures as early as gestation, such as maternal genetics, obesity, nutritional status, and environmental exposures, can alter the fetal nutrient supply, growth, and development (2). In early postnatal life, infant feeding practices including nutrient content of milk, duration of breastfeeding, and introduction of complementary foods can all shape a child's metabolism and microbiome (3). While the first 1,000 days (pregnancy and first two years of life) have received much attention in terms of early life interventions to mitigate risk of chronic disease, the periods of childhood and adolescence

represent an important bridge between this rapid period of growth and development and the stability of adulthood. This is a dynamic time during which lifelong behaviors (e.g., dietary patterns, development of taste and food preferences, eating behaviors, sedentary habits, changes in sex hormones and pubertal status, and increases in psychological and social stressors) develop, all of which can influence the development of adiposity and increase risk of chronic disease.

Childhood and adolescent overweight and obesity have emerged as one of the most important public health problems worldwide (4). Obesity is defined as an excess accumulation of adipose tissue, and its increase in prevalence during childhood has corresponded with the emergence of serious co-morbidities including type II diabetes, hypertension, non-alcoholic fatty liver disease, and dyslipidemia (4). The most common cause of obesity during childhood and adolescence is an inequity in energy balance (i.e., energy intake exceeds energy expenditure). It is well established that obesity and other risk factors for chronic disease can emerge early in life and track from childhood to adulthood. Studies have shown that approximately 55% of children with obesity go on to be obese as an adolescent, and 80% of adolescents with obesity go on to be obese as an adult (5). Therefore, identifying effective prevention strategies early in the life course is essential to mitigating overall disease burden (6).

2.1.2 Epidemiology of cardiometabolic disease: distribution and determinants

Cardiometabolic diseases, which include CVD, stroke, and type II diabetes, are leading causes of death globally. An estimated 17.9 million people globally died from cardiovascular disease alone in 2019, representing over 30% of all global deaths (7). Over three-quarters of CVD related deaths take place in low- and middle-income countries (7). Overwhelming evidence suggests that a substantial proportion of cases of cardiometabolic disease could be prevented via

lifestyle and behavioral changes such as a healthy diet, increasing physical activity, maintaining a healthy weight, avoiding tobacco products, and minimizing alcohol consumption (8).

The prevalence of cardiometabolic disease varies worldwide. Geographic and ethnic differences may be attributable to differences in genetic and environmental factors (e.g., diet, socioeconomic status, and inequities in access to healthcare). The increasing burden of obesity and non-communicable diseases in Mexico over the past thirty years have placed the country at the forefront of global health efforts to reduce disease burden. This increase is largely due to the nutrition transition, which is characterized by a shift from high infectious disease morbidity and mortality to increased prevalence of non-communicable disease, changes in dietary patterns and increased intake of processed foods high in fat and sugar, urbanization, and technological advancements that have led to increased sedentary time for both work and leisure (9). Additionally, the substantial increase in imports of food products from the United States has vastly re-shaped the food environment in Mexico, making processed foods readily available. In Mexico, more than 70% of adults are overweight or obese (BMI $\geq$ 25 kg/m^2) (10). Of additional concern, younger generations have higher cumulative exposure to an obesogenic environment shaped by processed foods and low diet quality (11). A recent national survey in Mexico (ENSANUT MC 2016) found that among school-age children (aged 5-18 years), 17.9% (95% CI: 15.2-21.1) were overweight and 15.3% (95%CI: 12.5–18.6) were obese, with the highest prevalence among children aged 10-11 years. The prevalence was higher among females compared to males and 5% higher in urban versus rural populations (12). Studies indicate that, aside from obesity, low HDL and high triglycerides are the most observed cardiometabolic risk factors present among Mexican children and adolescents (13-15).

2.1.3 Physiology of metabolic syndrome and cardiometabolic disease

Metabolic syndrome (MetS) is a cluster of conditions, which includes hypertension, hyperglycemia, obesity, and dyslipidemia, that increases risk of heart disease, stroke, and type II diabetes. The term MetS was coined from a framework (originally "syndrome X") used to describe the mechanisms underlying insulin resistance and its effects on glucose and lipid metabolism, blood pressure, and coronary artery disease risk (16). Obesity, insulin resistance, ectopic fat deposition, and inflammation play a central role in the pathophysiology of MetS. Healthy adipose tissue expansion is characterized by the formation of small new adipocytes that are vascularized and have minimal inflammation, while unhealthy expansion is characterized by inadequate angiogenesis, hypoxia, and storage of lipids in other tissues (e.g., liver, muscle) (3). The accumulation of free fatty acids, inflammatory cytokines, and lipid intermediates in non-adipose tissue (e.g., liver, muscles, and pancreas) leads to activation of inflammatory pathways (JNK, IKK, PKCs) and alterations in normal insulin signaling.

Under normal conditions, insulin stimulates glucose uptake in skeletal muscles and adipocytes, promotes glycogen synthesis in skeletal muscles, suppresses hepatic glucose production, and inhibits lipolysis in adipocytes (17). In a state of insulin resistance, there is impaired glycogen synthesis and protein catabolism in skeletal muscles and increased lipolysis in the adipocytes, resulting in an increased release of free fatty acids and inflammatory cytokines into the circulation. The excess release of free fatty acids and triglycerides into the circulation induces ectopic adipose deposition and dyslipidemias (e.g., high plasma triglycerides, low high-density lipoprotein (HDL) cholesterol, and increases in small, dense low-density lipoprotein (LDL) cholesterol), which are atherogenic and are known to increase cardiovascular risk. Insulin resistance also contributes to endothelial dysfunction by decreasing production of nitric oxide, resulting in vasoconstriction and elevated blood pressure, platelet aggregation, and enhanced LDL

oxidation. The buildup of oxidized LDL contributes to plaque formation, leading to atherosclerosis and CVD.

It is also important to consider the role of low-grade inflammation in the pathophysiology of cardiometabolic disease. C-reactive protein (CRP) is an established marker of systemic inflammation; elevated serum levels have been shown to be elevated in individuals with MetS and cardiovascular disease (18, 19). Excess adipose tissue is associated with increased levels of the pro-inflammatory acute phase reactant C-reactive protein (CRP) in the blood. The majority of adipokines released from adipose tissue, including leptin, resistin, interleukin-6 (IL-6), tumor necrosis factor α, and plasminogen activator inhibitor-1, have detrimental effects on cardiovascular health (20). With obesity, overexpansion of adipose tissue results in overproduction of these pro-inflammatory molecules, promoting CVD pathogenesis (21). In contrast, adiponectin is one of the few adipokines that is decreased in obesity, anti-inflammatory, and associated with increased insulin sensitivity (22).

Although obesity is generally considered a key contributor in the pathophysiology of MetS, it is important to consider that approximately one-third of individuals with obesity have metabolically healthy obesity (23), meaning that they meet the clinical definition of obesity but exhibit favorable cardiometabolic profiles (i.e., no dyslipidemia, hypertension, or hyperglycemia); however, it remains unclear whether this is a fixed or transient phenotype and warrants additional research (16, 20). Similarly, a subgroup of individuals with normal weight or underweight based on BMI definitions may present with clustering of cardiometabolic risk factors (24). Thus, it is important to consider the limitations of using measures of adiposity alone to classify cardiometabolic risk. One of the key concerns of using BMI is that it does not differentiate between lean and body fat mass; this can lead to misclassification among persons of muscular build or short

stature (23). Methods including dual-energy X-ray absorptiometry (gold standard), bioelectrical impedance analysis, and air displacement plethysmography provide more granular measures of body composition via measurements of fat mass and fat-free mass (25).

2.1.4 Defining CM risk in pediatric populations

Despite the increases in cardiometabolic risk factors early in the life course, defining cardiometabolic risk in pediatric populations remains a key challenge. In adult populations, metabolic syndrome is diagnosed by the presence of at least three or more of the following risk factors: central adiposity, hyperglycemia, hypertriglyceridemia, hypertension, and low HDL, although definitions vary slightly (26). However, for children and adolescents, over 40 MetS definitions including different risk factors have been proposed, and there is no consensus on which definition should be used (**Table 2-1**) (27). Additionally, there is high heterogeneity in the prevalence of MetS based on the diagnostic criteria used. One study used a sample of 508 Mexican children aged 9-13 years to compare five definitions of MetS and found that the prevalence varied from 2.4 – 45.4% based on the definition used (28). This clearly demonstrated the poor performance of current criteria to diagnose MetS in Mexican children and adolescents. While the overall prevalence of MetS is low, it is highly prevalent in overweight and obese children (20).

Table 2-1. Comparison of Select Published MetS Definitions for Pediatric and Adult Populations.

	Pediatric Definitions			Adult Definitions	
	Cook et al.	De Ferranti et al.	Zimmet et al. (IDF definition for ages 10-16)	Albierti et al. (IDF definition for Ages 16+)	Grundy et al. (AHA/NHLBI)
Criterion	≥ 3 criteria	≥ 3 criteria	Obesity and at least 2 of remaining 4 criteria	Obesity and at least 2 of remaining 4 criteria	≥ 3 criteria
Obesity	WC ≥ 90th percentile (age and sex specific, NHANES III)	WC > 75th percentile	WC ≥ 90th percentile or adult cutoff if lower	WC ≥ 94 cm for white men and ≥80 cm for white women	WC ≥ 102 cm in men and WC ≥ 88 cm in women
Glucose intolerance	Fasting glucose ≥ 110 mg/dL	Fasting glucose ≥ 110 mg/dL	Fasting glucose ≥ 100 mg/dL or known type 2 diabetes mellitus	Fasting glucose ≥ 100 mg/dL or known type 2 diabetes mellitus	Fasting glucose ≥ 100 mg/dL or drug treatment of elevated glucose
Triglycerides	Triglycerides ≥ 110 mg/dL	Triglycerides ≥ 100 mg/dL	Triglycerides ≥ 150 mg/dL	Triglycerides ≥ 150 mg/dL (1.7 mmol/L) or treatment of elevated triglycerides	Triglycerides ≥ 150 mg/dL (1.7 mmol/L) or treatment of elevated triglycerides
HDL-c	HDL-C ≤ 40 mg/dL	HDL-C ≤ 50 mg/dL	HDL-C < 40 mg/dL	HDL-C < 40 mg/dL in men and <50 mg/dL in women or specific treatment of low HDL-c	HDL-C < 40 mg/dL in men and < 50 mg/dL in women or on drug treatment of reduced HDL-C
High BP	BP ≥ 90th percentile (age, sex, and height specific)	BP > 90th percentile	Systolic BP ≥ 130 mmHg or diastolic BP ≥ 85 mmHg or treatment of previously diagnosed hypertension	Systolic BP ≥ 130 mmHg or diastolic BP ≥ 85 mmHg or treatment of previously diagnosed hypertension	Systolic BP ≥ 130 mmHg or diastolic BP ≥ 85 mmHg or treatment of previously diagnosed hypertension

Source: The Metabolic Syndrome in Children and Adolescents: Shifting the Focus to Cardiometabolic Risk Factor Clustering (Magge et al, 2017) (16)

Beyond the challenges of identifying optimal diagnostic criteria, the clinical utility of diagnostic criteria remains controversial. Using dichotomous thresholds to diagnose MetS likely only captures severe outliers. Furthermore, they may inadequately characterize early risk trajectories by ignoring the continuous nature of risk (29, 30). It is important to consider that adolescence is a period of considerable metabolic and hormonal change, characterized by the onset

of puberty, accumulation of lean and fat mass, and increases in sex hormones and insulin resistance (31). Several epidemiologic studies have demonstrated an association between early onset of puberty and increased future risk of obesity and cardiometabolic disease (32-34). Some evidence additionally suggests that leptin and adiponectin, two adipokines that are correlated with obesity, may influence the initiation of puberty via modulation of the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axes (35-37). These substantial physiological changes may contribute to instability in categorical diagnoses of MetS over time. As such, one study among adolescents in the United States (baseline age range: 12.2-19.3 years) reported that metabolic risk factor clustering is consistent over time, but there was instability in clinical categorization of MetS between baseline and follow up three years later (38). The American Academy of Pediatrics now recommends that, instead of focusing on a particular MetS definition or specific cut-off levels for individual risk factors, pediatricians should identify children with clustering of multiple risk components (39). Within the research setting, this has led to use of composite risk scores, which are typically calculated as the sum or mean of age-, sex-, and race/ethnicity-standardized z-scores for each risk component (40, 41). Typically, the most used score components include waist circumference, mean arterial pressure, Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), HDL, and triglycerides. However, there is heterogeneity across studies with respect to included components, and additionally, these scores assume that each component contributes equally to cardiometabolic risk. These limitations justify the need to evaluate alternate methods, such as principal component analysis (PCA), for deriving continuous MetS scores. Methodology will be further discussed in Chapter 3.

2.2 Overview of LC-PUFAs

2.2.1 Structure, function, and metabolism of PUFAs and LC-PUFAs

Essential fatty acids (EFAs) are polyunsaturated fatty acids (PUFAs) that are necessary for human growth and function but cannot be produced by the body, as the human body can only synthesize double bonds after the 9th carbon from the methyl end of a fatty acid (42). There are two classes of essential fatty acids: omega-6 fatty acids, which have a double bond in the omega-6 position of the hydrocarbon chain (includes linoleic acid (LA, 18:2n-6) and its derivatives), and omega-3 fatty acids, which have a double bond in the omega-3 position (alpha-linolenic acid (ALA, 18:3n-3) and its derivatives) (**Figure 2-1**).

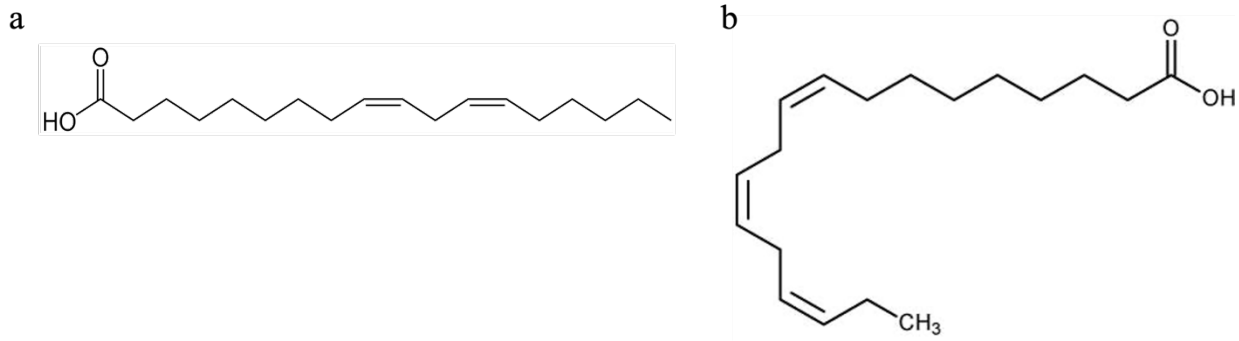

Figure 2-1. Chemical structure of a) n-6 linoleic acid and b) n-3 alpha-linolenic acid.

Dietary PUFAs are converted in the body to long-chain PUFAs by elongase enzymes, which add 2 carbons via acyl CoA, and desaturase enzymes, which add one double bond. With a steady supply of linoleic acid, arachidonic acid is produced in sufficient amounts; however, DHA and EPA can become conditionally essential due to a dietary deficiency or decreased enzyme activity. Most of the biological functions of PUFAs are exerted by the long-chain active forms: n-6 arachidonic acid (AA, 20:4n-6), n-3 eicosapentaenoic acid (EPA, 20:5n-3), and n-3 docosahexaenoic acid (DHA, 22:6n-3). While the dietary PUFAs n-6 LA and n-3 ALA are primarily used for beta oxidation to provide energy, the LC-PUFAs are involved in production of membrane phospholipids and membrane fluidity, cell differentiation, signal transduction, regulation of genes involved in fatty acid synthesis and oxidation, and synthesis of lipid

biomediators such as prostaglandins, leukotrienes, thromboxanes, and resolvins involved in physiological processes including platelet aggregation, vasoconstriction, and inflammation (43).

2.2.2 Role of LC-PUFAs in inflammation

Although both classes of fatty acids are necessary for normal cell membrane structure and function, cell signaling, and regulation of gene expression, they play opposing roles in modulating inflammation. Although there are some exceptions, the n-6 LC-PUFA arachidonic acid generally produces pro-inflammatory and more physiologically potent prostaglandins, leukotrienes, and thromboxanes, while n-3 EPA produces anti-inflammatory eicosanoids and n-3 DHA produces molecules that resolve inflammation (44). The anti-inflammatory effects of n-3 LC-PUFAs may be attributable to altered cell membrane phospholipid fatty acid composition, disruption of lipid rafts, reduced expression of inflammatory genes via inhibition of nuclear factor kappa B, and activation of anti-inflammatory peroxisome proliferator activated receptor (PPAR) γ (45). Further, n-3 LC-PUFAs are able to partly inhibit aspects of inflammation including production of inflammatory cytokines, production of eicosanoids such as prostaglandins and leukotrienes, T cell reactivity, adhesion molecule expression, and leucocyte chemotaxis (45). The n-3 LC-PUFAs compete with the n-6 LC-PUFAs for the same enzymes (COX and LOX) to synthesize different classes of eicosanoids. Thus, it is possible that excess intake of one class of fatty acids can lead to a decrease in conversion of the other class (i.e., excess intake of n-6 LA can lead to decreased formation of n-3 EPA and DHA). In the presence of large amounts of n-6 LC-PUFAs, conversion of ALA to EPA has been shown to be as low as 0.2 to 6%, and conversion to DHA is 0.05% or less (46). However, results from a mouse model showed that increasing dietary LA did not influence AA concentrations (47). While seemingly intuitive from a biochemical perspective,

research suggests that these pathways are more complex in the human body, especially because nutrients are not consumed in isolation.

2.2.3 Role of LC-PUFAs in lipid and glucose metabolism

n-3 LC-PUFAs have regulatory effects on enzymes involved in lipid and glucose metabolism (48). EPA and DHA are natural ligands for several nuclear receptors and transcriptional factors, including PPARα and PPARγ. PPARα is highly expressed in tissues with high fatty acid requirements, such as the liver, heart, and kidney, while PPARγ is highly expressed in adipose tissue. Activation of PPARα leads to reduced energy storage and enhanced fatty acid oxidation, resulting in reduced circulating triglyceride levels (49). PPARα activation may also have beneficial implications for glucose homeostasis, as some reports suggest that PPARα agonists may enhance insulin sensitivity in adipose tissue and muscles and preserve pancreatic β-cell function (50). Activation of PPARγ, meanwhile, leads to inhibition of nuclear factor κB (NF-κB) activity and inflammation (51). n-3 LC-PUFAs may also maintain glucose homeostasis via decreased activation of c-Jun N-terminal kinase (JNK) pathways and reduced insulin receptor substrate (IRS) phosphorylation. Additionally, n-3 LC-PUFAs have been shown to have anti-thrombotic properties, improve endothelial function, lower plasma triglycerides and LDL cholesterol, and inhibit cell growth factors (52).

2.2.4 Dietary sources and recommended dietary intakes

Unlike all other fatty acids, omega-3 and omega-6 fatty acids cannot be made by the body and must be obtained through the diet to support normal function. Omega-6 fatty acids are found in most seeds and vegetable oils, and the omega-6 LC-PUFA AA is abundant in the meat (e.g., chicken, beef) and eggs of animals fed grain diets high in omega-6 fatty acids. ALA is found in walnuts, flax, chia seeds, leafy greens, and plant oils, such as canola, soybean, and flaxseed oils,

and n-3 LC-PUFAs EPA and DHA are found in coldwater oily fish (e.g., salmon, sardines, and herring), algae, fish oils, and krill oils. Fish do not synthesize n-3 LC-PUFAs but are an excellent source of DHA + EPA because it accumulates in their flesh as they consume algae. While n-6 fatty acids are highly abundant in the food supply in most seeds, vegetable oils, animal products, and processed foods made using these products, omega-3s are found in far fewer sources (53).

The recommended dietary intakes of omega-3 and omega-6 fatty acids differ by age, sex, and health status. There is no specific recommended dietary intake of omega-6 fatty acids, but it is recommended that they make up between 5-10% of total daily calories (54). The Institute of Medicine (IOM) has not established specific dietary reference intake recommendations for EPA, DHA, or other n-3 LC-PUFAs, but adequate intakes (AIs) for ALA have been established. For children aged 9-13 years, recommended AIs are 1200 mg per day for males and 1000 mg per day for females (55). During pregnancy, the recommended AI is at least 1400 mg ALA. Consensus recommendations by the World Association of Perinatal Medicine state that pregnant women should aim to achieve average daily intakes of at least 200 mg DHA (56). The Dietary Guidelines for Americans recommends that pregnant women eat 8 to 12 oz (2 to 3 servings) per week of fish that are low in mercury. The American Heart Association recommends intakes of 1 g/day of DHA+EPA for adults with coronary heart disease and 3-4 oz per week for unaffected people as part of a heart-healthy diet (57). Some evidence suggests that the n-6/n-3 ratio may have important implications for pathogenesis of cardiovascular disease and cancer, but the optimal intake ratio, if any exists, has yet to be defined. While the focus of this dissertation is primarily on the potential cardioprotective benefits of n-3 DHA, it is important to consider that n-6 linoleic acid may also be beneficial for preventing cardiovascular disease (58). A study among nearly 900 healthy men and women in the United States reported that n-6 fatty acids do not inhibit the anti-inflammatory

actions of n-3 fatty acids, and a combination of both n-6 and n-3 fatty acids is associated with the lowest levels of inflammation. This work suggested that absolute intakes of n-3 and n-6 are more important than the ratio, but overall, examination of the relevance and clinical and public health implications of the n-6:n-3 ratio warrants further research. To improve the ratio of omega-3 to omega-6 in the body, it is currently recommended to consume more omega-3 fatty acids, not reduce intake of omega-6 fatty acids.

2.2.5 Changes in the food supply

The rise in risk factors for chronic disease among both adults and children has been paralleled by major changes in the food supply in the past 100 years. Advancements in agriculture and food technology have led to widescale production of vegetable oils high in n-6 fatty acids, changes in animal feed from grass to grain-based diets, and the widespread adoption of western diets (59). These changes have led to major shifts in dietary fat composition, especially n-6 and n-3 PUFAs, resulting in diets high in n-6 PUFAs. During paleolithic times, humans consumed a diet with a balanced ratio of n-6 to n-3 (1:1) in their diet; however, modern western diets are reported to have an n-6:n-3 ratio of approximately 16:1 (60). The simultaneous increase in inflammation-related chronic disease and consumption of omega-6 fatty acids has occurred despite more recent reductions in saturated fat and total fat intake.

2.3 Prenatal DHA supplementation and cardiometabolic health

The initial evidence supporting a potential cardioprotective role of n-3 LC-PUFAs came from the discovery that Greenland Eskimos, who had a diet rich in fatty fish, had lower mortality of coronary heart disease relative to Danish and American populations (61, 62). These initial findings motivated a wave of observational, experimental, and intervention studies to further understand this association and the underlying mechanisms.

Several early randomized controlled trials conducted among non-pregnant adults suggested beneficial effects of n-3 fatty acids on cardiovascular health in individuals with previous cardiovascular events; they reported that increasing consumption of fatty fish or n-3 LC-PUFA supplements led to reductions in CVD, coronary heart disease, myocardial infarction, stroke, and related fatalities (52, 63-65). Other studies have shown that supplementation with n-3 LC-PUFAs improves insulin resistance, blood pressure, and dyslipidemia via reduction of triglycerides (44, 60, 66-68). In contrast, numerous studies have reported no significant association with CVD or diabetes outcomes (52). Overall, evidence remains inconsistent and may be attributable to varying sources, doses, and type of n-3 PUFA, trial duration and follow-up time, population characteristics (e.g., co-morbidities, concurrent prescription drug use), primary vs. secondary prevention, sample size, and genetic variation (52).

2.3.1 Potential biological mechanisms

Due to their important roles in cell membrane synthesis, gene expression, and eicosanoid production, omega-3 fatty acid intake may be critical in determining long-term cardiometabolic health as early as gestation. Because the fetus primarily relies on the placental fatty acid supply, maternal DHA typically accumulates during the second half of pregnancy to support optimal fetal growth, development, and immune function (43). During this period of rapid growth and development, AA and DHA are readily incorporated into membrane lipids of growing tissues, including the liver, brain, and immune cells. The Developmental Origins of Health and Disease (DOHaD) theory suggests that some exposures, including nutrient intakes, during gestation may have permanent programming effects on offspring CMH (1, 2, 6, 69). Alterations in the prenatal fatty acid supply can contribute to structural changes in cells and organ development, epigenetic changes that regulate expression of genes involved in energy storage and oxidation, and disrupted

development of appetite regulation signals that control long term food intake and energy balance (2). Two mechanisms may mediate the benefits of prenatal n-3 LC-PUFAs for offspring CMH. First, especially among overweight or obese women, prenatal supplementation may improve maternal insulin sensitivity, circulating lipids, and placental inflammation, thereby reducing fetal overnutrition and adiposity (70). Second, prenatal n-3 LC-PUFA supplementation may directly affect fetal metabolic programming via altered cell and organ development, gene expression, and development of neuroendocrine signals, increasing long-term cardiometabolic risk (2, 69, 71, 72).

2.3.2 Evidence from animal models

Several experimental studies have demonstrated a relationship between prenatal DHA supplementation and offspring metabolic health. Evidence from animal models show that offspring born to dams who were fed diets supplemented with n-3 LC-PUFAs (e.g., fish oil) had lower levels of adiposity (73-76), insulin resistance (74-77), and dyslipidemia (74) compared to dams fed diets low in n-3 fatty acids but rich in saturated or n-6 fatty acids. Of interest, Sardinha et al. showed that fish oil intake during early pregnancy in rats reduced age-dependent insulin resistance and fat accretion in males, but not female offspring, suggesting possible sex-dependent nutritional programming (75).

2.3.3 Evidence from human populations

Some human observational studies report associations of higher maternal and/or cord n-3 LC-PUFA status with offspring measures of cardiometabolic health. One study performed in 4830 mother-child pairs participating in a population-based cohort in the Netherlands (The Generation R Study) found that a high n-6 PUFA pattern (derived using PCA) was associated with higher height, BMI, and fat-free mass index (FFMI) in offspring at age six years, while a high n-3 PUFA pattern was associated with lower fat mass index (FMI), higher FFMI, high HDL, and lower

triglycerides (78). Another study conducted in 4455 mother-child pairs enrolled in another population-based prospective cohort study in the Netherlands showed that higher maternal plasma n-3 PUFA and lower n-6 PUFA concentrations during pregnancy were associated with lower systolic blood pressure, but not diastolic blood pressure, during childhood (median: 6.0 years) (79). Another study of 1418 mother-child dyads in the Project Viva cohort in the United States showed that higher cord plasma DHA levels were associated with lower offspring BMI z-score, waist circumference, and leptin levels in early childhood (median age: 3.2 years), but not mid-childhood (median age: 7.7 years). Associations were strongest in offspring of women with isolated hyperglycemia (80). Another observational study in 388 participants of the German LISAplus study reported age-dependent associations of cord blood LC-PUFA composition with child body mass index (BMI) at 2 and 10 years, but not 6 years, highlighting the importance of age of outcome assessment (81). Within a sample of 237 participants in the same study, results suggested that nigher n-3 LC-PUFA concentrations and a lower n-6/n-3 ratio in cord blood was associated with higher adiponectin concentrations at 10 years (82). However, overall, associations remain inconsistent. A systematic review of 28 observational studies in total reported mixed associations between PUFA intake during pregnancy or early childhood and obesity (19 studies), no association with blood pressure (6 studies), and no association with lipid levels (9 studies) (72). Of the four studies that investigated the associations between PUFA levels in cord blood or intake during pregnancy and measures of insulin sensitivity, one study reported cord blood levels of n-6 GLA were inversely associated with fasting insulin and HOMA-IR at 7 y, another reported that DGLA, ALA, and DHA were inversely associated with proinsulin levels, and another study reported no associations between n-3 intake during pregnancy and insulin, glucose, or HOMA-IR at 20 y (72).

Although experimental studies consistently demonstrate a relationship between prenatal DHA supplementation and offspring measures of CMH, systematic reviews of RCTs report inconsistent effects of supplementation on offspring CMH outcomes (72, 83-86). A recent systematic review and meta-analysis of 6 RCTs reported no effects of maternal n-3 LC-PUFA supplementation in pregnancy or lactation on BMI in preschool (standardized mean difference (SMD)=0.07, 95% confidence interval (CI)=-0.22, 0.36, P=0.65) and school-age children (SMD=0.12, 95% CI=-0.06, 0.30, P=0.20) (83). A separate systematic review, which included 19 intervention trials, evaluated the effects of PUFA dietary intake and blood levels during pregnancy, lactation, and early childhood on obesity, blood pressure, lipids, and insulin resistance. The authors reported mixed effects of PUFA supplementation in early life on obesity (15 trials), cholesterol and lipid levels (5 trials), blood pressure (7 trials), and insulin resistance (2 studies) (72). To our knowledge, only one RCT has assessed the long-term effect of prenatal n-3 LC-PUFA supplementation on offspring metabolic health at age 19 years; no significant differences in blood pressure, insulin resistance, or lipids were observed, although the sample sizes were small (n = 180 for blood pressure outcomes, n = 243 for adiposity-related outcomes) (87, 88). Within the POSGRAD study in Mexico, supplementation modulated DNA methylation at *IGF2/H19* imprinted genes in overweight mothers (89) and increased DHA/ALA concentrations in breast milk at 1 month (90). However, no differences between treatment groups were observed for gestational age, birth weight (91), or non-fasting serum lipid and glucose concentrations at age 4 y (92).

Inconsistencies across studies may be attributed to heterogeneity in trial design with respect to types of omega-3s (e.g. fish oil vs. DHA only), dose, timing of supplementation, characteristics of mothers, variable length of follow-up, and variants in *FADS* genes. Mean follow-up time across

studies is 4 years; however, long-term effects of prenatal supplementation on CMH may manifest later in childhood or adolescence, particularly with the onset of puberty when cardiometabolic risk factors typically emerge (81, 93). Systematic reviews call for further follow-up of completed trials to assess longer-term outcomes and improve understanding of metabolic pathways involved (84).

2.4 Role of gene-nutrient interactions in CMH

2.4.1 Overview of gene-nutrient interactions

It is now well understood that individuals can respond differently to the same foods, nutrients, and supplements consumed. These differences may be attributable to factors including diversity in genetic makeup, metabolism, microbiota, prenatal nutrition, environmental exposures, and lifestyle. The field of "precision nutrition" leverages human individuality to design nutrition strategies to prevent, manage, and treat disease and optimize health, and "nutrigenetics" and "nutrigenomics" specifically focus on the interaction between genes, nutrition, and outcomes. Single nucleotide polymorphisms (SNPs) are variations in the genome at a single base pair that affect the way in which individuals absorb, metabolize, utilize, and excrete nutrients. Studies of human genomic variation show large global differences in allele frequencies of common SNPs involved in metabolism of common nutrients, including folate, choline, and polyunsaturated fatty acids (94). Additionally, evolutionary studies indicate genetic adaptations to ancestral diets and local environments across populations (95). For example, in response to a diet high in fatty meat and fish, ancestors of the Inuit people developed genetic adaptations associated with reduced metabolism of n-3 fatty acids (96). Thus, genetic diversity across populations may influence the bioavailability of certain nutrients and contribute to population-level differences in nutrient requirements. Improved understanding of gene-nutrient interactions, therefore, has relevance for the optimization of public health nutrition interventions worldwide.

2.4.2 *FADS* variants and PUFA metabolism

Tissue LC-PUFA concentrations are determined by both dietary intake of preformed LC-PUFAs and endogenous synthesis from their dietary precursors linoleic acid (LA; 18:2n-6) and α-linolenic acid (ALA; 18:3n-3. Conversion of dietary precursors to LC-PUFAs occurs through a series of consecutive desaturation and elongation steps, in which n-6 and n-3 fatty acids compete for conversion. The rate-limiting desaturase steps are mediated by Δ5 and Δ6-desaturase enzymes encoded in the *FADS* gene cluster (*FADS1, FADS2, FADS3*) located on human chromosome 11 in the region 11q12 – 11q13.1 (**Figure 2-2**).

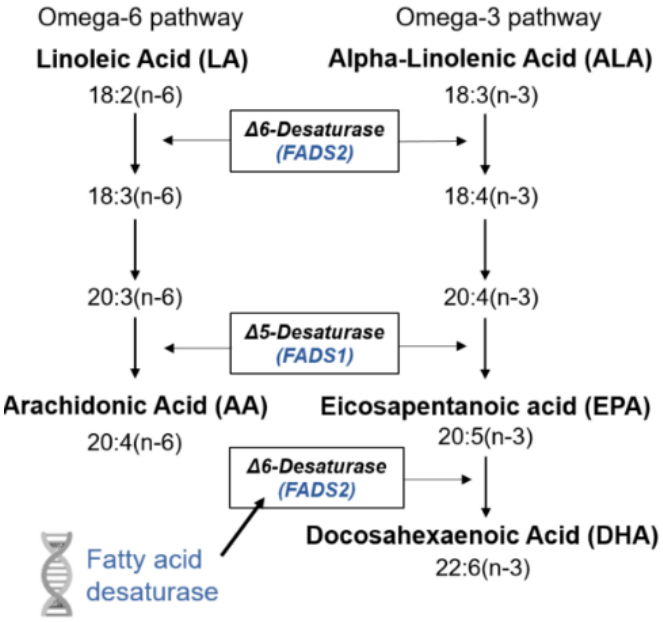

Figure 2-2. Long Chain Polyunsaturated Fatty Acid (LC-PUFA) Metabolism.
LA and ALA compete for conversion to LC-PUFAs by Δ6 and Δ5-desaturase enzymes encoded in the *FADS* gene cluster.

SNPs in *FADS* genes have been previously associated with lower transcription levels and/or diminished enzyme conversion rates, resulting in reduced conversion of precursors (43, 97). In a population of 727 healthy Caucasian adults, participants carrying common alleles of *FADS*

SNPs had higher blood levels of products of PUFA desaturation, whereas carriers of minor alleles had higher blood levels of precursors (98). This study revealed that a five-locus *FADS* haplotype SNPs in *FADS* genes explained 27.2, 5.2, and 1.4% of variability in AA, EPA, and DHA concentrations in serum phospholipids, respectively (98). This association has since been replicated in several candidate gene and genome-wide association studies. While there is preferential metabolism of n-3 fatty acids, excess intake of n-6 fatty acids drives the pathways and outcompetes n-3 fatty acids. Thus, diets high in n-6 LA may interfere with synthesis or accumulation of EPA/DHA in human tissues. As such, recent evidence suggests that the influence of dietary PUFAs on CMH may differ by variations in fatty acid desaturase (*FADS*) genes across populations (99, 100). *FADS* polymorphisms can therefore affect the balance between omega-3 and omega-6 fatty acid concentrations in the body, which may have implications for inflammation and the immune response.

2.4.3 *FADS* variants and CMH: evidence in adult populations

Several genetic and genome-wide association studies in adults have explored the relationship between *FADS* variants, plasma and tissue LC-PUFA concentrations, and risk of cardiovascular disease, showing that *FADS* polymorphisms influence lipid profiles and glucose homeostasis (101-105). In European adults, *FADS* alleles that predict inactive PUFA conversion are associated with lower inflammation, total cholesterol, LDL, HDL, and risk of coronary artery disease (43). For example, Hester et al. showed that participants with the major allele for rs174537 had significantly higher levels of pro-inflammatory eicosanoids LTB4 and 5-HETE compared to minor allele carriers (106). However, some studies have shown contradictory results, which may be attributable to either differences in ethnicity or LC-PUFA content of diet. Recent work has shown that diet composition can influence the relationship between *FADS* genotype and plasma

fatty acid concentrations. Hellstrand et al. found that the minor allele for SNP rs174547 was associated with lower LDL-C in the lowest tertile of LC n-3 PUFA intakes. They also observed interaction between the SNP and ratio of ALA:LA on HDL-c (107). A 14-year follow up study showed an inverse association between ALA:LA and CVD risk only among homozygous minor allele carriers for SNP rs174547 (108). More recently, the influence of *FADS* genetic variants in response to LC-PUFA supplementation has been explored, but evidence of genotype x supplement interaction across studies is conflicting (99, 100, 109, 110). Ultimately, additional research is needed to determine whether diet modifies the association between *FADS* genotype, fatty acid concentrations, and CVD risk in adults.

2.4.4 *FADS* variants and CMH: evidence in pediatric populations

While there has been substantial work done exploring the association of *FADS* polymorphisms with lipid levels in adult populations, a growing body of literature also supports this association in pediatric populations. Studies show that multiple variants in *FADS* genes are associated with lower lipid profiles and inflammation in European children (111-113). Specifically, one study showed that homozygous minor allele carriers had lower levels of total cholesterol and LDL, and heterozygous carriers had higher triglycerides and lower HDL relative to homozygous major allele carriers; however, no gene-diet interaction was observed (111). Similarly, one study performed in a large sample of Mexican adolescents showed that *FADS1* SNP rs174546 was a major contributor of plasma triglyceride and VLDL concentrations; however, no significant interaction effects were detected between dietary intake and genotype, warranting further research (114). Although a few observational studies show that maternal *FADS* genotype influences child LC-PUFA status and lipid profiles, this relationship remains underexamined in RCTs (115). One RCT in the United States showed that only among individuals with *FADS* SNPs

associated with lower conversion of precursors, prenatal DHA supplementation increased DHA concentrations and reduced AA:DHA ratios at delivery, suggesting a selective beneficial impact of supplementation among carriers of variants for some *FADS* SNPs (116). A recent birth cohort study in China also reported significant interaction between DHA supplementation and maternal SNP rs174602 on DHA concentrations in colostrum (117). Within the POSGRAD study, it was previously demonstrated that maternal SNP rs174602 modified the effect of prenatal DHA supplementation on birth weight (118) and the 3-month metabolome (119). However, whether these differences persist and influence long-term CMH remain unexplored.

2.4.5 Genetic variability in *FADS* SNPs across populations

Most studies assessing the role of maternal and offspring *FADS* genes in child health have been conducted in European populations (120, 121). However, *FADS* genotype distributions greatly vary across human populations. Ameur et al. showed that humans have two common but distinct *FADS* haplotypes, defined by 28 closely linked SNPs across the *FADS1* and *FADS2* regions. The two haplotypes, derived (D) and ancestral (A), vastly differ in their ability to generate LC-PUFAs. The more common haplotype D, seen in European and African populations, predicts more rapid conversion of LCPUFA precursors and higher lipid levels compared to the less common ancestral haplotype, observed in Native American and Mexican populations. The D haplotype is thought to be a genetic adaptation to shifts in diets from a hunter gatherer lifestyle high in EFAs to an agricultural diet high in LA but low in preformed LC-PUFAs such as AA and EPA. In a recent review, Koletzko and colleagues expanded upon these population level differences in *FADS* genotype, showing that there were substantially different frequencies of alleles associated with rapid PUFA conversion across populations. In Indonesia and Mexico, these alleles were prevalent in only a quarter of the population but two-thirds to three-fourths of the

population in Europe and Australia (43). Ultimately, this demonstrates that it may not be possible to extrapolate the effects of PUFAs in a specific population to populations with different genotype distributions.

2.4.6 Relative contribution of maternal and offspring genotype

Although it was previously assumed that the fetus solely relies on the mother's LC-PUFA supply, results from several recent studies have demonstrated evidence of desaturase enzyme activity in the liver of the developing fetus, suggesting that fetal synthesis of LC-PUFAs does indeed contribute to overall LC-PUFA status. The Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort study conducted in the United Kingdom explored the contribution of maternal and fetal genotypes on cord blood fatty acids, which represent placental transfer and fetal metabolism of PUFAs, in a subset of 2,035 mother-child pairs (122). After adjusting for maternal and child genotype, both maternal and child *FADS* genotypes and haplotypes influenced concentrations of cord plasma LC-PUFAs and fatty acid ratios. Most of the maternal SNPs were associated with cord levels of precursor n-6 PUFAs, child genotypes were mostly associated with products of n-6 desaturation, including AA, and both maternal and child genotypes were associated with DHA status. Evidence largely indicates that child genotype for several SNPs including rs174575, rs1535, rs174561, rs3834458, rs102275, and rs174448 influences cord n-6 PUFA concentrations (122-124). In another study conducted in the United States, *FADS2* rs174575 genotyping was performed on a subset of mothers and their 16-month-old toddlers. Results showed that the effect of maternal genotype on declarative memory task performance was above and beyond the child's own genotype (125). While these studies collectively indicate the importance of maternal genotype in predicting offspring phenotype, the relative contribution of maternal and offspring genotype in determining cord LC-PUFA

concentrations and later outcomes, particularly in non-European populations, merits further examination.

2.5 Summary and Overall Significance

Despite extensive knowledge that cardiometabolic risk factors emerge early in the life course and typically cluster, defining cardiometabolic risk in pediatric populations remains a key challenge. Evidence for the long-term effects of prenatal DHA supplementation on offspring CMH remains inconsistent. Results from RCTs are limited primarily by large between-study population heterogeneity and variable timing of study follow-ups. Additionally, most studies have failed to incorporate *FADS* genotype when assessing outcomes, and those that do were typically conducted in European populations. Given the ethnic variation in *FADS* genotype distributions and PUFA dietary intake, studies in distinct settings (i.e., limited migration, similar dietary quality), are needed to improve understanding of gene-nutrient interactions. Finally, the respective contributions of maternal and offspring *FADS* genetic profiles towards CMH are poorly understood. Supplementation is not a one-size-fits-all approach; there is a need to identify which individuals will benefit most from nutrition intervention strategies. This dissertation seeks to address these research gaps in Chapters 4-6.

2.6 References

1. Koletzko B, Brands B, Poston L, Godfrey K, Demmelmair H. Early nutrition programming of long-term health. Proc Nutr Soc 2012;71(3):371-8. doi: 10.1017/s0029665112000596.
2. Innis SM. Metabolic programming of long-term outcomes due to fatty acid nutrition in early life. Matern Child Nutr 2011;7 Suppl 2(Suppl 2):112-23. doi: 10.1111/j.1740-8709.2011.00318.x.
3. Orsso CE, Colin-Ramirez E, Field CJ, Madsen KL, Prado CM, Haqq AM. Adipose Tissue Development and Expansion from the Womb to Adolescence: An Overview. Nutrients 2020;12(9). doi: 10.3390/nu12092735.
4. Kumar S, Kelly AS. Review of Childhood Obesity: From Epidemiology, Etiology, and Comorbidities to Clinical Assessment and Treatment. Mayo Clinic Proceedings 2017;92(2):251-65. doi: https://doi.org/10.1016/j.mayocp.2016.09.017.
5. Simmonds M, Llewellyn A, Owen CG, Woolacott N. Predicting adult obesity from childhood obesity: a systematic review and meta-analysis. Obes Rev 2016;17(2):95-107. doi: 10.1111/obr.12334.
6. Guardamagna O, Abello F, Cagliero P, Lughetti L. Impact of nutrition since early life on cardiovascular prevention. Italian Journal of Pediatrics 2012;38(1):73. doi: 10.1186/1824-7288-38-73.
7. World Health Organization. Internet: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds) (accessed June 27 2023).
8. Rippe JM. Lifestyle Strategies for Risk Factor Reduction, Prevention, and Treatment of Cardiovascular Disease. Am J Lifestyle Med 2019;13(2):204-12. doi: 10.1177/1559827618812395.

9. Rivera JA, Barquera S, González-Cossío T, Olaiz G, Sepúlveda J. Nutrition transition in Mexico and in other Latin American countries. Nutr Rev 2004;62(7 Pt 2):S149-57. doi: 10.1111/j.1753-4887.2004.tb00086.x.

10. Martin RP GB, Erick RA, Eduardo AG, et al. Obesity and Cardiovascular Risk in Mexico: Pathophysiology and Prevention. . Obes Control Ther 2017;4(3):1-9.

11. Johnson W, Li L, Kuh D, Hardy R. How Has the Age-Related Process of Overweight or Obesity Development Changed over Time? Co-ordinated Analyses of Individual Participant Data from Five United Kingdom Birth Cohorts. PLoS Med 2015;12(5):e1001828; discussion e. doi: 10.1371/journal.pmed.1001828.

12. Cuevas-Nasu L, Levy TS, Arcos MAA, Gomez IM, Dommarco JR. Overweight and obesity in Mexican children and adolescents: Data from National Nutrition and Health Surveys in 2016. The FASEB Journal 2017;31(S1):640.35-.35. doi: https://doi.org/10.1096/fasebj.31.1_supplement.640.35.

13. Cardoso-Saldaña GC, Yamamoto-Kimura L, Medina-Urrutia A, Posadas-Sánchez R, Caracas-Portilla NA, Posadas-Romero C. [Obesity or overweight and metabolic syndrome in Mexico City teenagers]. Arch Cardiol Mex 2010;80(1):12-8.

14. Cárdenas-Villareal VM, López Alvarenga JC, Bastarrachea RA, Rizo-Baeza MM, Cortés-Castell E. [Metabolic syndrome prevalence in teenagers of Monterrey, Nuevo Leon]. Arch Cardiol Mex 2010;80(1):19-26.

15. Evia-Viscarra ML, Rodea-Montero ER, Apolinar-Jiménez E, Quintana-Vargas S. Metabolic syndrome and its components among obese (BMI >=95th) Mexican adolescents. Endocr Connect 2013;2(4):208-15. doi: 10.1530/ec-13-0057.

16. Magge SN, Goodman E, Armstrong SC. The Metabolic Syndrome in Children and Adolescents: Shifting the Focus to Cardiometabolic Risk Factor Clustering. Pediatrics 2017;140(2):e20171603. doi: 10.1542/peds.2017-1603.

17. Ormazabal V, Nair S, Elfeky O, Aguayo C, Salomon C, Zuñiga FA. Association between insulin resistance and the development of cardiovascular disease. Cardiovascular Diabetology 2018;17(1):122. doi: 10.1186/s12933-018-0762-4.

18. Kaptoge S, Di Angelantonio E, Lowe G, Pepys MB, Thompson SG, Collins R, et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet 2010;375(9709):132-40. doi: 10.1016/s0140-6736(09)61717-7.

19. Thorand B, Löwel H, Schneider A, Kolb H, Meisinger C, Fröhlich M, et al. C-Reactive Protein as a Predictor for Incident Diabetes Mellitus Among Middle-aged Men: Results From the MONICA Augsburg Cohort Study, 1984-1998. Archives of Internal Medicine 2003;163(1):93-9. doi: 10.1001/archinte.163.1.93.

20. Samson SL, Garber AJ. Metabolic Syndrome. Endocrinology and Metabolism Clinics of North America 2014;43(1):1-23. doi: https://doi.org/10.1016/j.ecl.2013.09.009.

21. Al-Hamodi Z, Al-Habori M, Al-Meeri A, Saif-Ali R. Association of adipokines, leptin/adiponectin ratio and C-reactive protein with obesity and type 2 diabetes mellitus. Diabetol Metab Syndr 2014;6(1):99. doi: 10.1186/1758-5996-6-99.

22. Hui X, Lam KS, Vanhoutte PM, Xu A. Adiponectin and cardiovascular health: an update. Br J Pharmacol 2012;165(3):574-90. doi: 10.1111/j.1476-5381.2011.01395.x.

23. Phillips CM. Metabolically healthy obesity: Definitions, determinants and clinical implications. Reviews in Endocrine and Metabolic Disorders 2013;14(3):219-27. doi: 10.1007/s11154-013-9252-x.

24. Ruderman NB, Schneider SH, Berchtold P. The "metabolically-obese," normal-weight individual. The American Journal of Clinical Nutrition 1981;34(8):1617-21. doi: https://doi.org/10.1093/ajcn/34.8.1617.

25. Duren DL, Sherwood RJ, Czerwinski SA, Lee M, Choh AC, Siervogel RM, et al. Body composition methods: comparisons and interpretation. J Diabetes Sci Technol 2008;2(6):1139-46. doi: 10.1177/193229680800200623.

26. Ford ES. Prevalence of the Metabolic Syndrome Defined by the International Diabetes Federation Among Adults in the U.S. Diabetes Care 2005;28(11):2745-9. doi: 10.2337/diacare.28.11.2745.

27. Ford ES, Li C. Defining the metabolic syndrome in children and adolescents: will the real definition please stand up? J Pediatr 2008;152(2):160-4. doi: 10.1016/j.jpeds.2007.07.056.

28. Peña-Espinoza BI, Granados-Silvestre M, Sánchez-Pozos K, Ortiz-López MG, Menjivar M. Metabolic syndrome in Mexican children: Low effectiveness of diagnostic definitions. Endocrinol Diabetes Nutr 2017;64(7):369-76. doi: 10.1016/j.endinu.2017.04.004.

29. Beckstead JW, Beckie TM. How much information can metabolic syndrome provide? An application of information theory. Med Decis Making 2011;31(1):79-92. doi: 10.1177/0272989x10373401.

30. Tropeano A, Corica D, Li Pomi A, Pepe G, Morabito LA, Curatola SL, et al. The metabolic syndrome in pediatrics: do we have a reliable definition? A systematic review. Eur J Endocrinol 2021;185(2):265-78. doi: 10.1530/eje-21-0238.

31. Kelsey MM, Zeitler PS. Insulin Resistance of Puberty. Curr Diab Rep 2016;16(7):64. doi: 10.1007/s11892-016-0751-5.

32. Canoy D, Beral V, Balkwill A, Wright FL, Kroll ME, Reeves GK, et al. Age at menarche and risks of coronary heart and other vascular diseases in a large UK cohort. Circulation 2015;131(3):237-44. doi: 10.1161/circulationaha.114.010070.

33. Elks CE, Ong KK, Scott RA, van der Schouw YT, Brand JS, Wark PA, et al. Age at Menarche and Type 2 Diabetes Risk: The EPIC-InterAct study. Diabetes Care 2013;36(11):3526-34. doi: 10.2337/dc13-0446.

34. Widén E, Silventoinen K, Sovio U, Ripatti S, Cousminer DL, Hartikainen A-L, et al. Pubertal Timing and Growth Influences Cardiometabolic Risk Factors in Adult Males and Females. Diabetes Care 2012;35(4):850-6. doi: 10.2337/dc11-1365.

35. Kiess W, Reich A, Meyer K, Glasow A, Deutscher J, Klammt J, et al. A role for leptin in sexual maturation and puberty? Horm Res 1999;51 Suppl 3:55-63. doi: 10.1159/000053163.

36. Mantzoros CS, Flier JS, Rogol AD. A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. V. Rising leptin levels may signal the onset of puberty. J Clin Endocrinol Metab 1997;82(4):1066-70. doi: 10.1210/jcem.82.4.3878.

37. Nieuwenhuis D, Pujol-Gualdo N, Arnoldussen IAC, Kiliaan AJ. Adipokines: A gear shift in puberty. Obesity Reviews 2020;21(6):e13005. doi: https://doi.org/10.1111/obr.13005.

38. Goodman E, Daniels SR, Meigs JB, Dolan LM. Instability in the diagnosis of metabolic syndrome in adolescents. Circulation 2007;115(17):2316-22. doi: 10.1161/circulationaha.106.669994.

39. Magge SN, Goodman E, Armstrong SC, Committee On N, Section On E, Section On O, et al. The Metabolic Syndrome in Children and Adolescents: Shifting the Focus to Cardiometabolic Risk Factor Clustering. Pediatrics 2017;140(2):e20171603. doi: 10.1542/peds.2017-1603.

40. Bugge A, El-Naaman B, McMurray RG, Froberg K, Nielsen CH, Müller K, et al. Inflammatory markers and clustered cardiovascular disease risk factors in Danish adolescents. Horm Res Paediatr 2012;78(5-6):288-96. doi: 10.1159/000345546.

41. Okosun IS, Lyn R, Davis-Smith M, Eriksen M, Seale P. Validity of a continuous metabolic risk score as an index for modeling metabolic syndrome in adolescents. Ann Epidemiol 2010;20(11):843-51. doi: 10.1016/j.annepidem.2010.08.001.

42. Ross AC, Caballero BH, Cousins RJ, Tucker KL, Ziegler TR. Modern nutrition in health and disease: Eleventh edition.: Wolters Kluwer Health Adis (ESP), 2012.

43. Koletzko B, Reischl E, Tanjung C, Gonzalez-Casanova I, Ramakrishnan U, Meldrum S, et al. FADS1 and FADS2 Polymorphisms Modulate Fatty Acid Metabolism and Dietary Impact on Health. Annu Rev Nutr 2019;39:21-44. doi: 10.1146/annurev-nutr-082018-124250.

44. Lorente-Cebrián S, Costa AG, Navas-Carretero S, Zabala M, Martínez JA, Moreno-Aliaga MJ. Role of omega-3 fatty acids in obesity, metabolic syndrome, and cardiovascular diseases: a review of the evidence. J Physiol Biochem 2013;69(3):633-51. doi: 10.1007/s13105-013-0265-4.

45. Calder PC. Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology? Br J Clin Pharmacol 2013;75(3):645-62. doi: 10.1111/j.1365-2125.2012.04374.x.

46. Goyens PL, Spilker ME, Zock PL, Katan MB, Mensink RP. Conversion of alpha-linolenic acid in humans is influenced by the absolute amounts of alpha-linolenic acid and linoleic acid in the diet and not by their ratio. Am J Clin Nutr 2006;84(1):44-53. doi: 10.1093/ajcn/84.1.44.

47. Weldon KA, Whelan J. Allometric scaling of dietary linoleic acid on changes in tissue arachidonic acid using human equivalent diets in mice. Nutr Metab (Lond) 2011;8(1):43. doi: 10.1186/1743-7075-8-43.

48. Poudyal H, Panchal SK, Diwan V, Brown L. Omega-3 fatty acids and metabolic syndrome: Effects and emerging mechanisms of action. Progress in Lipid Research 2011;50(4):372-87.

49. Grygiel-Górniak B. Peroxisome proliferator-activated receptors and their ligands: nutritional and clinical implications--a review. Nutr J 2014;13:17. doi: 10.1186/1475-2891-13-17.

50. Lin Y, Wang Y, Li P-f. PPARα: An emerging target of metabolic syndrome, neurodegenerative and cardiovascular diseases. Frontiers in Endocrinology 2022;13. doi: 10.3389/fendo.2022.1074911.

51. Belchior T, Paschoal VA, Magdalon J, Chimin P, Farias TM, Chaves-Filho AB, et al. Omega-3 fatty acids protect from diet-induced obesity, glucose intolerance, and adipose tissue inflammation through PPARγ-dependent and PPARγ-independent actions. Molecular Nutrition & Food Research 2015;59(5):957-67. doi: https://doi.org/10.1002/mnfr.201400914.

52. Sahye-Pudaruth S, Ma DWL. Assessing the Highest Level of Evidence from Randomized Controlled Trials in Omega-3 Research. Nutrients 2023;15(4):1001.

53. Parra-Cabrera S, Stein AD, Wang M, Martorell R, Rivera J, Ramakrishnan U. Dietary intakes of polyunsaturated fatty acids among pregnant Mexican women. Matern Child Nutr 2011;7(2):140-7. doi: 10.1111/j.1740-8709.2010.00254.x.

54. Harris WS, Mozaffarian D, Rimm E, Kris-Etherton P, Rudel LL, Appel LJ, et al. Omega-6 Fatty Acids and Risk for Cardiovascular Disease. Circulation 2009;119(6):902-7. doi: 10.1161/CIRCULATIONAHA.108.191627.

55. Institute of Medicine. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Washington, DC: The National Academies Press, 2005.

56. Koletzko B, Lien E, Agostoni C, Böhles H, Campoy C, Cetin I, et al. The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations. J Perinat Med 2008;36(1):5-14. doi: 10.1515/jpm.2008.001.

57. Siscovick DS, Barringer TA, Fretts AM, Wu JH, Lichtenstein AH, Costello RB, et al. Omega-3 Polyunsaturated Fatty Acid (Fish Oil) Supplementation and the Prevention of Clinical Cardiovascular Disease: A Science Advisory From the American Heart Association. Circulation 2017;135(15):e867-e84. doi: 10.1161/cir.0000000000000482.

58. Sacks FM, Lichtenstein AH, Wu JHY, Appel LJ, Creager MA, Kris-Etherton PM, et al. Dietary Fats and Cardiovascular Disease: A Presidential Advisory From the American Heart Association. Circulation 2017;136(3):e1-e23. doi: 10.1161/CIR.0000000000000510.

59. Blasbalg TL, Hibbeln JR, Ramsden CE, Majchrzak SF, Rawlings RR. Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th

century. The American Journal of Clinical Nutrition 2011;93(5):950-62. doi: https://doi.org/10.3945/ajcn.110.006643.

60. Simopoulos AP. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp Biol Med (Maywood) 2008;233(6):674-88. doi: 10.3181/0711-mr-311.

61. Bang HO, Dyerberg J, Hjøorne N. The composition of food consumed by Greenland Eskimos. Acta Med Scand 1976;200(1-2):69-73. doi: 10.1111/j.0954-6820.1976.tb08198.x.

62. Leaf A. Historical overview of n-3 fatty acids and coronary heart disease. Am J Clin Nutr 2008;87(6):1978s-80s. doi: 10.1093/ajcn/87.6.1978S.

63. Burr ML, Fehily AM, Gilbert JF, Rogers S, Holliday RM, Sweetnam PM, et al. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). Lancet 1989;2(8666):757-61. doi: 10.1016/s0140-6736(89)90828-3.

64. Singh RB, Niaz MA, Sharma JP, Kumar R, Rastogi V, Moshiri M. Randomized, double-blind, placebo-controlled trial of fish oil and mustard oil in patients with suspected acute myocardial infarction: the Indian experiment of infarct survival--4. Cardiovasc Drugs Ther 1997;11(3):485-91. doi: 10.1023/a:1007757724505.

65. Marchioli R, Barzi F, Bomba E, Chieffo C, Di Gregorio D, Di Mascio R, et al. Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. Circulation 2002;105(16):1897-903. doi: 10.1161/01.cir.0000014682.14181.f2.

66. Saravanan P, Davidson NC, Schmidt EB, Calder PC. Cardiovascular effects of marine omega-3 fatty acids. Lancet 2010;376(9740):540-50. doi: 10.1016/s0140-6736(10)60445-x.

67. Amatruda M, Ippolito G, Vizzuso S, Vizzari G, Banderali G, Verduci E. Epigenetic Effects of n-3 LCPUFAs: A Role in Pediatric Metabolic Syndrome. Int J Mol Sci 2019;20(9). doi: 10.3390/ijms20092118.

68. Eslick GD, Howe PR, Smith C, Priest R, Bensoussan A. Benefits of fish oil supplementation in hyperlipidemia: a systematic review and meta-analysis. Int J Cardiol 2009;136(1):4-16. doi: 10.1016/j.ijcard.2008.03.092.

69. Rinaudo P, Wang E. Fetal programming and metabolic syndrome. Annu Rev Physiol 2012;74:107-30. doi: 10.1146/annurev-physiol-020911-153245.

70. Satokar VV, Cutfield WS, Cameron-Smith D, Albert BB. Omega-3 fats in pregnancy: could a targeted approach lead to better metabolic health for children? Nutr Rev 2020. doi: 10.1093/nutrit/nuaa071.

71. Kabaran S, Besler HT. Do fatty acids affect fetal programming? J Health Popul Nutr 2015;33:14. doi: 10.1186/s41043-015-0018-9.

72. Voortman T, van den Hooven EH, Braun KV, van den Broek M, Bramer WM, Chowdhurry R, et al. Effects of polyunsaturated fatty acid intake and status during pregnancy, lactation, and early childhood on cardiometabolic health: A systematic review. Prog Lipid Res 2015;59:67-87. doi: 10.1016/j.plipres.2015.05.001.

73. D'Asti E, Long H, Tremblay-Mercier J, Grajzer M, Cunnane SC, Di Marzo V, et al. Maternal dietary fat determines metabolic profile and the magnitude of endocannabinoid

inhibition of the stress response in neonatal rat offspring. Endocrinology 2010;151(4):1685-94. doi: 10.1210/en.2009-1092.

74. Hussain A, Nookaew I, Khoomrung S, Andersson L, Larsson I, Hulthén L, et al. A maternal diet of fatty fish reduces body fat of offspring compared with a maternal diet of beef and a post-weaning diet of fish improves insulin sensitivity and lipid profile in adult C57BL/6 male mice. Acta Physiol (Oxf) 2013;209(3):220-34. doi: 10.1111/apha.12130.

75. Sardinha FL, Fernandes FS, Tavares do Carmo MG, Herrera E. Sex-dependent nutritional programming: fish oil intake during early pregnancy in rats reduces age-dependent insulin resistance in male, but not female, offspring. Am J Physiol Regul Integr Comp Physiol 2013;304(4):R313-20. doi: 10.1152/ajpregu.00392.2012.

76. Hollander KS, Tempel Brami C, Konikoff FM, Fainaru M, Leikin-Frenkel A. Dietary enrichment with alpha-linolenic acid during pregnancy attenuates insulin resistance in adult offspring in mice. Arch Physiol Biochem 2014;120(3):99-111. doi: 10.3109/13813455.2014.940352.

77. Siemelink M, Verhoef A, Dormans JA, Span PN, Piersma AH. Dietary fatty acid composition during pregnancy and lactation in the rat programs growth and glucose metabolism in the offspring. Diabetologia 2002;45(10):1397-403. doi: 10.1007/s00125-002-0918-2.

78. Voortman T, Tielemans MJ, Stroobant W, Schoufour JD, Kiefte-de Jong JC, Steenweg-de Graaff J, et al. Plasma fatty acid patterns during pregnancy and child's growth, body composition, and cardiometabolic health: The Generation R Study. Clin Nutr 2018;37(3):984-92. doi: 10.1016/j.clnu.2017.04.006.

79. Vidakovic AJ, Gishti O, Steenweg-de Graaff J, Williams MA, Duijts L, Felix JF, et al. Higher Maternal Plasma n–3 PUFA and Lower n–6 PUFA Concentrations in Pregnancy Are Associated with Lower Childhood Systolic Blood Pressure. The Journal of Nutrition 2015;145(10):2362-8. doi: 10.3945/jn.115.210823.

80. Maslova E, Rifas-Shiman SL, Olsen SF, Gillman MW, Oken E. Prenatal n-3 long-chain fatty acid status and offspring metabolic health in early and mid-childhood: results from Project Viva. Nutr Diabetes 2018;8(1):29. doi: 10.1038/s41387-018-0040-2.

81. Standl M, Thiering E, Demmelmair H, Koletzko B, Heinrich J. Age-dependent effects of cord blood long-chain PUFA composition on BMI during the first 10 years of life. British Journal of Nutrition 2014;111(11):2024-31. doi: 10.1017/S0007114514000105.

82. Standl M, Demmelmair H, Koletzko B, Heinrich J. Cord blood n-3 LC-PUFA is associated with adiponectin concentrations at 10 years of age. Prostaglandins Leukot Essent Fatty Acids 2015;96:51-5. doi: 10.1016/j.plefa.2015.02.003.

83. Stratakis N, Gielen M, Chatzi L, Zeegers MP. Effect of maternal n-3 long-chain polyunsaturated fatty acid supplementation during pregnancy and/or lactation on adiposity in childhood: a systematic review and meta-analysis of randomized controlled trials. Eur J Clin Nutr 2014;68(12):1277-87. doi: 10.1038/ejcn.2014.158.

84. Middleton P, Gomersall JC, Gould JF, Shepherd E, Olsen SF, Makrides M. Omega-3 fatty acid addition during pregnancy. Cochrane Database Syst Rev 2018;11(11):Cd003402. doi: 10.1002/14651858.CD003402.pub3.

85. He S, Stein AD. Early-Life Nutrition Interventions and Associated Long-Term Cardiometabolic Outcomes: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Adv Nutr 2021;12(2):461-89. doi: 10.1093/advances/nmaa107.

86. Vahdaninia M, Mackenzie H, Dean T, Helps S. The effectiveness of ω-3 polyunsaturated fatty acid interventions during pregnancy on obesity measures in the offspring: an up-to-date systematic review and meta-analysis. Eur J Nutr 2019;58(7):2597-613. doi: 10.1007/s00394-018-1824-9.

87. Rytter D, Christensen JH, Bech BH, Schmidt EB, Henriksen TB, Olsen SF. The effect of maternal fish oil supplementation during the last trimester of pregnancy on blood pressure, heart rate and heart rate variability in the 19-year-old offspring. Br J Nutr 2012;108(8):1475-83. doi: 10.1017/s0007114511006799.

88. Rytter D, Schmidt EB, Bech BH, Christensen JH, Henriksen TB, Olsen SF. Fish Oil Supplementation During Late Pregnancy Does Not Influence Plasma Lipids or Lipoprotein Levels in Young Adult Offspring. Lipids 2011;46(12):1091-9. doi: 10.1007/s11745-011-3606-5.

89. Lee HS, Barraza-Villarreal A, Biessy C, Duarte-Salles T, Sly PD, Ramakrishnan U, et al. Dietary supplementation with polyunsaturated fatty acid during pregnancy modulates DNA methylation at IGF2/H19 imprinted genes and growth of infants. Physiol Genomics 2014;46(23):851-7. doi: 10.1152/physiolgenomics.00061.2014.

90. Imhoff-Kunsch B, Stein AD, Villalpando S, Martorell R, Ramakrishnan U. Docosahexaenoic acid supplementation from mid-pregnancy to parturition influenced breast milk fatty acid concentrations at 1 month postpartum in Mexican women. The Journal of nutrition 2011;141(2):321-6. doi: 10.3945/jn.110.126870.

91. Ramakrishnan U, Stein AD, Parra-Cabrera S, Wang M, Imhoff-Kunsch B, Juarez-Marquez S, et al. Effects of docosahexaenoic acid supplementation during pregnancy on gestational

age and size at birth: randomized, double-blind, placebo-controlled trial in Mexico. Food and nutrition bulletin 2010;31(2 Suppl):S108-16. doi: 10.1177/15648265100312s203.

92. Gutierrez-Gomez Y, Stein AD, Ramakrishnan U, Barraza-Villarreal A, Moreno-Macias H, Aguilar-Salinas C, et al. Prenatal Docosahexaenoic Acid Supplementation Does Not Affect Nonfasting Serum Lipid and Glucose Concentrations of Offspring at 4 Years of Age in a Follow-Up of a Randomized Controlled Clinical Trial in Mexico. J Nutr 2017;147(2):242-7. doi: 10.3945/jn.116.238329.

93. Weihrauch-Blüher S, Schwarz P, Klusmann JH. Childhood obesity: increased risk for cardiometabolic disease and cancer in adulthood. Metabolism 2019;92:147-52. doi: 10.1016/j.metabol.2018.12.001.

94. Mead MN. Nutrigenomics: the genome--food interface. Environ Health Perspect 2007;115(12):A582-9. doi: 10.1289/ehp.115-a582.

95. Luca F, Perry GH, Di Rienzo A. Evolutionary adaptations to dietary changes. Annu Rev Nutr 2010;30:291-314. doi: 10.1146/annurev-nutr-080508-141048.

96. Fumagalli M, Moltke I, Grarup N, Racimo F, Bjerregaard P, Jørgensen ME, et al. Greenlandic Inuit show genetic signatures of diet and climate adaptation. Science 2015;349(6254):1343-7. doi: 10.1126/science.aab2319.

97. Conway MC, McSorley EM, Mulhern MS, Strain JJ, van Wijngaarden E, Yeates AJ. Influence of fatty acid desaturase (FADS) genotype on maternal and child polyunsaturated fatty acids (PUFA) status and child health outcomes: a systematic review. Nutrition Reviews 2020;78(8):627-46. doi: 10.1093/nutrit/nuz086.

98. Schaeffer L, Gohlke H, Muller M, Heid IM, Palmer LJ, Kompauer I, et al. Common genetic variants of the FADS1 FADS2 gene cluster and their reconstructed haplotypes are

associated with the fatty acid composition in phospholipids. Human molecular genetics 2006;15(11):1745-56. doi: 10.1093/hmg/ddl117.

99. Gillingham LG, Harding SV, Rideout TC, Yurkova N, Cunnane SC, Eck PK, et al. Dietary oils and FADS1-FADS2 genetic variants modulate [13C]α-linolenic acid metabolism and plasma fatty acid composition. Am J Clin Nutr 2013;97(1):195-207. doi: 10.3945/ajcn.112.043117.

100. Roke K, Mutch DM. The role of FADS1/2 polymorphisms on cardiometabolic markers and fatty acid profiles in young adults consuming fish oil supplements. Nutrients 2014;6(6):2290-304. doi: 10.3390/nu6062290.

101. Huang MC, Chang WT, Chang HY, Chung HF, Chen FP, Huang YF, et al. FADS Gene Polymorphisms, Fatty Acid Desaturase Activities, and HDL-C in Type 2 Diabetes. Int J Environ Res Public Health 2017;14(6). doi: 10.3390/ijerph14060572.

102. Aulchenko YS, Ripatti S, Lindqvist I, Boomsma D, Heid IM, Pramstaller PP, et al. Loci influencing lipid levels and coronary heart disease risk in 16 European population cohorts. Nat Genet 2009;41(1):47-55. doi: 10.1038/ng.269.

103. Sabatti C, Service SK, Hartikainen AL, Pouta A, Ripatti S, Brodsky J, et al. Genome-wide association analysis of metabolic traits in a birth cohort from a founder population. Nat Genet 2009;41(1):35-46. doi: 10.1038/ng.271.

104. Chasman DI, Paré G, Mora S, Hopewell JC, Peloso G, Clarke R, et al. Forty-three loci associated with plasma lipoprotein size, concentration, and cholesterol content in genome-wide analysis. PLoS Genet 2009;5(11):e1000730. doi: 10.1371/journal.pgen.1000730.

105. Mathias RA, Pani V, Chilton FH. Genetic Variants in the FADS Gene: Implications for Dietary Recommendations for Fatty Acid Intake. Curr Nutr Rep 2014;3(2):139-48. doi: 10.1007/s13668-014-0079-1.

106. Hester AG, Murphy RC, Uhlson CJ, Ivester P, Lee TC, Sergeant S, et al. Relationship between a common variant in the fatty acid desaturase (FADS) cluster and eicosanoid generation in humans. J Biol Chem 2014;289(32):22482-9. doi: 10.1074/jbc.M114.579557.

107. Hellstrand S, Sonestedt E, Ericson U, Gullberg B, Wirfält E, Hedblad B, et al. Intake levels of dietary long-chain PUFAs modify the association between genetic variation in FADS and LDL-C. J Lipid Res 2012;53(6):1183-9. doi: 10.1194/jlr.P023721.

108. Hellstrand S, Ericson U, Gullberg B, Hedblad B, Orho-Melander M, Sonestedt E. Genetic variation in FADS1 has little effect on the association between dietary PUFA intake and cardiovascular disease. J Nutr 2014;144(9):1356-63. doi: 10.3945/jn.114.192708.

109. Cormier H, Rudkowska I, Paradis A-M, Thifault E, Garneau V, Lemieux S, et al. Association between Polymorphisms in the Fatty Acid Desaturase Gene Cluster and the Plasma Triacylglycerol Response to an n-3 PUFA Supplementation. Nutrients 2012;4:1026-41. accessed Date Accessed)|.

110. Huang PC, Cheng H, Su YT, Huang MC, Hsu CC, Hwang SJ, et al. Interaction among dietary n-3 and n-6 polyunsaturated fatty acid intake, fatty acid desaturase 2 genetic variants, and low-density lipoprotein cholesterol levels in type 2 diabetes patients. J Diabetes Investig 2023;14(2):297-308. doi: 10.1111/jdi.13944.

111. Standl M, Lattka E, Stach B, Koletzko S, Bauer CP, von Berg A, et al. FADS1 FADS2 gene cluster, PUFA intake and blood lipids in children: results from the GINIplus and LISAplus studies. PLoS One 2012;7(5):e37780. doi: 10.1371/journal.pone.0037780.

112. Bokor S, Dumont J, Spinneker A, Gonzalez-Gross M, Nova E, Widhalm K, et al. Single nucleotide polymorphisms in the FADS gene cluster are associated with delta-5 and delta-6 desaturase activities estimated by serum fatty acid ratios. J Lipid Res 2010;51(8):2325-33. doi: 10.1194/jlr.M006205.

113. Wolters M, Dering C, Siani A, Russo P, Kaprio J, Risé P, et al. The role of a FADS1 polymorphism in the association of fatty acid blood levels, BMI and blood pressure in young children-Analyses based on path models. PLoS One 2017;12(7):e0181485. doi: 10.1371/journal.pone.0181485.

114. Vazquez-Vidal I, Voruganti VS, Hannon BA, Andrade FCD, Aradillas-García C, Nakamura MT, et al. Serum Lipid Concentrations and FADS Genetic Variants in Young Mexican College Students: The UP-AMIGOS Cohort Study. Lifestyle Genom 2018;11(1):40-8. doi: 10.1159/000488085.

115. Molto-Puigmarti C, Jansen E, Heinrich J, Standl M, Mensink RP, Plat J, et al. Genetic variation in FADS genes and plasma cholesterol levels in 2-year-old infants: KOALA Birth Cohort Study. PLoS One 2013;8(5):e61671. doi: 10.1371/journal.pone.0061671.

116. Scholtz SA, Kerling EH, Shaddy DJ, Li S, Thodosoff JM, Colombo J, et al. Docosahexaenoic acid (DHA) supplementation in pregnancy differentially modulates arachidonic acid and DHA status across FADS genotypes in pregnancy. Prostaglandins Leukot Essent Fatty Acids 2015;94:29-33. doi: 10.1016/j.plefa.2014.10.008.

117. Li P, Chen Y, Song J, Yan L, Tang T, Wang R, et al. Maternal DHA-rich n-3 PUFAs supplementation interacts with FADS genotypes to influence the profiles of PUFAs in the colostrum among Chinese Han population: a birth cohort study. Nutr Metab (Lond) 2022;19(1):48. doi: 10.1186/s12986-022-00683-3.

118. Gonzalez-Casanova I, Rzehak P, Stein AD, Garcia Feregrino R, Rivera Dommarco JA, Barraza-Villarreal A, et al. Maternal single nucleotide polymorphisms in the fatty acid desaturase 1 and 2 coding regions modify the impact of prenatal supplementation with DHA on birth weight. Am J Clin Nutr 2016;103(4):1171-8. doi: 10.3945/ajcn.115.121244.

119. Tandon S, Gonzalez-Casanova I, Barraza-Villarreal A, Romieu I, Demmelmair H, Jones D, et al. Infant Metabolome in Relation to Prenatal DHA Supplementation and Maternal Single Nucleotide Polymorphism rs174602: Secondary Analysis of a Randomized Controlled Trial in Mexico. The Journal of Nutrition 2021(In press).

120. Ameur A, Enroth S, Johansson A, Zaboli G, Igl W, Johansson AC, et al. Genetic adaptation of fatty-acid metabolism: a human-specific haplotype increasing the biosynthesis of long-chain omega-3 and omega-6 fatty acids. American journal of human genetics 2012;90(5):809-20. doi: 10.1016/j.ajhg.2012.03.014.

121. Mathias RA, Fu W, Akey JM, Ainsworth HC, Torgerson DG, Ruczinski I, et al. Adaptive evolution of the FADS gene cluster within Africa. PLoS One 2012;7(9):e44926. doi: 10.1371/journal.pone.0044926.

122. Lattka E, Koletzko B, Zeilinger S, Hibbeln JR, Klopp N, Ring SM, et al. Umbilical cord PUFA are determined by maternal and child fatty acid desaturase (FADS) genetic variants in the Avon Longitudinal Study of Parents and Children (ALSPAC). Br J Nutr 2013;109(7):1196-210. doi: 10.1017/s0007114512003108.

123. Steer CD, Hibbeln JR, Golding J, Davey Smith G. Polyunsaturated fatty acid levels in blood during pregnancy, at birth and at 7 years: their associations with two common FADS2 polymorphisms. Human molecular genetics 2012;21(7):1504-12. doi: 10.1093/hmg/ddr588.

124. Barman M, Nilsson S, Torinsson Naluai Å, Sandin A, Wold AE, Sandberg AS. Single Nucleotide Polymorphisms in the FADS Gene Cluster but not the ELOVL2 Gene are Associated with Serum Polyunsaturated Fatty Acid Composition and Development of Allergy (in a Swedish Birth Cohort). Nutrients 2015;7(12):10100-15. doi: 10.3390/nu7125521.

125. Cheatham CL, Lupu DS, Niculescu MD. Genetic and epigenetic transgenerational implications related to omega-3 fatty acids. Part II: maternal FADS2 rs174575 genotype and DNA methylation predict toddler cognitive performance. Nutr Res 2015;35(11):948-55. doi: 10.1016/j.nutres.2015.09.005.

Chapter 3 : Expanded Methods

This chapter provides details on the data source and statistical methods used for all analyses included in this dissertation. Detail is provided on the study setting and participants, trial and follow-up study design, and data collection methods. Additionally, this chapter includes an extended discussion of statistical methods used that were beyond the scope of inclusion in a journal publication.

3.1 Study setting and participants

This dissertation uses data that have been collected through the Prenatal Omega-3 Fatty Acid Supplementation and Child Growth and Development (POSGRAD) study, a randomized, double-blind, placebo-controlled trial that was originally designed to assess the effect of prenatal DHA supplementation on offspring growth and development. This study is a collaboration between Emory University and the Instituto Nacional de Salud Pública (INSP), located in Cuernavaca, Mexico. Cuernavaca is in the state of Morelos, located in central Mexico approximately 50 miles south of Mexico City. Study participants were recruited at the Mexican Institute of Social Security (Instituto Mexicano del Seguro Social, IMSS) General Hospital I, a large hospital, and three small healthcare clinics within the IMSS healthcare system. IMSS provides employed persons access to medical care; the rural poor and self-employed do not have access, and the wealthy generally choose private medical care (1). At the time of study enrollment in 2005, IMSS provided healthcare to approximately 50% of the Mexican population. Women who receive medical care from IMSS are predominantly medium-to-low socioeconomic class and either they or their husbands, or both, are employed. Generally, the IMSS patient pays one-third of healthcare costs, and the employer and federal government cover the remaining cost.

3.2 Trial design

3.2.1 Recruitment and eligibility criteria

Between February 2005 to February 2007, pregnant women were approached by study personnel during routine prenatal visits and were screened for eligibility if they were interested in participating in the study. Women were considered for inclusion in the study if they were in gestation weeks 18-22, were 18-35 years old, planned to deliver at the IMSS hospital, predominantly breastfeed for at least 3 months, and continue living in the area for ≥ 2 years following delivery. Women were excluded if any of the following criteria were present: high-risk pregnancy (history and prevalence of pregnancy complications, including abruptio placentae, preeclampsia, pregnancy-induced hypertension, any serious bleeding episode in the current pregnancy, and/or physician referral); lipid metabolism or absorption disorders; regular intake of fish oil or DHA supplements; or chronic use of certain medications (e.g., medications for epilepsy).

3.2.2 Enrollment and randomization

Once eligibility was confirmed by the study physician, participants were contacted at home and were provided with a thorough explanation of the study protocol. Written informed consent was obtained from each participant, and dietary intake and socioeconomic status were assessed using previously tested questionnaires (2). Women who agreed to participate were scheduled for a hospital visit, during which they were assigned to receive either treatment or placebo and received the first week's supply of supplements. Block randomization was used to randomize study participants into groups of equal sample size using a block size of eight. All study participants and members of the study team were blinded to treatment allocations throughout the intervention period of the study. Data were unblinded for the analytical study team after the last baby was born

and reached 6 mo. of age. The participants and fieldworkers remain blinded to the intervention, as the study is still ongoing for follow-up.

The study was conducted according to the guidelines of the Declaration of Helsinki. The Emory University Institutional Review Board and the INSP Biosafety and Ethics Committee approved all procedures involving human subjects, including study protocols and informed consent documents. An external Data and Safety Monitoring Committee reviewed study data periodically. This is a registered clinical trial (registered at INSP in Mexico: #CI-011, and at clinicaltrials.gov: NCT00646360).

3.2.3 Intervention

Of 1,762 eligible women, a total of 1,094 pregnant women were randomized, and 1,040 began treatment; 54 women who had agreed to participate and were randomized withdrew from the study prior to beginning treatment. An additional 67 women withdrew from the study after beginning treatment due to various reasons including lack of family support, moving away from the area, and side effects of the supplement. The intervention consisted of either two capsules containing either 200 mg algal DHA per capsule (treatment) or a corn/soy oil blend (placebo) daily from mid-pregnancy through delivery. The fatty acid composition of the DHA supplement and placebo (produced by Martek BioSciences) are shown below (**Table 3-1**). Following the enrollment visit, the supplements were distributed by trained fieldworkers during weekly visits to the participant's home or workplace. Participants received 14 capsules at a time, and capsules remaining from the prior visit were counted. Compliance was expressed as a percentage of total number of capsules expected to be consumed. Data were analyzed according to the intent-to-treat principle, meaning that all randomized participants were included in analyses, regardless of the

treatment they actually received, protocol deviations, noncompliance, or withdrawal from the study (3).

Table 3-1. Fatty acid profile of DHA and placebo capsules administered twice daily to mothers enrolled in POSGRAD trial.

Fatty acid profile (weight percent)	DHA[1]	Placebo[2]
Caproic acid (6:0)	< 0.1	1.46
Caprylic acid (8:0)	1.48	0.57
Capric acid (10:0)	1.82	0.89
Undecylic acid (11:0)	0.28	< 0.1
Lauric acid (12:0)	5.07	0.18
Myristic acid (14:0)	15.20	< 0.1
Myristoleic acid (14:1)	0.13	< 0.1
Palmitic acid (16:0)	12.82	10.42
Palmitoleic acid (16:1)	1.68	< 0.1
Stearic acid (18:0)	0.62	3.07
Oleic acid (18:1n-9)	15.63	25.27
Vaccenic acid (18:1n-7)	< 0.1	1.15
Linoleic acid (18:2n-6)	0.83	50.59
Alpha-linolenic acid (18:3n-3)	< 0.1	3.44
Gamma-linolenic acid (18:3n-6)	None	0.19
Arachidic acid (20:0)	0.11	0.39
Heneicosylic acid (21:0)	None	0.44
Eicosenoic acid (20:1n-9)	< 0.1	0.39
Eicosapentaenoic acid (20:5n-3)	< 0.1	< 0.1
Behenic acid (22:0)	0.17	0.24
Docosapentaenoic acid (22:5n-3)	0.22	< 0.1
Docosahexaenoic acid (22:6n-3)	42.44	< 0.1
Lignoceric acid (24:0)	< 0.1	0.15
Others	1.50	1.16

[1]NEUROMINS® capsules (500 mg) contained DHASCO® with flavoring. Each capsule contained 196.2 mg docosahexaenoic acid; analysis provided by Martek Biosciences Corporation and rounded to the nearest 0.1%.
[2]Placebo capsules (500 mg) contained corn/soy oil with orange flavoring.

3.3 Follow-up study at age 11 years

The cross-sectional follow up study at age 11 years occurred from 2016-2018 as a collaboration between INSP, IMSS General Hospital, Salvador Zubirán National Institute of Medical Sciences and Nutrition, and Emory University. The main objective of this study was to examine the relationship between feeding and growth patterns during the first 7 years of life and

measures of adiposity, body composition, and cardiometabolic health of the children at age 11 years. All mothers of children in the birth cohort were invited to attend a clinic visit with their child and participate in the study. During the visit, mothers provided written informed consent and children provided written assent.

Of the 961 children contacted at age 11 years, 566 children completed a follow-up visit (58% of birth cohort). Reasons for loss to follow-up included: declined to participate (n = 47), missed visit (n = 12), or could not be located (n = 336). Cardiometabolic indicators were collected in a sub-sample of the first 500 individuals enrolled, of whom 485 provided a venous blood sample. Plasma and red blood cells were separated and stored in the laboratory of the Research Center for Infectious Diseases at INSP at -70°C until analysis at the Salvador Zubirán National Institute of Medical Sciences and Nutrition. All questionnaires were administered by trained personnel to the mother in the presence of the child, with additional information provided by the child. Data collection methods for adiposity, cardiometabolic indicators, diet, and covariates (e.g., household socioeconomic status, infant feeding practices, sedentary time) are described in detail in the methods sections of Chapters 4-6.

3.4 Statistical Methods

The main data analysis procedures performed for the three original research studies included in this dissertation are described in Chapters 4 to 6. The analytical strategy and statistical methods used in this dissertation, including evaluation of selection bias, handling of missing data, principal component analysis, processing of genetic data, haplotype estimation, and adjustment for energy intake are described in greater detail in this chapter.

3.4.1 Evaluation of selection bias

RCTs are considered the gold standard for assessing the effects of an intervention; their design also offers a valuable opportunity to assess effects of the intervention long after the intervention period has concluded. Block randomization helps ensure the balance of known and unknown confounders between the comparison groups in clinical trials. The randomized design of the POSGRAD study, along with extensive follow up of the children since birth, allows us the opportunity to study the influence of the intervention on long-term outcomes.

The conceptual framework of potential covariates and effect modifiers that may influence the relationship of prenatal DHA supplementation and offspring CMH is shown in **Figure 3-1**. Several factors early in the life course may influence offspring CMH measures, including maternal age, BMI, and breastmilk composition and offspring sex, birth weight, and infant feeding practices. Time-varying characteristics over the life course include household SES, physical activity, and dietary intake. Childhood and adolescence are a period of substantial change during which different lifestyle behaviors develop; these lifestyle factors may be a consequence of the exposure or modify the effect of the intervention but are not otherwise confounders.

We accounted for differences in fixed and time-varying characteristics over the follow-up period by confirming that these characteristics were balanced by treatment group in the analytic samples for each aim. For each analysis, we also determined the influence of selection bias by checking whether any imbalances in maternal characteristics at study enrollment and offspring characteristics at birth between children with data at 11 y and those lost to follow up altered effect estimates by including them in statistical models using a stepwise approach. These methods are outlined in greater detail for each research aim in Chapters 4-6.

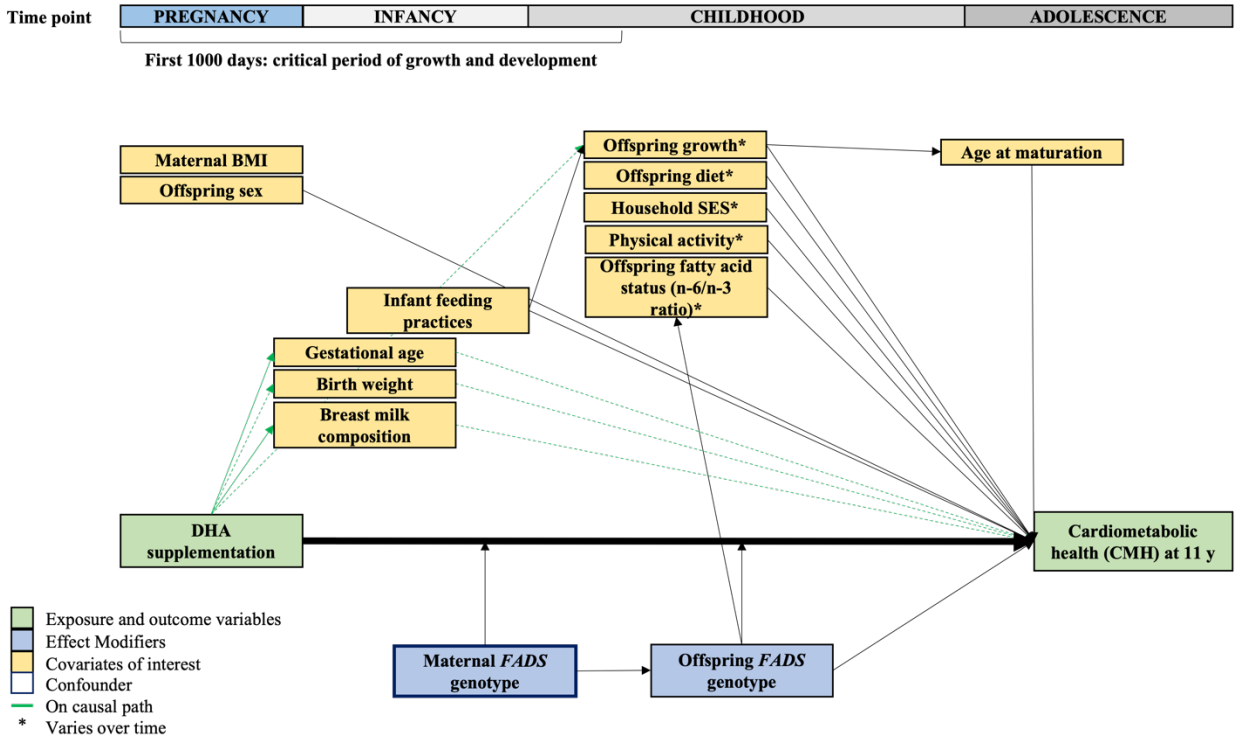

Figure 3-1. Conceptual framework of covariates and effect modifiers influencing the association of prenatal DHA supplementation with offspring CMH at 11 y.

Abbreviations: BMI, body mass index; DHA, docosahexaenoic acid; FADS, fatty acid desaturase; SES, socioeconomic status.

3.4.2 Imputation of missing data

Missing data are a common occurrence in research, especially within studies with long-term follow up. Missing data can be categorized as: 1) missing completely at random, meaning that there are no systematic differences between missing and observed data; 2) missing at random, meaning that there might be systematic differences between missing and observed data, but they can be explained by other observed data; and 3) missing not at random, meaning that missingness is dependent on unobserved values (4). Common approaches to address missing data include complete case analysis (removing any subjects with missing data) and single value imputation (e.g., mean-value imputation, last observation carried forward, and random imputation); however,

these approaches are suboptimal. Restricting samples to complete cases can lead to exclusions of a considerable proportion of the original sample, resulting in a substantial loss of precision and power, while single value imputation can lead to biased results and artificial precision.

Statistical methods to address missing data include maximum likelihood estimation, Bayesian estimation, and multiple imputation, all of which can be used assuming that data are missing completely at random or missing at random (5). Of these approaches, multiple imputation is relatively straightforward to apply using standard statistical software. Multiple imputation better handles missing data by allowing for uncertainty regarding missing values by creating several different plausible imputed data sets and combining the results (6). The process of multiple imputation involves two stages: 1) generating imputed values for missing data using information from other variables and repeating the procedure multiple times, resulting in many data sets with slightly varied imputed values; and 2) analyzing the many imputed data sets and combining the results (4). Statistical analyses are run separately on each imputed data set, and the parameter estimates from all the imputed data sets are combined into a single estimate. Multiple imputation by fully conditional specification specifies the multivariate imputation model on a variable-by-variable basis (5). This allows a flexible approach for addressing missing data, especially in complex data sets with both continuous and categorical variables. For the analyses included in this dissertation, complete case analysis was applied for cardiometabolic indicators (primary outcomes of interest) and multiple imputation techniques were applied for covariates (e.g., dietary intake, sedentary time, sexual maturation, and breastfeeding type and duration). The R ‘mice’ package was used to generate 20 imputed datasets using fully conditional specification with 50 iterations (7). For each analysis, we conducted general linear models for each of the 20 models and pooled the estimates.

3.4.3 Principal component analysis

Principal component analysis (PCA) is an unsupervised learning method used to simplify the complexity in high-dimensional data while preserving patterns in the data. This is accomplished by transforming the input variables into a new set of uncorrelated variables called principal components (PCs). Each PC (C_1, C_2, … C_p) is a linear combination of the input variables (X_1, X_2, …, X_p) that explains the largest possible variance in the data (**Figure 3-2**). The coefficients a_{11}, a_{12}, …, a_{1p} are the loadings of the first PC. These coefficients represent the correlation of the input variables with the PC and are chosen to: 1) maximize the variance of C_1; 2) ensure that the value of any two PCs are uncorrelated; and 3) ensure that, for any PC, the sum of squares of the coefficients equals 1 (i.e., $a_{i1}^2 + a_{i2}^2 + \ldots + a_{ip}^2 = 1$). The second PC ($C_2$) is a linear combination of input variables that is uncorrelated with the first PC but accounts for the next highest possible variance, and so on.

$$C_1 = a_{11}X_1 + a_{12}X_2 + \cdots a_{1p}X_p$$

$$C_2 = a_{21}X_1 + a_{22}X_2 + \cdots a_{2p}X_p$$

$$C_p = a_{p1}X_1 + a_{p2}X_2 + \cdots a_{pp}X_p$$

Figure 3-2. Equations to calculate principal components.
Principal components (C_1, C_2, … C_p) are linear combinations of the input variables X_1, X_2, …, X_p, weighted by coefficients a_{11}, a_{12}, …, a_{1p}.

Once the PCs and loadings are obtained, the PC scores for each observation (i.e., individual) can be calculated. The PC1 score for the first individual is calculated by plugging in the observed values for an individual into the first PC linear combination. The process is then repeated to obtain PC scores for the second PC for each individual, and so on.

For the analyses included in this dissertation, PCA was used to derive continuous MetS and CMH scores using the "FactoMineR" R package (8). We examined the distributions of input variables and log-transformed variables with skewed distributions. To ensure that each variable contributed equally to the analysis, we standardized the range of continuous variables by subtracting the mean and dividing by the standard deviation for each value of each variable. We used the inverse of cardiometabolic risk factors that are inversely associated with cardiometabolic health (e.g., HDL, adiponectin, fat free mass index) as input for PCA, so a higher loading score would have a similar interpretation to other measures in the model. Next, we examined the correlation matrix to identify correlations between variables in the dataset. We then computed the eigenvectors and eigenvalues of the covariance matrix. Eigenvectors are the direction of data that explain a maximal amount of variance, and eigenvalues are the coefficients associated with the eigenvectors, which measure the amount of variance explained by each PC. Eigenvectors are ranked by their eigenvalues in descending order. The top PCs were identified visually using scree plots and quantitatively using the Kaiser criterion (eigenvalue > 1) (**Figure 3-3**). Eigenvalues >1 indicate that PCs account for more variance than one of the input variables.

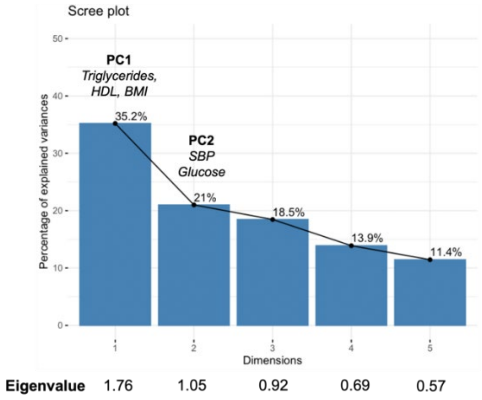

Figure 3-3. PCA scree plot.

Abbreviations: PC, principal component; HDL, High Density Lipoprotein; BMI, Body Mass Index.

Two PCs were selected based on the Kaiser criterion, and MetS scores were calculated for each individual as the sum of the top two PC scores, weighted by variance explained. The score can be interpreted as a z-score, with higher scores representing increased cardiometabolic risk.

MetS Score = [% variance explained by PC 1(PC1)] + [% variance explained by PC 2(PC2)]

We used PCA to calculate a separate MetS score for each specific aim given differences in sample size for each aim. Because there are established sex differences in cardiometabolic risk factors, we derived sex-specific MetS scores by calculating MetS scores in males and females separately. We then performed a sensitivity analysis to compare sex-specific and overall MetS scores and found that their correlation was 0.98, so we elected to use the overall MetS score in all subsequent analyses.

While PCA is a powerful dimensionality reduction tool, some limitations should also be considered. PCA may miss nonlinear data patterns, nonorthogonal patterns may not be well characterized, and the goal is to maximize variance and not necessarily identify clusters (9). Furthermore, loading coefficients of individual cardiometabolic factors from PCA are only applicable to the population from which they are derived. An alternate approach to PCA for creating continuous MetS score is using composite risk scores, which are calculated as the sum or mean of age-, sex-, and race/ethnicity-standardized z-scores for each risk component (10, 11). However, the utility of these scores may be limited by assumptions that each component contributes equally to cardiometabolic risk.

3.4.4 Processing of genetic data

An important consideration in genetic association analyses is proper use of quality control procedures to avoid bias and false signals. PLINK version 1.9 was used to perform quality control to filter out samples and SNPs with minor allele frequency (MAF<0.1), SNP call rate < 95%, and

sample call rate < 90%. For this analysis, genetic data were subset to chromosome 11 (where the *FADS1, FADS2,* and *FADS3* genes are located) and mapped to the human genome reference build GRCh37/hg19 for strand, id names, positions, alleles, and reference/alternative alleles assignment (12). The data were subset to available SNPs in the *FADS* gene region (rs174545 to rs1000778), resulting in 31 *FADS* variants for 626 children. Pairwise linkage disequilibrium (LD) among the 31 SNPs were calculated as both D' and r^2 using Haploview software, version 4.2 (13). D' represents the difference between observed and expected frequencies of a given haplotype, while r^2 represents the correlation between a pair of loci. If two loci are independent (i.e., in linkage equilibrium), the D' value will be 0. Ultimately, 5 SNPs that captured all 31 SNPs at $r^2 \geq 0.3$ (rs174578, rs2727271, rs174602, rs174605, rs174450) were selected via Tagger (**Figure 3-4**).

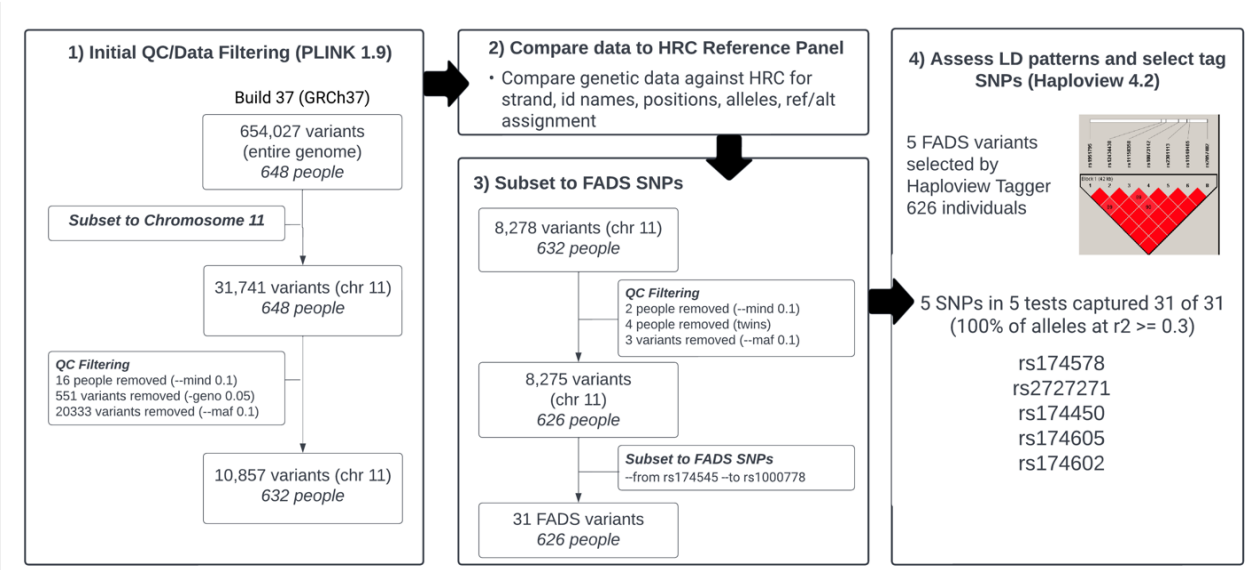

Figure 3-4. Summary of genetic data processing.

Abbreviations: QC, Quality Control; HRC, Haplotype Reference Consortium; FADS, Fatty Acid Desaturase; chr, chromosome; SNP, Single Nucleotide Polymorphism; LD, Linkage Disequilibrium

3.4.5 Haplotype estimation

When there is substantial linkage disequilibrium in a region, multiple SNPs within the region may be associated with the disease phenotype; however, testing each SNP would introduce multiple testing problems. Haplotypes are a combination of alleles on the same chromosome that are inherited together as a unit (14). Examining associations between haplotypes and disease phenotypes can provide additional power for mapping disease genes and understanding dependencies between genetic markers beyond any single SNP analysis. When subjects are unrelated, haplotypes can be directly observed when there is no more than one heterozygous site; however, if there are k heterozygous sites, the number of pairs of possible haplotypes is 2^{k-1} (15). Thus, multilocus haplotypes often cannot be directly determined from genotype data in humans, and statistical approaches are needed. We used the R "haplo.stats" package version 1.8.7 (https://cran.r-project.org/web/packages/haplo.stats/index.html) to estimate haplotype frequencies. This package uses an expectation maximization (EM) algorithm to calculate maximum likelihood estimates of probabilities of haplotype pairs for each participant (15). Haplotypes with frequency <5% were grouped as rare haplotypes. We used the "haplo.glm" function to assess the association of offspring *FADS* haplotype with offspring MetS score (adjusted for sex), using the most common haplotype as the referent. The function tests the difference between someone with 2 copies of the reference group haplotype and someone with 1 copy of the other haplotypes. For example, having a single TTCGT is associated with 0.60 increase in MetS Score compared to someone with 2 copies of the base haplotype ATCGG (reference).

3.4.6 Energy adjustment for dietary intake

One of the biggest challenges in nutritional epidemiology research is separating the effects of an individual dietary component from the overall diet. Typically, those who consume more of a nutrient will also consume more food in general and have a greater overall energy intake. Those

who consume more food tend to be different in other important ways, including body size and composition (16). Ultimately, total energy intake is the best proxy for determinants of dietary intake and body composition. Four models are typically used to adjust for energy intake in nutritional epidemiology research: (1) the 'standard model' of energy adjustment, which adjusts for total energy intake as an additional variable in the model; (2) the 'energy partition model', which adjusts for remaining energy intake (i.e., total caloric intake excluding the nutrient of interest); (3) the 'nutrient density model', which rescales the nutrient as a proportion of total energy; and (4) the residual model, which indirectly adjusts for total energy by calculating a residual (16). For the analyses included in this dissertation, we examined several approaches for energy adjustment, including the standard model, residual model, and adjusting for omega-3 and omega-6 intake alone. There were no significant differences in effect estimates using these approaches; therefore, we elected to use the standard model of energy adjustment for all analyses.

3.5 References

1. Parra-Cabrera S, Stein AD, Wang M, Martorell R, Rivera J, Ramakrishnan U. Dietary intakes of polyunsaturated fatty acids among pregnant Mexican women. Matern Child Nutr 2011;7(2):140-7. doi: 10.1111/j.1740-8709.2010.00254.x.
2. Parra MS, Schnaas L, Meydani M, Perroni E, Martínez S, Romieu I. Erythrocyte cell membrane phospholipid levels compared against reported dietary intakes of polyunsaturated fatty acids in pregnant Mexican women. Public Health Nutr 2002;5(6a):931-7. doi: 10.1079/phn2002381.
3. Gupta SK. Intention-to-treat concept: A review. Perspect Clin Res 2011;2(3):109-12. doi: 10.4103/2229-3485.83221.
4. Li P, Stuart EA, Allison DB. Multiple Imputation: A Flexible Tool for Handling Missing Data. Jama 2015;314(18):1966-7. doi: 10.1001/jama.2015.15281.
5. Liu Y, De A. Multiple Imputation by Fully Conditional Specification for Dealing with Missing Data in a Large Epidemiologic Study. Int J Stat Med Res 2015;4(3):287-95. doi: 10.6000/1929-6029.2015.04.03.7.
6. Sterne JAC, White IR, Carlin JB, Spratt M, Royston P, Kenward MG, et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. BMJ 2009;338:b2393. doi: 10.1136/bmj.b2393.
7. van Buuren S, Groothuis-Oudshoorn K. mice: Multivariate Imputation by Chained Equations in R. Journal of Statistical Software 2011;45(3):1 - 67. doi: 10.18637/jss.v045.i03.
8. Lê S, Josse J, Husson F. FactoMineR: An R Package for Multivariate Analysis. Journal of Statistical Software 2008;25(1):1 - 18. doi: 10.18637/jss.v025.i01.

9. Lever J, Krzywinski M, Altman N. Principal component analysis. Nature Methods 2017;14(7):641-2. doi: 10.1038/nmeth.4346.

10. Bugge A, El-Naaman B, McMurray RG, Froberg K, Nielsen CH, Müller K, et al. Inflammatory markers and clustered cardiovascular disease risk factors in Danish adolescents. Horm Res Paediatr 2012;78(5-6):288-96. doi: 10.1159/000345546.

11. Okosun IS, Lyn R, Davis-Smith M, Eriksen M, Seale P. Validity of a continuous metabolic risk score as an index for modeling metabolic syndrome in adolescents. Ann Epidemiol 2010;20(11):843-51. doi: 10.1016/j.annepidem.2010.08.001.

12. McCarthy S, Das S, Kretzschmar W, Delaneau O, Wood AR, Teumer A, et al. A reference panel of 64,976 haplotypes for genotype imputation. Nat Genet 2016;48(10):1279-83. doi: 10.1038/ng.3643.

13. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21(2):263-5. doi: 10.1093/bioinformatics/bth457.

14. Schaid DJ. Genetic epidemiology and haplotypes. Genet Epidemiol 2004;27(4):317-20. doi: 10.1002/gepi.20046.

15. Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA. Score tests for association between traits and haplotypes when linkage phase is ambiguous. American journal of human genetics 2002;70(2):425-34. doi: 10.1086/338688.

16. Tomova GD, Arnold KF, Gilthorpe MS, Tennant PWG. Adjustment for energy intake in nutritional research: a causal inference perspective. The American Journal of Clinical Nutrition 2022;115(1):189-98. doi: https://doi.org/10.1093/ajcn/nqab266.

Chapter 4 : Clustering of Cardiometabolic Risk Factors in Mexican Pre-Adolescents

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Format adapted for this dissertation

4.1 Abstract

Objective

To examine clustering of cardiometabolic markers in Mexican children at age 11 years and compare a metabolic syndrome (MetS) score to an exploratory cardiometabolic health (CMH) score.

Methods

We used data from children enrolled in the POSGRAD birth cohort with cardiometabolic data available (n = 413). We used principal component analysis (PCA) to derive a Metabolic Syndrome (MetS) score and an exploratory cardiometabolic health (CMH) score, which additionally included adipokines, lipids, inflammatory markers, and adiposity. We assessed reliability of individual cardiometabolic risk as defined by MetS and CMH by calculating % agreement and Cohen's kappa statistic.

Results

At least one cardiometabolic risk factor was present in 42 % of study participants; the most common risk factors were low High-Density Lipoprotein (HDL) cholesterol (31.9 %) and elevated triglycerides (18.2 %). Measures of adiposity and lipids explained the most variation in cardiometabolic measures for both MetS and CMH scores. Two-thirds of individuals were categorized in the same risk category by both MetS and CMH scores ($\kappa = 0.42$).

Conclusions

MetS and CMH scores capture a similar amount of variation. Additional follow-up studies comparing predictive abilities of MetS and CMH scores may enable improved identification of children at risk for cardiometabolic disease.

4.2 Introduction

Cardiometabolic diseases, which include cardiovascular disease, stroke, and type II diabetes mellitus, are among the leading causes of death globally (1). Risk factors including hypertension, insulin resistance, obesity, and abnormal lipid levels are emerging earlier in the life course and track from childhood to adulthood (2, 3). Childhood obesity is a consistent predictor of adulthood obesity and cardiometabolic disease (4, 5). However, not all individuals with the same degree of obesity exhibit equivalent risk of disease.

Early and comprehensive characterization of cardiometabolic risk offers an important opportunity to identify high-risk individuals, tailor clinical management strategies, and prevent disease development. Metabolic syndrome (MetS), a cluster of conditions that predicts future risk of cardiometabolic disease, is typically diagnosed in adult populations by the presence of at least three or more of the following risk factors: central adiposity, hyperglycemia, hypertriglyceridemia, hypertension, and low HDL, although definitions vary slightly (6). In pediatric populations, different MetS definitions have been proposed; however, the clinical utility of diagnostic criteria remains controversial, as using dichotomous thresholds to diagnose MetS likely only captures severe outliers and may inadequately characterize early risk trajectories by ignoring the continuous nature of risk (7, 8). The American Academy of Pediatrics now recommends that pediatricians identify children with clustering of multiple risk components rather than focusing on specific cut-off levels for individual risk factors or a particular MetS definition (9). This has led to use of composite risk scores, which are typically calculated as the sum or mean of age-, sex-, and race/ethnicity-standardized z-scores for each risk component (10, 11). However, the utility of these scores is likely limited by assumptions that each component contributes equally to cardiometabolic risk as well as heterogeneity across studies with respect to the included components.

More recently, studies in pediatric populations have applied principal component analysis (PCA), a dimensionality reduction technique used to minimize the number of variables that explain correlations across observed measures, to derive a continuous MetS score (12-14). PCA-derived MetS scores during childhood and early adolescence have been associated with increased risk of cardiometabolic disease in adulthood; one study recently reported that a 1 SD increase in a continuous MetS score during adolescence was associated with 30% increased risk of type II diabetes and 20% increased risk of high carotid intima-media thickness in adulthood (15).

Current MetS scores do not account for all factors that may play an important role in the early pathogenesis of type II diabetes mellitus and CVD, such as insulin resistance and inflammation. Excess visceral adipose tissue secretes large amounts of adipokines, including cytokines and hormones, promoting a state of systemic low-grade inflammation, which contributes to oxidative stress and subsequent endothelial and pancreatic beta-cell damage.(10, 16) High levels of high-sensitivity c-reactive protein (hs-CRP) and low levels of adiponectin, in particular, have previously been associated with systemic inflammation and insulin resistance, respectively; evidence suggests that leptin, adiponectin, and hs-CRP may be used as predictors of cardiometabolic risk in pediatric populations (17). Utilizing additional biomarkers to characterize overall cardiometabolic risk may enable earlier and improved detection of cardiometabolic disease development among pediatric populations.

Finally, it is important to consider ethnic and genetic variation in the etiology of cardiometabolic disease. Some studies suggest that ethnic and genetic differences emerge early in the life course, with populations of Hispanic origin at increased risk (18, 19). In Mexico, 33% of school-age children are overweight or obese, with the highest prevalence among children aged 10-

11 years (20). Therefore, detailed characterization of cardiometabolic risk in Mexican populations at this age may better inform overall disease prevention strategies.

The objectives of this study are to describe the clustering of cardiometabolic markers in a Mexican population at age 11 years and compare use of a MetS score to an exploratory CMH score, which additionally includes markers of insulin resistance and inflammation, to assess cardiometabolic profiles.

4.3 Methods

4.3.1 Study design and participants

We conducted a secondary analysis of data from children born to women who participated in the Prenatal Omega-3 fatty acid Supplementation and Child Growth and Development (POSGRAD) trial in Cuernavaca, Mexico. A detailed description of the trial design and protocol has been published previously (21). Briefly, from 2005-2007, women were randomized at 18-22 weeks gestation to receive a daily dose of 400 mg of algal DHA or placebo during pregnancy; children have been followed prospectively since birth. Of 961 children contacted at age 11 years, 566 (58%) completed a follow-up visit. Due to budgetary limitations, CMH indicators were collected in a sub-sample of the first 500 individuals enrolled, of whom 485 provided a venous blood sample. The analytic sample includes children with complete data on cardiometabolic factors at age 11 (n = 413) (**Supplementary Figure 4-1**).

The study was conducted according to the guidelines of the Declaration of Helsinki. The Emory University Institutional Review Board and the Mexican National Public Health Institute (INSP) ethics committee approved all procedures involving human subjects.

4.3.2 Anthropometry and Body Composition

Body weight (kg) and height (cm) were collected from offspring in duplicate by trained personnel using standardized procedures.(22) Children were weighed wearing light clothing with a portable electronic pediatric scale (Tanita model 1582) with a precision of 100 g, which was calibrated daily with a known reference weight. Height was measured using a stadiometer with a precision of 0.1 cm. Abdominal circumference was measured with a fiberglass tape with a precision of 0.1 cm. Average values of all measurements were calculated. The nutritional status of children was classified according to age- and sex-specific cut-offs from the International Obesity Task Force (IOTF) (23) and World Health Organization (WHO) reference standards; BMI-for-age z-scores were calculated according to the 2007 WHO Growth Reference Standards using the 'zscorer' R package (24). Blood pressure (mmHg) was measured using a digital device (OMRON model HEM-711ACINT) when the child was at rest (>5 minutes after the child arrived at the study visit). In each arm, 4 measurements were made with 2-minute intervals; the average of the last three readings was taken.

Body volume was measured by air displacement plethysmography (ADP) using BODPOD ® instrumentation (Bod Pod Express, COSMED USA Inc., Concord, CA, USA), which has been shown to be highly correlated with measures derived from 4-component (4-C) models of body composition in Mexican children and adolescents (25). Measurements were taken while the children remained sitting and breathing normally; fat mass (kg) was calculated using the Siri equation, and fat-free mass (kg) was calculated by subtracting fat-mass from body weight (26-28). Fat mass index and fat-free mass index were calculated (kg/m^2). The BODPOD was calibrated before each measurement according to the manufacturer's recommendations.

4.3.3 Determination of Cardiometabolic Markers

Serum was obtained after a 12-hour fasting period, divided into aliquots, frozen in liquid nitrogen and stored at −80°C until further analysis at the National Institute of Medical Sciences and Nutrition Salvador Zubirán in Mexico City, Mexico.

Cholesterol, triglyceride, and glucose concentrations (mg/dL) were assessed using the Beckman Coulter SYNCHRON CX 5 Delta automated kit. Insulin concentrations (***μ***M/mL) were assessed using the ELISA kit (EZHI-14K/EZHI-14BK). Homeostasis model assessment (HOMA-IR) was calculated as an estimate of insulin resistance: HOMA-IR = insulin (μU/ml) x glucose (mg/dL)/405 (29). Data collection methods to determine high sensitivity C reactive protein (hs-CRP), IL-6, leptin, and adiponectin concentrations have been previously published (30). The adiponectin/leptin ratio was calculated in terms of ng/mL. Cardiometabolic risk factor thresholds were defined as follows: triglycerides ≥ 150 mg/dL, HDL < 40 mg/dL, Systolic Blood Pressure (SBP) ≥ 90th percentile for sex and height, and fasting glucose ≥ 100 mg/dL (31, 32). Metabolically Healthy Normal Weight (MHNW) was defined as BMI-for-age < 1 SD above WHO Growth Reference Median with 0 cardiometabolic risk factors, Metabolically Unhealthy Normal Weight (MUNW) was defined as above but with 1 or more cardiometabolic risk factors, Metabolically Healthy Overweight/obesity (MHO) was defined as BMI-for-age > 1 SD above the WHO Growth Reference median (85^{th} percentile) with 0 cardiometabolic risk factors, and Metabolically Unhealthy Overweight/obesity (MUO) was defined as above with 1 or more cardiometabolic risk factors (33).

4.3.4 Covariates

Data on infant feeding practices were obtained by maternal interview at 3 mo of age and used to categorize breastfeeding (BF) status at 3 mo of age as exclusive BF (EBF), predominantly BF (PreBF), partial BF (PaBF), and non-BF (NBF) according to the WHO classification (34, 35).

Dietary intake at 11 years was assessed via multiple-pass 24-hour dietary recall adapted for Mexican populations (36). Trained personnel administered the diet recall tool to the child's primary caregiver (in presence of the child). Nutrient and energy estimations were obtained using the 2012 and 2016 Mexican Food Databases, which are maintained by INSP (37). Sedentary time was estimated using data that were collected through the administration of a self-reported physical activity and inactivity questionnaire that was developed for and validated among children ages 10-14 in Mexico (38). Sexual maturation was assessed by proxy using testosterone levels for males and attainment of menarche for females that was ascertained by a self-reported questionnaire.

4.3.5 Statistical Analysis

Normality of data was assessed using histograms and quantile-quantile plots. Means and standard deviations or medians and interquartile ranges (IQR) of variables were calculated and presented by sex and body size phenotype, as appropriate. Differences between groups were assessed using t-tests, ANOVA, and Wilcoxon rank-sum tests as appropriate; pairwise comparisons were assessed using Tukey-Kramer tests.

4.3.6 MetS Score

We used PCA to calculate a MetS score using systolic blood pressure, HDL, triglycerides, BMI-z, and glucose. We used BMI-z instead of waist circumference because it has more reliability and has been used in other similar studies, which allows for better comparison across studies (12, 15). We log-transformed triglycerides and glucose and used the inverse of HDL when standardizing, so a higher factor loading score would have a similar interpretation to other measures in the model. The top principal components (PCs) were identified visually using scree plots and quantitatively using the Kaiser criterion (eigenvalues > 1) (39). MetS was calculated as the sum of the top two PCs, weighted by variance explained. The score can be interpreted as a z-

score, with higher scores representing increased cardiometabolic risk. PCA was performed using the 'FactoMineR' R package (40).

4.3.7 Exploratory CMH Score

We also used PCA to create an exploratory cardiometabolic health (CMH) score using 13 measures of cardiometabolic health: SBP, DBP, adiponectin, leptin, insulin, glucose, IL-6, CRP, Low Density Lipoprotein (LDL) cholesterol, HDL cholesterol, triglycerides, fat mass index, and fat-free mass index. The inverses of HDL, adiponectin, and fat-free mass index were used to ensure that loadings would have similar interpretations to other variables. Analyses were performed using the 'FactoMineR' and 'missMDA' R packages (40, 41). We estimated the number of dimensions by K-fold cross validation and imputed missing values in the dataset with a PCA model bootstrapped 1000 times. Only factors with <5% missing data were used as input for MIPCA (missingness of cardiometabolic factors ranged from 0-4.8% (IL-6)). We performed a sensitivity analysis of imputed values, in which we assessed the difference in CMH scores as computed from the complete dataset and from the imputed dataset. Nearly 95% of individuals were classified similarly using the imputed and complete case scores; thus, we retained the scores derived from the imputed values to increase available sample size.

4.3.8 Reliability of MetS and CMH Scores

We used quintiles of MetS and CMH score distributions to categorize individuals in Q1 as 'low' risk, Q2-Q4 as 'moderate' risk, and Q5 as 'high' risk and compared risk categorizations of individuals using both scores by calculating % agreement and Cohen's kappa statistic (42). We used ANOVA to compare adjusted mean MetS and CMH scores by body size phenotype using the R 'emmeans' package and adjusted estimates for maternal (prenatal treatment group, SES, age, and BMI at trial enrollment) and offspring (sex and age at examination) factors.

4.3.9 Association of Continuous MetS Score with CMH Markers

We used multivariable generalized linear models to assess associations between the continuous MetS score and markers of insulin resistance, inflammation, and energy homeostasis. Given their skewed distributions, variables were log-transformed prior to analysis. Models were adjusted for prenatal treatment group, maternal BMI at 18-22 weeks gestation, household socioeconomic status close to the time of birth, child sex, birth weight, breastfeeding type and duration, age at examination, diet (energy intake and total omega-3 fatty acid intake), and sedentary time at age 11 years. Models were stratified by sex and additionally adjusted for sexual maturation (defined in girls as attainment of menarche and in boys by testosterone concentrations). We tested for interaction by including sex as an interaction term in the models. We used multiple imputation to account for missing covariates (diet, sedentary time, sexual maturation, and breastfeeding type and duration). The R 'mice' package was used to generate 20 imputed datasets using fully conditional specification with 50 iterations (43). We conducted GLMs for each of the 20 models and pooled the estimates. All statistical analyses were performed using R version 4.0.4 (R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was held at $p < 0.05$.

4.4 Results

Sample characteristics for the study population are shown in **Table 4-1**. Briefly, the mean age at follow up was 11.1 years (SD=0.18), and 47% of the sample were female. The sample was balanced by prenatal treatment group for the original trial design. Nearly 9% of children were born preterm and 5% were born with low birth weight. At 3 months of age, 82% of children were consuming breastmilk as part of their diets, but only 14% were exclusively breastfed. Only 34 females (17.6%) had attained menarche by the time of examination at 11 years. Compared to

children missing cardiometabolic data, children included in the study tended to have mothers who were older, less likely to be primiparous, breastfeed for longer durations, and have higher household SES at study enrollment (**Supplementary Table 4-1**).

4.4.1 Clustering Patterns of Cardiometabolic Factors

When using International Diabetes Federation (IDF) criteria, 31.9% of participants had low HDL, 18.2% had elevated triglycerides, 3.6% had elevated glucose, and no children had high blood pressure. Prevalence and clustering of cardiometabolic factors, stratified by weight category, are shown in **Table 4-2**. Nearly 45% and 35% of children had overweight or obesity, according to WHO criteria and IOTF criteria, respectively. Over 40% of the population had at least one cardiometabolic risk factor besides overweight/obesity. Among children with normal weight, 30% had at least one cardiometabolic risk factor. The most common cardiometabolic risk factors were low HDL, followed by high triglycerides and high fasting glucose. High triglycerides and low HDL tended to cluster together most frequently among individuals with ≥2 cardiometabolic risk factors. Nearly all cardiometabolic factors, aside from adiponectin and blood pressure, differed by body size phenotype (**Table 4-3**). Relative to individuals with MHNW, MUNW, and MHO, individuals with MUO had higher fasting glucose, insulin, HOMA-IR, triglycerides, fat mass index, fat-free mass index, inflammatory markers (IL-6, hs-CRP), and leptin concentrations, along with lower HDL (all $p < 0.01$). Cardiometabolic factors stratified by sex are shown in **Supplementary Table 4-2**. Most cardiometabolic factors did not differ across male and female children, although fasting glucose was lower, and triglycerides and sum of skinfolds were significantly higher among females compared to males.

4.4.2 Derivation of MetS Score

Contributions of MetS components to the PCA-derived MetS score are shown in **Figure 4-1a**. The top two principal components (PCs) collectively explained 56.2% of the variance in the measured data. Triglycerides, BMI-z, and HDL contributed to the first PC (35.2% variance explained), while SBP and fasting glucose contributed to the second PC (21.0% variance explained). Given the observed sex differences in triglycerides and fasting glucose, we performed a sensitivity analysis comparing sex-specific and overall MetS scores; their correlation was 0.98 (**Supplementary Figure 4-2**); so we use the overall score in subsequent analyses. To improve comparability between studies, we also assessed correlation between MetS scores derived using PCA and a sum of z-scores (r = 0.99).

4.4.3 Associations of MetS Score with Markers of Inflammation and Insulin Resistance

The associations between the continuous MetS score and additional cardiometabolic markers are shown in **Table 4-4**. HOMA-IR (β (95% CI) = 0.79 (0.59 - 0.99)) and hs-CRP (0.75 (0.52-0.98)) were significantly positively associated with continuous MetS scores among both sexes ($p<0.05$), although interaction by sex was only significant for hs-CRP. Positive associations with leptin and inverse associations with the adiponectin/leptin ratio were observed among males only, while no association was observed for adiponectin or Il-6 for either sex.

4.4.4 Derivation of Exploratory CMH Score

Correlations among all variables included in the exploratory CMH score are shown in **Supplementary Figure 4-3**. After applying the Kaiser criterion, five PCs were retained and collectively explained 62.1% of variance in the measured data. Contributions of each cardiometabolic factor to the PCA-derived CMH score are shown in **Figure 4-1b** and are summarized as follows: fat mass index, hs-CRP, triglycerides, and HDL contributed to the first PC (18.6%), SBP and DBP contributed to the second PC (13.7%), insulin, leptin, and IL-6

contributed to the third PC (12.6%), LDL and fat-free mass index contributed to the fourth PC (9.4%), and adiponectin and glucose contributed to the fifth PC (7.8%). The correlation between overall and sex-specific CMH scores was 0.83 (**Supplementary Figure 4-4**).

4.4.5 Comparison of MetS and CMH Scores

There was a positive correlation between the MetS and CMH scores ($r = 0.75$, $p < 0.0001$). Approximately 43% of individuals were categorized in the same quintile by both scores ($\kappa = 0.29$), while more than 67% of individuals were categorized in the same risk category (Q1: low, Q2 – Q4: medium, Q5: high) by both MetS and CMH scores ($\kappa = 0.42$). Mean MetS and CMH scores, stratified by body size phenotype, are shown in **Figure 4-1c**. Children with MUNW, MHO, and MUO had higher MetS scores ($\beta = 0.29$, 95% CI: 0.27-0.33) and CMH scores ($\beta = 0.17$, 95% CI: 0.14-0.20) relative to the MHNW group, although differences in the CMH score were less pronounced. MetS and CMH scores were significantly higher in the MUO group relative to the MHO group, but only MetS scores were significantly higher in the MUNW group relative to the MHNW group. While there were significant differences in CMH scores between MUNW and MHO groups ($\Delta = 0.25$ (95% CI: 0.09, 0.39)), no significant differences were observed in MetS scores between MUNW and MHO groups ($\Delta = 0.07$ (-0.08, 0.22))

4.5 Discussion

In this study, we describe the clustering of cardiometabolic health markers among pre-adolescent Mexican children and examine use of a MetS score and exploratory CMH score to assess cardiometabolic profiles. We found that, across both the MetS and CMH scores, adiposity and lipids explained most of the variance in cardiometabolic measures among the study population. The MetS score captured similar variation in cardiometabolic measures as the CMH score, with nearly 70% of the study population categorized in the same risk category using both scores.

Children with overweight/obesity had worse MetS and CMH scores relative to children with normal weight. Furthermore, a linear trend was observed by body size phenotype; children with MUO, MHO, and MUNW had worse MetS and CMH scores relative to children with MHNW. Among children with normal weight, having at least one cardiometabolic factor placed individuals at nearly equivalent MetS risk as individuals with MHO. These findings suggest that even minor perturbations in cardiometabolic profiles (i.e., developing overweight/obesity or one cardiometabolic risk factor) place individuals on a poor trajectory that may ultimately contribute to higher risk of future disease.

Derivation of the CMH score via PCA showed that fat mass index, hs-CRP, triglycerides, and HDL had the highest contributions to variance observed across cardiometabolic measures. Although no individuals in this sample had high blood pressure, measures of blood pressure (MetS score: SBP only, CMH score: SBP and DBP) contributed substantially (> 75%) to the second PC for both MetS and CMH scores. Insulin, leptin, and IL-6 tended to cluster together in the third PC, and fat-free mass index, adiponectin, LDL, and glucose tended to cluster together across the fourth and fifth PCs. Of interest, across both MetS and CMH scores, fasting glucose concentrations did not account for substantial variance in cardiometabolic measures; the study population was likely managing glucose well at the time of examination, as less than 5% of the study population had high glucose levels.

While PCA has predominantly been used to derive MetS scores in most previous studies (12), one study recently used PCA to examine the variance components of 13 cardiometabolic factors among pre-adolescent children in New Zealand (14). Findings showed that 4 factors (blood pressure, adiposity, lipids, and vascular, respectively) explained 60% of the variance in the measured variables. Although clustering of factors was similar between our study and this study,

blood pressure explained a majority of variance as compared to adiposity and lipids in our study. Like our study, children with overweight or obesity had worse cardiometabolic risk scores relative to children with normal weight. A key difference is the populations studied; most study participants identified as New Zealand European whereas our study was conducted among children of Hispanic origin. Multiple studies conducted in the United States indicate that ethnic differences in the prevalence of cardiometabolic risk factors exist early in life (18, 19). Previous studies have shown that low HDL and high triglycerides are the most commonly observed cardiometabolic risk factors present among Mexican populations (44-46); our results align with these findings, as HDL and triglycerides loaded onto the first PC for both MetS and CMH scores. Further examination of ethnic and genetic differences may have implications for targeted prevention and treatment of cardiometabolic disease.

Another recent study in Denmark evaluated the association between inflammatory markers and a clustered z-score of CVD risk, which included TC/HDL-c, TGs, HOMA-IR, SBP, sum of 4 skinfold thickness (S4SF), and inverse of $VO_{2peak;}$ they reported that CVD risk was associated with alterations in adiponectin, TNFα, hs-CRP, and IL-6 (10). Although components included in the composite scores varied, our multivariable regression models showed that hs-CRP, a marker of systemic inflammation, and HOMA-IR, a marker of insulin resistance, were significantly positively associated with MetS scores. Of interest, positive associations with leptin and inverse associations with the adiponectin/leptin ratio were observed among males only, which may be attributable to sex differences in pubertal development trajectories. Leptin, a hormone secreted from white adipose tissue, plays a key role in regulation of feeding behavior, metabolic rate, and energy balance and is positively correlated with obesity, but some studies suggest that leptin may also signal the initiation of puberty (47, 48). Among females, leptin concentrations typically

increase throughout puberty, while among males, levels increase prior to the onset of puberty and subsequently decrease as puberty progresses (47). Alterations in circulating leptin may partially explain the associations between obesity, onset of puberty, and future cardiometabolic risk, but additional research is warranted (49).

Several limitations should be considered when interpreting our findings. We used secondary data from a previously conducted RCT. Thus, sample sizes were limited to individuals who were not lost to follow-up over the 11-year period; selection bias may influence our findings. Compared with individuals lost to follow-up, participants in our study sample tended to have mothers who were older, less likely to be primiparous, breastfeed for longer durations, and have higher household SES at study enrollment. To account for this, we adjusted for these factors in all multivariable regression models. Additionally, while PCA is a powerful dimensionality-reduction tool, loading coefficients of individual cardiometabolic factors from PCA are only applicable to the population from which they are derived. Furthermore, results cannot be easily compared across studies because a variety of input measures (e.g., sum of skinfolds, TC: HDL, mean blood pressure) have been used (50). Finally, although we adjusted models for total sedentary time (including screen time), we did not have access to a gold-standard measure of physical activity. Overall, generalizability of results may be limited; this work should be replicated with consistent sets of cardiometabolic measures in large, nationally representative datasets in Mexico, as well as populations with diverse racial and ethnic backgrounds.

It is important to note that this study utilizes a cross-sectional sample of children at age 11 years. This age represents a dynamic time of growth development, either immediately prior to or during the onset of puberty, which is characterized by complex physical, hormonal, and emotional changes including weight gain and increases in insulin resistance (33). In our study, pubertal status

was assessed by documenting attainment of menarche and testosterone concentrations among males in a subset of the population, as opposed to more robust methods such as assessing Tanner stages (51). Although the children are well-characterized early in the life course, due to the cross-sectional study design, it is not possible to assess the risk of adult cardiometabolic disease or determine the sequence of risk factor development over time. While multiple studies have shown that PCA-derived MetS scores are associated with increased risks of CVD and type II diabetes in adulthood, longitudinal studies with a comprehensive set of cardiometabolic factors measured consistently across the life course are needed to establish the temporality of risk factor development and disease progression.

Several strengths of this study should be acknowledged: most notably, the availability of multiple measures of cardiometabolic health measured simultaneously, including traditional MetS risk factors, adiposity, adipokines, and measures of inflammation and insulin resistance. We used data from a large, well-characterized study with information on both mother and child. Children have been followed since birth with a rich set of data characterizing their health and lifestyle across the life course. Data collection and laboratory assays were standardized, validated, and conducted by trained personnel within a clinical setting. Additionally, the age period of 11 years old offers a stable time for lipid assessment in children prior to the onset of puberty for most of the study population.

As more widely available resources enable less expensive testing of blood samples, it is becoming increasingly feasible to collect more comprehensive data to better characterize CMH risk and prevent future disease. Therefore, it is important to understand if, given additional cost considerations, there is added value in collecting more biomarkers to better characterize cardiometabolic health during this life stage, and additionally, whether there is added value in

collecting these data in low-resource environments. Our findings suggest that MetS scores capture a similar amount of variation compared to CMH scores that incorporate additional biological measures. Here, the more complex CMH score captured little additional variance relative to the MetS score (62% variance explained by the top 5 PCs vs. 56% variance explained by the top 2 PCs, respectively). Across both scores, adiposity seems to account for most of the variation in cardiometabolic health at this age. Particularly within resource-limited settings, it may be sufficient to identify individuals with at least one cardiometabolic risk factor (obesity or other MetS components) for more intensive disease prevention strategies.

In summary, our findings provide new insights into the clustering patterns of cardiometabolic factors during a period of growth and development that currently lacks extensive characterization but offers an important opportunity to intervene prior to the development of cardiometabolic disease. A remaining question is whether CMH scores offer useful predictions of adult cardiometabolic risk. Additional follow-up studies comparing predictive abilities of MetS and CMH scores in diverse populations may enable early identification of children and adolescents at risk for cardiometabolic disease.

4.6 References

1. Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, et al. Global, Regional, and National Burden of Cardiovascular Diseases for 10 Causes, 1990 to 2015. J Am Coll Cardiol 2017;70(1):1-25. doi: 10.1016/j.jacc.2017.04.052.
2. Weiss R, Bremer AA, Lustig RH. What is metabolic syndrome, and why are children getting it? Ann N Y Acad Sci 2013;1281(1):123-40. doi: 10.1111/nyas.12030.
3. Nicklas TA, von Duvillard SP, Berenson GS. Tracking of serum lipids and lipoproteins from childhood to dyslipidemia in adults: the Bogalusa Heart Study. Int J Sports Med 2002;23 Suppl 1:S39-43. doi: 10.1055/s-2002-28460.
4. Juonala M, Magnussen CG, Berenson GS, Venn A, Burns TL, Sabin MA, et al. Childhood Adiposity, Adult Adiposity, and Cardiovascular Risk Factors. New England Journal of Medicine 2011;365(20):1876-85. doi: 10.1056/NEJMoa1010112.
5. Franks PW, Hanson RL, Knowler WC, Sievers ML, Bennett PH, Looker HC. Childhood obesity, other cardiovascular risk factors, and premature death. N Engl J Med 2010;362(6):485-93. doi: 10.1056/NEJMoa0904130.
6. Ford ES. Prevalence of the Metabolic Syndrome Defined by the International Diabetes Federation Among Adults in the U.S. Diabetes Care 2005;28(11):2745-9. doi: 10.2337/diacare.28.11.2745.
7. Beckstead JW, Beckie TM. How much information can metabolic syndrome provide? An application of information theory. Med Decis Making 2011;31(1):79-92. doi: 10.1177/0272989x10373401.

8. Tropeano A, Corica D, Li Pomi A, Pepe G, Morabito LA, Curatola SL, et al. The metabolic syndrome in pediatrics: do we have a reliable definition? A systematic review. Eur J Endocrinol 2021;185(2):265-78. doi: 10.1530/eje-21-0238.

9. Magge SN, Goodman E, Armstrong SC, Committee On N, Section On E, Section On O, et al. The Metabolic Syndrome in Children and Adolescents: Shifting the Focus to Cardiometabolic Risk Factor Clustering. Pediatrics 2017;140(2):e20171603. doi: 10.1542/peds.2017-1603.

10. Bugge A, El-Naaman B, McMurray RG, Froberg K, Nielsen CH, Müller K, et al. Inflammatory markers and clustered cardiovascular disease risk factors in Danish adolescents. Horm Res Paediatr 2012;78(5-6):288-96. doi: 10.1159/000345546.

11. Okosun IS, Lyn R, Davis-Smith M, Eriksen M, Seale P. Validity of a continuous metabolic risk score as an index for modeling metabolic syndrome in adolescents. Ann Epidemiol 2010;20(11):843-51. doi: 10.1016/j.annepidem.2010.08.001.

12. Lycett K, Juonala M, Magnussen CG, Norrish D, Mensah FK, Liu R, et al. Body Mass Index From Early to Late Childhood and Cardiometabolic Measurements at 11 to 12 Years. Pediatrics 2020;146(2):e20193666. doi: 10.1542/peds.2019-3666.

13. Eisenmann JC. On the use of a continuous metabolic syndrome score in pediatric research. Cardiovascular Diabetology 2008;7(1):17. doi: 10.1186/1475-2840-7-17.

14. Stoner L, Weatherall M, Skidmore P, Castro N, Lark S, Faulkner J, et al. Cardiometabolic Risk Variables in Preadolescent Children: A Factor Analysis. J Am Heart Assoc 2017;6(10). doi: 10.1161/jaha.117.007071.

15. Magnussen CG, Cheriyan S, Sabin MA, Juonala M, Koskinen J, Thomson R, et al. Continuous and Dichotomous Metabolic Syndrome Definitions in Youth Predict Adult

Type 2 Diabetes and Carotid Artery Intima Media Thickness: The Cardiovascular Risk in Young Finns Study. The Journal of Pediatrics 2016;171:97-103.e3. doi: 10.1016/j.jpeds.2015.10.093.

16. Chan JC, Tong PC, Critchley JA. The insulin resistance syndrome: mechanisms of clustering of cardiovascular risk. Semin Vasc Med 2002;2(1):45-57. doi: 10.1055/s-2002-23095.

17. Stroescu RF, Mărginean O, Bizerea T, Gafencu M, Voicu A, Doroș G. Adiponectin, leptin and high sensitivity C-reactive protein values in obese children - important markers for metabolic syndrome? J Pediatr Endocrinol Metab 2019;32(1):27-31. doi: 10.1515/jpem-2018-0378.

18. Messiah SE, Arheart KL, Lopez-Mitnik G, Lipshultz SE, Miller TL. Ethnic group differences in cardiometabolic disease risk factors independent of body mass index among American youth. Obesity (Silver Spring) 2013;21(3):424-8. doi: 10.1002/oby.20343.

19. Tortolero SR, Goff DC, Nichaman MZ, Labarthe DR, Grunbaum JA, Hanis CL. Cardiovascular Risk Factors in Mexican-American and Non-Hispanic White Children. Circulation 1997;96(2):418-23. doi: 10.1161/01.CIR.96.2.418.

20. Martin RP GB, Erick RA, Eduardo AG, et al. Obesity and Cardiovascular Risk in Mexico: Pathophysiology and Prevention. . Obes Control Ther 2017;4(3):1-9.

21. Ramakrishnan U, Stein AD, Parra-Cabrera S, Wang M, Imhoff-Kunsch B, Juarez-Marquez S, et al. Effects of docosahexaenoic acid supplementation during pregnancy on gestational age and size at birth: randomized, double-blind, placebo-controlled trial in Mexico. Food and nutrition bulletin 2010;31(2 Suppl):S108-16. doi: 10.1177/15648265100312s203.

22. Lohman TG, Roche AF, Martorell R. Anthropometric standardization reference manual. Champaign, IL: Human Kinetics Books, 1988.

23. Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. Pediatr Obes 2012;7(4):284-94. doi: 10.1111/j.2047-6310.2012.00064.x.

24. Myatt M, E G. Internet: https://nutriverse/zscorer/, https://github.com/nutriverse/zscorer.

25. Lopez-Gonzalez D, Wells JCK, Parra-Carriedo A, Bilbao G, Mendez M, Clark P. Body Composition Assessment in Mexican Children and Adolescents. Part 1: Comparisons between Skinfold-Thickness, Dual X-ray Absorptiometry, Air-Displacement Plethysmography, Deuterium Oxide Dilution, and Magnetic Resonance Imaging with the 4-C Model. Nutrients 2022;14(5). doi: 10.3390/nu14051073.

26. Higgins PB, Fields DA, Hunter GR, Gower BA. Effect of Scalp and Facial Hair on Air Displacement Plethysmography Estimates of Percentage of Body Fat. Obesity Research 2001;9(5):326-30. doi: https://doi.org/10.1038/oby.2001.41.

27. Fields DA, Higgins PB, Hunter GR. Assessment of body composition by air-displacement plethysmography: influence of body temperature and moisture. Dynamic Medicine 2004;3(1):3. doi: 10.1186/1476-5918-3-3.

28. Siri WE. Body composition from fluid spaces and density: analysis of methods. 1961. Nutrition 1993;9(5):480-91; discussion , 92.

29. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28(7):412-9. doi: 10.1007/bf00280883.

30. Barragán-Vázquez S, Ariza AC, Ramírez Silva I, Pedraza LS, Rivera Dommarco JA, Ortiz-Panozo E, et al. Pro-Inflammatory Diet Is Associated with Adiposity during Childhood and with Adipokines and Inflammatory Markers at 11 Years in Mexican Children. Nutrients 2020;12(12). doi: 10.3390/nu12123658.

31. Zimmet P, Alberti KGMM, Kaufman F, Tajima N, Silink M, Arslanian S, et al. The metabolic syndrome in children and adolescents – an IDF consensus report. Pediatric Diabetes 2007;8(5):299-306. doi: https://doi.org/10.1111/j.1399-5448.2007.00271.x.

32. Cossio-Bolaños M, Vidal-Espinoza R, de Campos FCC, Sulla-Torres J, Cossio-Bolaños W, Andruske CL, et al. Establishing percentiles for blood pressure based on absolute height for children and adolescents. BMC Pediatr 2021;21(1):26. doi: 10.1186/s12887-020-02489-9.

33. Damanhoury S, Newton AS, Rashid M, Hartling L, Byrne JLS, Ball GDC. Defining metabolically healthy obesity in children: a scoping review. Obes Rev 2018;19(11):1476-91. doi: 10.1111/obr.12721.

34. Ramirez-Silva I, Rivera JA, Trejo-Valdivia B, Martorell R, Stein AD, Romieu I, et al. Breastfeeding Status at Age 3 Months Is Associated with Adiposity and Cardiometabolic Markers at Age 4 Years in Mexican Children. The Journal of Nutrition 2015;145(6):1295-302. doi: 10.3945/jn.114.198366.

35. OMS/UNICEF/USA. Indicators for assessing infant and young child feeding practices. Geneva, Switzerland: World Health Organization, 2008.

36. Ramírez-Silva I, Rodríguez-Ramírez S, Barragán-Vázquez S, Castellanos-Gutiérrez A, Reyes-García A, Martínez-Piña A, et al. Prevalence of inadequate intake of vitamins and

minerals in the Mexican population correcting by nutrient retention factors, Ensanut 2016. Salud Publica Mex 2020;62(5):521-31. doi: 10.21149/1109.

37. Ramírez Silva I B-VS, Rodríguez Ramírez S, Rivera Dommarco JA, Mejía-Rodríguez F, Barquera Cervera S, Tolentino Mayo L, Flores Aldana M, Villalpando Hernández S, Ancira Moreno M, et al. . Base de Alimentos de México (BAM): Compilación de la Composición de los Alimentos Frecuentemente Consumidos en el país, Version 18.1.1. 2021.

38. Hernández B, Gortmaker SL, Laird NM, Colditz GA, Parra-Cabrera S, Peterson KE. [Validity and reproducibility of a questionnaire on physical activity and non-activity for school children in Mexico City]. Salud Publica Mex 2000;42(4):315-23.

39. Kaiser HF. The Application of Electronic Computers to Factor Analysis. Educational and Psychological Measurement 1960;20(1):141-51. doi: 10.1177/001316446002000116.

40. Lê S, Josse J, Husson F. FactoMineR: An R Package for Multivariate Analysis. Journal of Statistical Software 2008;25(1):1 - 18. doi: 10.18637/jss.v025.i01.

41. Josse J, Husson F. missMDA: A Package for Handling Missing Values in Multivariate Data Analysis. Journal of Statistical Software 2016;70(1):1 - 31. doi: 10.18637/jss.v070.i01.

42. McHugh ML. Interrater reliability: the kappa statistic. Biochem Med (Zagreb) 2012;22(3):276-82.

43. van Buuren S, Groothuis-Oudshoorn K. mice: Multivariate Imputation by Chained Equations in R. Journal of Statistical Software 2011;45(3):1 - 67. doi: 10.18637/jss.v045.i03.

44. Cardoso-Saldaña GC, Yamamoto-Kimura L, Medina-Urrutia A, Posadas-Sánchez R, Caracas-Portilla NA, Posadas-Romero C. [Obesity or overweight and metabolic syndrome in Mexico City teenagers]. Arch Cardiol Mex 2010;80(1):12-8.

45. Cárdenas-Villareal VM, López Alvarenga JC, Bastarrachea RA, Rizo-Baeza MM, Cortés-Castell E. [Metabolic syndrome prevalence in teenagers of Monterrey, Nuevo Leon]. Arch Cardiol Mex 2010;80(1):19-26.

46. Evia-Viscarra ML, Rodea-Montero ER, Apolinar-Jiménez E, Quintana-Vargas S. Metabolic syndrome and its components among obese (BMI >=95th) Mexican adolescents. Endocr Connect 2013;2(4):208-15. doi: 10.1530/ec-13-0057.

47. Kiess W, Reich A, Meyer K, Glasow A, Deutscher J, Klammt J, et al. A role for leptin in sexual maturation and puberty? Horm Res 1999;51 Suppl 3:55-63. doi: 10.1159/000053163.

48. Mantzoros CS, Flier JS, Rogol AD. A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. V. Rising leptin levels may signal the onset of puberty. J Clin Endocrinol Metab 1997;82(4):1066-70. doi: 10.1210/jcem.82.4.3878.

49. Shalitin S, Phillip M. Role of obesity and leptin in the pubertal process and pubertal growth—a review. International Journal of Obesity 2003;27(8):869-74. doi: 10.1038/sj.ijo.0802328.

50. Katzmarzyk PT, Pérusse L, Malina RM, Bergeron J, Després JP, Bouchard C. Stability of indicators of the metabolic syndrome from childhood and adolescence to young adulthood: the Québec Family Study. J Clin Epidemiol 2001;54(2):190-5. doi: 10.1016/s0895-4356(00)00315-2.

51. Emmanuel M, Bokor BR. Tanner Stages. Edtion ed. StatPearls. Treasure Island (FL): StatPearls Publishing

4.7 Tables and Figures

Table 4-1. Maternal and offspring characteristics (n = 413).

Maternal factors at enrollment into POSGRAD	
Age, years	26.8 (4.81)
BMI, kg/m^2	26.3 (4.36)
Height, cm	155 (5.61)
Primiparous (n, %)	135 (32.7)
SES Score	0.12 (0.97)
Schooling, y	12.2 (3.45)
Offspring factors	
Female (n, %)	194 (47.0)
Early life characteristics	
Birth weight, g	3221 (466)
Gestational age at birth, weeks	39.1 (1.78)
Breastfeeding status, 3 mo (n, %)	
EBF	51 (13.6)
PreBF	49 (13.1)
PaBF	207 (55.3)
NBF	67 (17.9)
Duration of BF, mo	9.41 (7.66)
At age 11 years	
Cardiometabolic factors	
BMI z-score	0.7 (1.3)
HDL, mg/dL	45.8 (9.9)
Triglycerides, mg/dL	108.3 (57.6)
SBP, mmHg	102.1 (8.1)
Glucose, mg/dL	88.3 (8.5)
MetS Score	0.0 (0.5)
CMH Score	0.0 (0.4)
Lifestyle factors	
Sedentary time per day, min	287 (134)
Total fat intake, g	74.8 (34.1)
Total energy intake, kcal	1996 (674)

Values presented are mean (SD) unless otherwise stated.
BMI, body mass index; SES, socioeconomic status score; EBF, Exclusive Breastfeeding; PreBF, Predominantly Breastfeeding; PaBF, Partially Breastfeeding; NBF, No Breastfeeding; HDL, High Density Lipoprotein Cholesterol; SBP, Systolic Blood Pressure; MetS, Metabolic Syndrome; CMH, Cardiometabolic Health

Table 4-2. Prevalence and clustering of cardiometabolic risk factors among POSGRAD children at 11 years of age.

	Normal weight n = 231	Overweight/obese n = 182	Overall n = 413
Individual risk factors			
Low HDL	53 (22.9%)	79 (43.4%)	132 (31.9%)
High Triglycerides	18 (7.8%)	57 (31.3%)	75 (18.2%)
High Glucose	7 (3.0%)	8 (4.4%)	15 (3.6%)
High BP	0 (0%)	0 (0%)	0 (0%)
Clustering of CM factors			
0 CM factors	162 (70.1%)	77 (42.3%)	239 (57.9%)
1 CM factor	60 (26.0%)	68 (37.4%)	128 (31.0%)
2 CM factors	9 (3.9%)	35 (19.2%)	44 (10.7%)
3 CM factors	0 (0%)	2 (1.1%)	2 (0.5%)

Values presented are n (%).
CM, Cardiometabolic; HDL, High Density Lipoprotein Cholesterol; BP, Blood Pressure.
Overweight/obesity was defined as BMI-for-age > 1 SD above the WHO Growth Reference median (85th percentile) and cardiometabolic risk factors were defined as follows: low HDL (<40 mg/dL), high triglycerides (≥150 mg/dL), high glucose (≥100 mg/dL), and high BP (SBP ≥90th percentile for sex and height).

Table 4-3. Cardiometabolic factors stratified by body size phenotype (n = 413).

Cardiometabolic factor	MHNW N =162	MUNW N = 69	MHO N=77	MUO N=105
Glucose Homeostasis				
Insulin (μM/mL)	4.13 (2.58, 15.5)	7.10 (3.36, 14.9)	11.6 (3.87, 21.9)	15.7 (4.80, 34.2)
Glucose (mg/dL)	87.0 (82.2, 91.0)	88.0 (84.0, 92.0)	88.0 (84.0, 91.0)	90.0 (86.0, 95.0)
HOMA-IR	0.89 (0.55, 3.40)	1.51 (0.73, 3.48)	2.55 (0.78, 4.81)	3.74 (1.10, 7.66)
Lipids				
Non-HDL (mg/dL)	108 (94.0, 125)	107 (91.0, 120)	117 (101, 131)	125 (106, 144)
HDL (mg/dL)	50.0 (46.0, 57.0)	38.0 (35.0, 40.0)	47.0 (44.0, 53.0)	38.0 (34.0, 40.0)
Triglycerides (mg/dL)	76.5 (54.5, 95.0)	105 (83.0, 150)	93.0 (70.0, 120)	153 (105, 197)
LDL (mg/dL)	94.5 (80.0, 107)	84.5 (68.5, 98.0)	99.0 (87.0, 116)	93.0 (77.2, 109)
Inflammation				
IL-6 (pg/mL)	0.41 (0.22, 0.87)	0.50 (0.24, 0.90)	0.61 (0.22, 1.37)	0.68 (0.30, 1.60)
hs-CRP (mg/L)	0.38 (0.22, 0.82)	0.54 (0.25, 0.98)	1.54 (0.66, 3.16)	1.75 (0.51, 3.51)
Adipokines				
Adiponectin (ng/mL)[a]	13.9 (11.7, 17.4)	14.6 (12.4, 17.8)	14.6 (11.7, 18.8)	14.6 (11.5, 18.2)
Leptin (ng/mL)	5.74 (2.72, 10.5)	5.67 (2.61, 11.4)	6.71 (2.65, 14.7)	10.0 (4.02, 16.2)
Adiponectin:Leptin Ratio	2.42 (1.15, 5.42)	2.74 (1.35, 5.48)	2.31 (1.19, 4.90)	1.71 (0.77, 3.42)
Blood Pressure				
SBP (mmHg)[a]	102 (96.2, 108)	101 (95.8, 105)	102 (98.2, 105)	103 (97.6, 108)
DBP (mmHg)[a]	61.5 (56.8, 65.0)	60.4 (58.0, 63.2)	61.2 (58.2, 65.8)	62.3 (58.5, 65.6)
Adiposity				
BMI (kg/m^2)	17.0 (15.5, 18.1)	17.4 (15.9, 18.5)	21.7 (20.7, 23.8)	23.1 (20.9, 25.5)
FMI (kg/m^2)	4.15 (3.26, 5.29)	4.24 (3.42, 5.22)	8.03 (7.02, 10.1)	8.89 (7.21, 10.8)
FFMI (kg/m^2)	12.4 (11.7, 13.4)	12.6 (11.9, 13.5)	14.0 (13.1, 14.9)	14.3 (13.3, 15.4)
Sum of skinfolds (mm)	35.0 (28.5, 43.5)	36.8 (29.0, 43.5)	68.0 (59.2, 81.5)	75.0 (62.8, 89.5)
Abdominal Circumference (cm)	66.1 (62.3, 69.5)	66.0 (63.2, 70.2)	78.9 (76.9, 83.6)	83.7 (78.2, 88.8)

Values presented for cardiometabolic factors are median (IQR). Differences in cardiometabolic factors were assessed using Wilcoxon rank-sum tests and corrected for pairwise comparisons.

HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; Non-HDL, Non-High-Density Lipoprotein (Non-HDL = TC – HDL); HDL, High Density Lipoprotein; LDL, Low Density Lipoprotein; IL-6, Interleukin 6; hs-CRP, high sensitivity C-reactive protein; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; BMI, Body Mass Index; FMI, Fat Mass Index; FFMI, Fat-free Mass Index; MetS, Metabolic Syndrome; MHNW, Metabolically Healthy Normal Weight; MUNW, Metabolically Unhealthy Normal Weight; MHO, Metabolically Healthy Overweight/Obesity; MUO, Metabolically Unhealthy Overweight/Obesity.

[a] Cardiometabolic factors did not significantly differ by body size phenotype ($p > 0.05$).

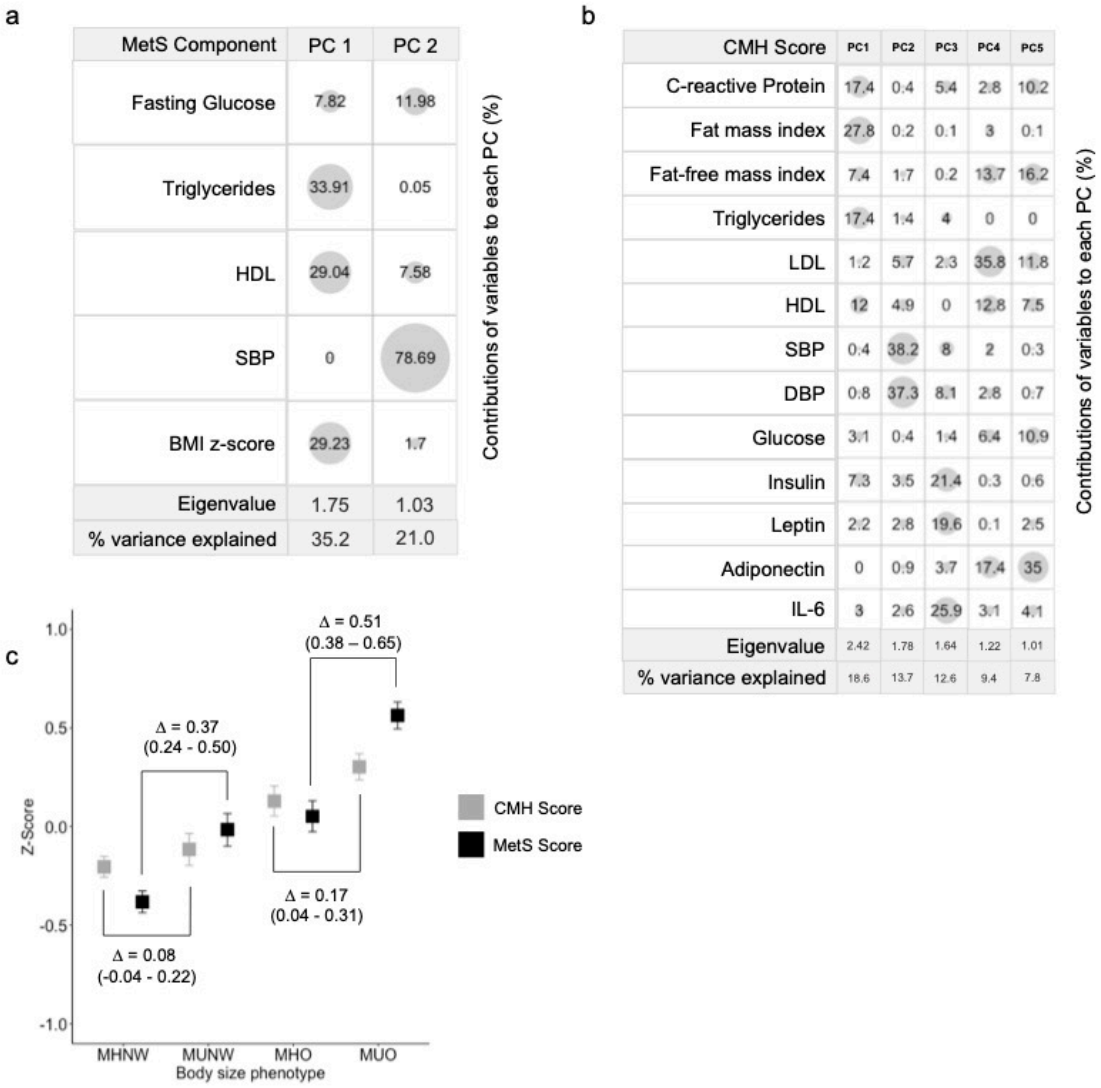


Figure 4-1. Contribution of cardiometabolic factors to PCA-derived continuous MetS score and CMH score and differences by body size phenotype.

Principal components analysis (PCA) was used to calculate the **a)** MetS score using five components used to diagnose MetS in pediatric populations and **b)** exploratory CMH score using 13 cardiometabolic factors. Top PCs were identified visually using scree plots and quantitatively using eigenvalues > 1. Both scores were calculated as the sum of the top components, weighted by variance explained. The x axes represent the top PCs used to create the MetS score and percentage of variance in the data explained by each PC via PCA. The diameter of each bubble represents the magnitude of contributions (%) of individual MetS components to each PC. Cardiometabolic factors with the highest contributions represent the most important variables within each PC. The highest contributing components in the first PC contribute most to the derived scores. **C)** adjusted mean differences (95% CI) in MetS and CMH scores, stratified by body size phenotype.

Abbreviations: CMH, Cardiometabolic Health; MetS, Metabolic Syndrome; MHNW, Metabolically Healthy Normal Weight; MUNW, Metabolically Unhealthy Normal Weight; MHO, Metabolically Healthy Overweight/Obesity; MUO, Metabolically Unhealthy Overweight/Obesity; PC, Principal Component

Table 4-4. Association of MetS score with markers of inflammation and insulin resistance.

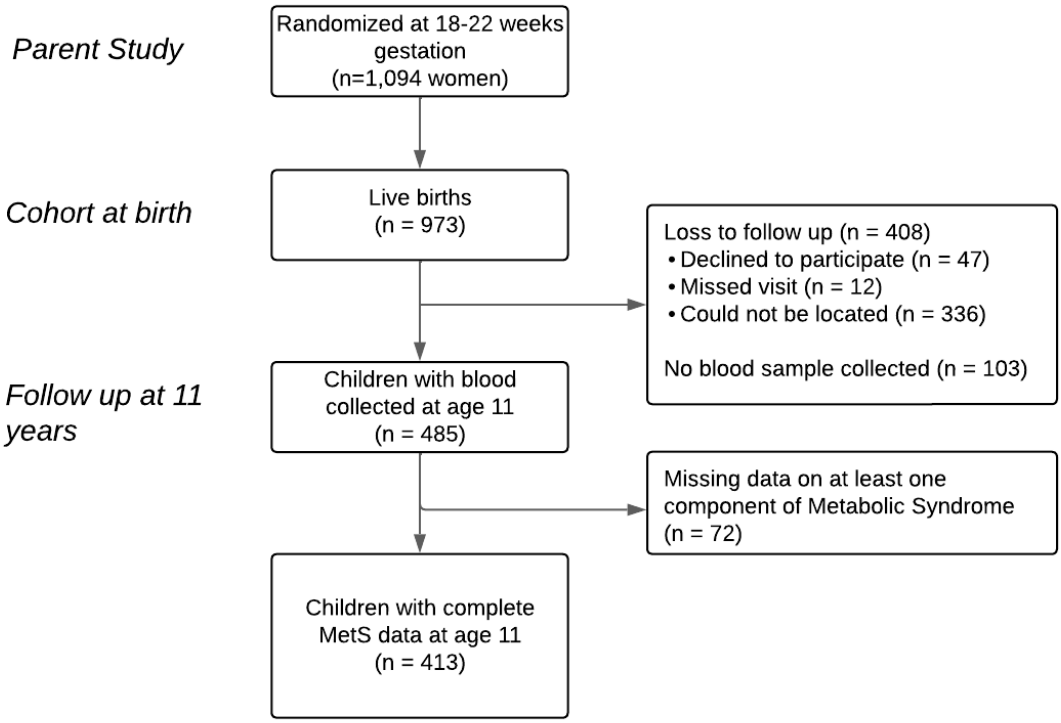
	Overall N = 413	Males N = 219	Females N = 194
Biomarker (log-transformed)	$\boldsymbol{\beta}$ (95% CI)	$\boldsymbol{\beta}$ (95% CI)	$\boldsymbol{\beta}$ (95% CI)
Leptin*	0.19 (-0.02, 0.41)	**0.39 (0.08, 0.69)**	0.03 (-0.29, 0.35)
Adiponectin	-0.003 (-0.08, 0.08)	-0.04 (-0.01, 0.07)	0.04 (-0.09, 0.17)
Adiponectin/Leptin ratio*	-0.18 (-0.41, 0.04)	**-0.41 (-0.73, -0.08)**	0.006 (-0.33, 0.34)
IL-6	0.14 (-0.09, 0.37)	0.22 (-0.08, 0.53)	0.08 (-0.29, 0.44)
Hs-CRP *	**0.75 (0.52, 0.98)**	**0.93 (0.62, 1.24)**	**0.56 (0.20, 0.92)**
HOMA-IR	**0.79 (0.59, 0.99)**	**0.60 (0.34, 0.86)**	**0.99 (0.68, 1.30)**

Values represent $\boldsymbol{\beta}$ (95% CI) from generalized linear models testing the association between the PCA-derived MetS score and biomarkers of inflammation and insulin resistance. Biomarkers with skewed distributions were log-transformed prior to analysis. Models were adjusted for prenatal treatment group, maternal age, BMI at 18-22 weeks gestation, household socioeconomic status close to the time of birth, child sex, birth weight, type of breastfeeding at age 3 months and total duration, age at measurement, diet (energy intake and total omega-3 fatty acid intake), and sedentary time at age 11 years. Models were stratified by sex and additionally adjusted for sexual maturation (defined in girls as attainment of menarche and in boys by testosterone concentrations). We used multiple imputation to account for missing covariates (diet, screen time, sexual maturation, and breastfeeding type and duration).

Interaction by sex was tested by including an interaction term in the overall model. The asterisk denotes significant interaction by sex ($p < 0.05$).

IL-6, Interleukin 6; hs-CRP, high-sensitivity C-reactive protein; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; MetS, Metabolic Syndrome.

4.8 Supplementary Tables and Figures



Supplementary Figure 4-1. Flow of study sample selection.
Abbreviations: MetS, Metabolic Syndrome

Supplementary Table 4-1. Comparison of maternal factors at POSGRAD study enrollment and offspring characteristics at birth in analytic and missing samples.

	Analytic sample N=413	Missing sample N=560	p-value
Maternal factors at enrollment into POSGRAD			
Age, years	26.8 (4.81)	25.9 (4.60)	*0.003*
BMI, kg/m^2	26.3 (4.36)	25.9 (4.13)	*0.136*
Height, cm	155 (5.61)	155 (5.78)	*0.934*
Primiparous (n, %)	135 (32.7%)	235 (42.0%)	*0.004*
SES Score	0.12 (0.97)	-0.03 (1.01)	*0.02*
Schooling, y	12.2 (3.45)	11.8 (3.59)	*0.153*
Received prenatal DHA intervention	212 (51.3%)	275 (49.1%)	*0.535*
Offspring factors			
Female (n, %)	194 (47.0%)	270 (48.2%)	*0.537*
Birth weight, g	3221 (466)	3193 (457)	*0.353*
Gestational age at birth, weeks	39.1 (1.78)	39.1 (1.79)	*0.901*
Breastfeeding status, 3 mo (n, %)			*0.439*
EBF	51 (13.6)	53 (11.4)	
PreBF	49 (13.1)	50 (10.8)	
PaBF	207 (55.3)	280 (60.5)	
NBF	67 (17.9)	80 (17.3)	
Duration of BF, mo	9.41 (7.66)	8.05 (7.67)	*0.007*

Values presented are mean (SD) unless otherwise stated.
BMI, body mass index; SES, socioeconomic status score; DHA, Docosahexaenoic Acid; EBF, Exclusive Breastfeeding; PreBF, Predominantly Breastfeeding; PaBF, Partially Breastfeeding; NBF, No Breastfeeding

Supplementary Table 4-2. Cardiometabolic factors stratified by sex.

Cardiometabolic factor	Overall N=413	Males N=219	Females N=194
Glucose Homeostasis			
Insulin (μM/mL)	8.25 (3.06, 21.6)	7.25 (3.05, 20.4)	11.3 (3.12, 24.3)
Glucose (mg/dL)*	88.0 (84.0, 92.0)	89.0 (85.5, 94.0)	87.0 (83.0, 91.0)
HOMA-IR	1.83 (0.65, 4.79)	1.59 (0.67, 4.53)	2.44 (0.62, 5.12)
Lipids			
Non-HDL (mg/dL)	113 (97.0, 132)	112 (96.0, 131)	113 (99.0, 133)
HDL (mg/dL)	45.0 (39.0, 52.0)	45.0 (39.0, 52.0)	45.0 (39.0, 51.0)
Triglycerides (mg/dL)*	94.0 (69.0, 131)	88.0 (64.0, 123)	100 (76.0, 141)
LDL (mg/dL)	93.0 (78.0, 107)	93.5 (78.8, 109)	93.0 (78.0, 104)
Inflammation			
IL-6 (pg/mL)	0.51 (0.24, 1.10)	0.55 (0.25, 1.09)	0.50 (0.24, 1.12)
Hs-CRP (mg/L)	0.72 (0.32, 2.13)	0.78 (0.33, 2.35)	0.62 (0.28, 1.67)
Adipokines			
Adiponectin (ng/mL)	14.4 (11.8, 17.9)	14.3 (11.7, 17.5)	14.6 (12.1, 18.2)
Leptin (ng/mL)	6.64 (2.76, 13.0)	6.59 (2.92, 14.7)	6.86 (2.62, 12.1)
Adiponectin:Leptin Ratio	2.27 (1.08, 5.08)	2.25 (1.02, 5.19)	2.32 (1.13, 4.85)
Blood Pressure			
SBP (mmHg)	102 (96.6, 107)	102 (96.5, 106)	103 (96.8, 108)
DBP (mmHg)	61.6 (57.6, 65.0)	61.6 (57.8, 64.6)	61.6 (57.6, 65.6)
Adiposity			
BMI (kg/m^2)	18.9 (16.8, 22.0)	18.9 (16.7, 22.2)	19.1 (17.1, 21.6)
FMI (kg/m^2)	5.89 (3.99, 8.12)	5.72 (3.79, 8.57)	5.97 (4.12, 7.70)
FFMI (kg/m^2)	13.2 (12.2, 14.5)	13.2 (12.2, 14.3)	13.2 (12.0, 14.7)
Sum of skinfolds (mm)*	49.2 (34.0, 70.0)	43.5 (31.8, 67.9)	52.5 (36.9, 71.5)
Abdominal Circumference (cm)	72.5 (65.0, 80.5)	71.9 (64.8, 81.3)	72.9 (66.0, 79.0)
Cardiometabolic Risk			
MetS Score	-0.04 (-0.33, 0.36)	-0.04 (-0.34, 0.38)	-0.04 (-0.33, 0.36)
CMH Score	-0.05 (-0.27, 0.26)	-0.06 (-0.33, 0.23)	-0.04 (-0.25, 0.30)

Values presented are median (IQR).

BMI, body mass index; SES, socioeconomic status score; DHA, Docosahexaenoic Acid; EBF, Exclusive Breastfeeding; PreBF, Predominantly Breastfeeding; PaBF, Partially Breastfeeding; NBF, No Breastfeeding; HDL, High Density Lipoprotein Cholesterol; SBP, Systolic Blood Pressure; MetS, Metabolic Syndrome; CMH, Cardiometabolic Health

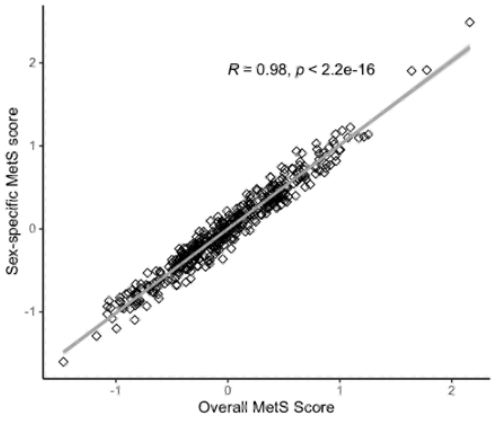
*Significantly differs by sex ($p < 0.05$)

a

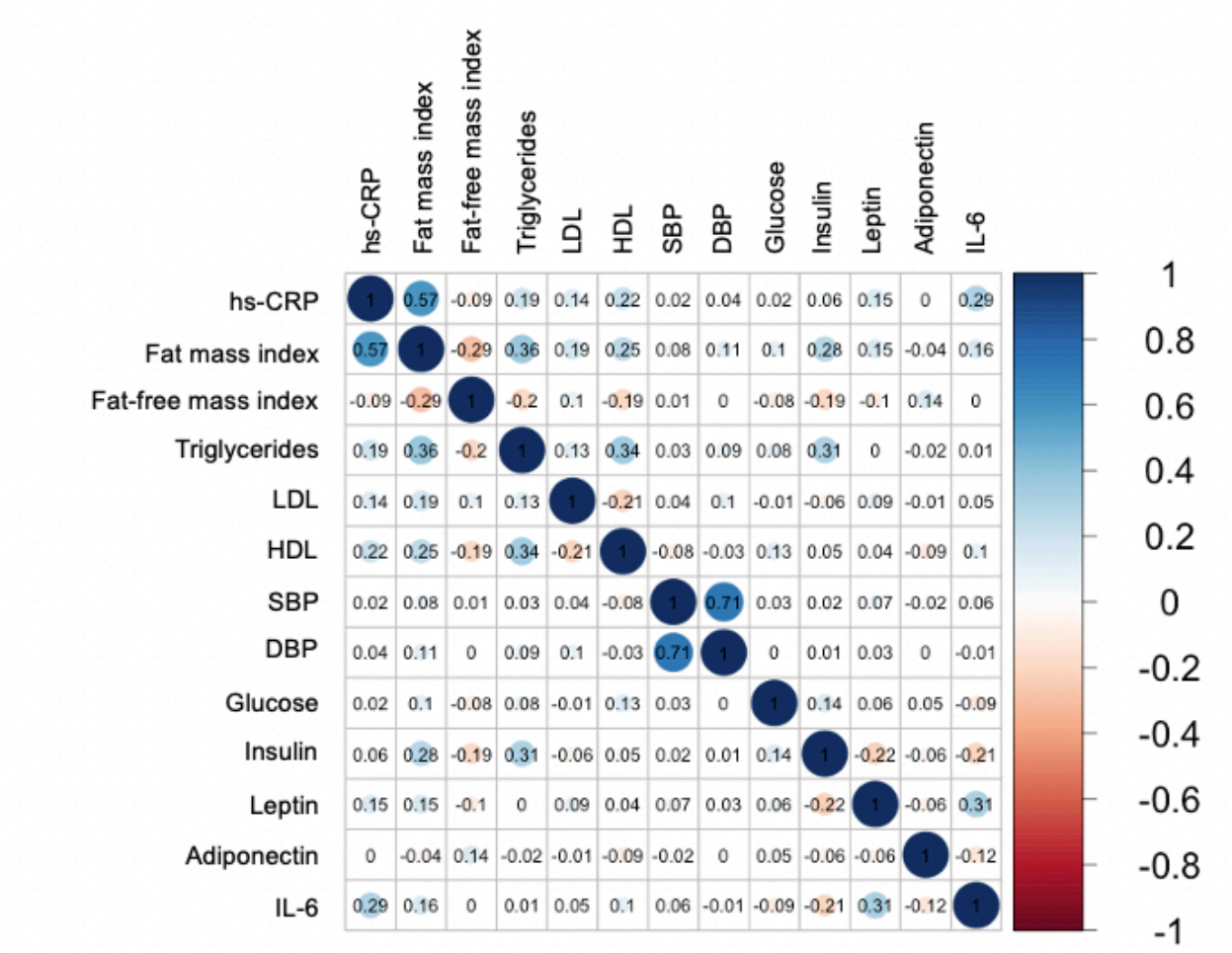
Sex	Males		Females	
MetS Component	PC 1	PC 2	PC 1	PC 2
Fasting Glucose	9.17	17.85	7.23	17.7
Triglycerides	30.75	5.4	33.75	2.08
HDL	27.33	8.24	28.04	7.98
SBP	4.05	68.43	3.83	67.5
BMI z-score	28.71	0.08	27.15	4.74
Eigenvalue	1.76	1.01	1.88	1.05
% variance explained	35.2	20.2	37.6	21.0

Contributions of variables to each PC (%)

b



Supplementary Figure 4-2. Contribution of cardiometabolic factors to PCA-derived sex-specific continuous MetS score.
Principal components analysis (PCA) was used to calculate the continuous MetS risk score using five components used to define metabolic syndrome in pediatric populations among a) males and females. Top principal components (PCs) were identified visually using scree plots and quantitatively using eigenvalues > 1. Both scores were calculated as the sum of the top components, weighted by variance explained. The x axes represent the top principal components (PCs) used to create the MetS score and percentage of variance in the data explained by each PC via PCA. The diameter and color of each bubble represent the magnitude of contributions (%) of individual MetS components to each PC. Cardiometabolic factors with the highest contributions represent the most important variables within each PC. The highest contributing components in the first PC contribute most to the derived scores. b) Pearson correlation between the overall MetS score and sex-specific MetS score.



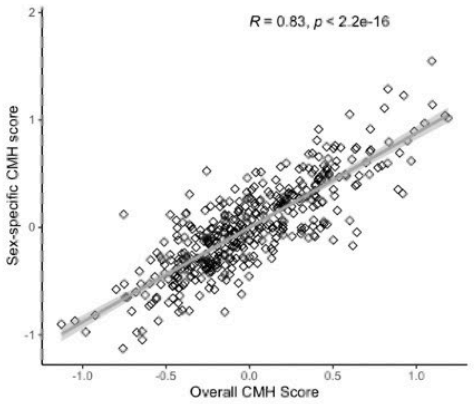
Supplementary Figure 4-3. Spearman correlations between cardiometabolic factors included in CMH score.
hs-CRP, high-sensitivity C-reactive protein; LDL, Low Density Lipoprotein Cholesterol; HDL, High Density Lipoprotein Cholesterol; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; IL-6, Interleukin-6.

a

Sex	Males					Females				
CMH Score	PC1	PC2	PC3	PC4	PC5	PC1	PC2	PC3	PC4	PC5
C-reactive Protein	17.65	0.81	2.07	0.05	4.15	11.4	13.47	1.04	3.49	12.09
Fat mass index	24.64	0.93	0.3	0.94	3.54	21.81	2.21	6.76	0.82	0.81
Fat-free mass index	8.59	4.53	0.25	0.02	0.08	5.79	4.69	0	28.67	2.06
Triglycerides	11.74	0.63	7.9	0.06	0.71	23.76	1.35	0.88	0.07	0.63
LDL	2.08	3.38	1.63	48.42	1.77	0.14	15.26	4.05	14.01	9.32
HDL	8.6	3.54	0.52	27.8	3.19	16.89	0	1.95	3.27	3.1
SBP	5.01	40.89	0.15	2.17	0.11	3.63	3.56	36.66	0.13	1.77
DBP	3.52	41.69	0.29	4.17	0.02	1.06	1.67	43.18	0	2.08
Glucose	2.6	0.42	10.08	0.56	40.41	3.37	0.04	0.52	4.92	48.57
Insulin	4.47	1.1	25.07	0.73	8.53	12.01	17.09	0.37	1.17	0.22
Leptin	4.99	0.12	10.89	10.84	24.69	0.05	17.39	0.36	10.14	0.24
Adiponectin	0.41	0.8	10.76	3.58	12.56	0.03	1.42	3.69	27.87	14.74
IL-6	5.7	1.17	30.08	0.67	0.27	0.04	21.84	0.54	5.46	4.37
Eigenvalue	2.69	1.70	1.51	1.22	1.03	2.39	1.92	1.76	1.30	1.06
% variance explained	20.7	13.1	11.6	9.4	7.9	18.4	14.8	13.6	9.9	8.2

Contributions of variables to each PC (%)

b



Supplementary Figure 4-4. Contribution of cardiometabolic factors to PCA-derived sex-specific continuous CMH score.

Principal components analysis (PCA) was used to calculate the CMH score among a) males and females. Top principal components (PCs) were identified visually using scree plots and quantitatively using eigenvalues > 1. The CMH score was calculated as the sum of the top components, weighted by variance explained. The x axes represent the top principal components (PCs) used to create the CMH score and percentage of variance in the data explained by each PC via PCA. The diameter and color of each bubble represent the magnitude of contributions (%) of individual cardiometabolic components to each PC. Cardiometabolic factors with the highest contributions represent the most important variables within each PC. The highest contributing components in the first PC contribute most to the derived scores. b) Pearson correlation between the overall CMH score and sex-specific CMH score.

Chapter 5 : Maternal single nucleotide polymorphism rs174602 modified the effect of prenatal DHA supplementation on offspring cardiometabolic health at 11 years: follow-up of a randomized controlled trial in Mexico

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5.1 Abstract

Background: There is limited evidence regarding long-term effects of prenatal docosahexaenoic acid (DHA) supplementation on offspring cardiometabolic health (CMH). Inconsistent results may be attributable to variants of fatty acid desaturase (*FADS*) genes.

Objective: We evaluated the effect of prenatal DHA supplementation on offspring CMH and investigated effect modification by maternal *FADS2* single nucleotide polymorphism (SNP) rs174602.

Methods: We used follow-up data from a double-blind randomized controlled trial in Mexico in which pregnant females received 400 mg/d of algal DHA or placebo from mid-gestation until delivery. The study sample included 314 offspring who had data at age 11 years and maternal *FADS* genetic data (DHA: n = 160; Placebo: n = 154). We derived a Metabolic Syndrome (MetS) score from body mass index, HDL, triglycerides, systolic blood pressure, and fasting glucose. Generalized linear models were used to evaluate the effect of the intervention on offspring MetS score and test interactions between treatment group and genotype, adjusting for maternal, offspring, and household factors.

Results: Offspring MetS score did not differ significantly by treatment group. We observed evidence of effect modification by maternal SNP rs174602 (p = 0.001); offspring of maternal TT genotype who received DHA had lower MetS score relative to the placebo group (DHA (mean ± standard error of the mean (SEM)): -0.21 ± 0.11, n = 21; Placebo: 0.05 ± 0.11, n = 23; Δ= -0.26 (95% CI: -0.55, 0.04), p = 0.09); among CC maternal genotype carriers, offspring of mothers who received DHA had higher MetS score (0.18 ± 0.06, n = 62) relative to the placebo group(-0.05 ± 0.06, n = 65, Δ=0.24 (0.06, 0.41), p < 0.01).

Conclusion: The effect of prenatal DHA supplementation on offspring MetS score differed by maternal *FADS* SNP rs174602. These findings further support the need to incorporate genetic analysis of *FADS* polymorphisms in DHA supplementation trials.

5.2 Introduction

Nutritional exposures may have long-term implications for offspring metabolic health, including risk of cardiovascular disease and type II diabetes (1-4). Docosahexaenoic Acid (DHA) is an n-3 long-chain polyunsaturated fatty acid (LC-PUFA) that typically accumulates during the second half of pregnancy to support optimal fetal DHA tissue deposition, development, and immune function (5). Animal models and epidemiological studies suggest that alterations in the prenatal DHA supply may also influence long-term offspring cardiometabolic risk via altered cell and organ development, gene expression, and development of neuroendocrine signals (1, 4, 6, 7). For example, studies in rat and mouse models have shown that prenatal n-3 LC-PUFA supplementation results in lower adiposity, insulin resistance, and dyslipidemia among offspring (8-11).

In humans, observational studies report associations of higher maternal n-3 LC-PUFA status during pregnancy with lower adiposity, dyslipidemia, and leptin concentrations among offspring in early and mid-childhood (12, 13). Additional evidence suggests that especially among females with overweight or obesity, mother-offspring dyads may benefit from prenatal DHA supplementation via improvements in maternal insulin sensitivity, circulating lipids, and placental inflammation, thus reducing fetal overnutrition and adiposity (14). However, systematic reviews of results from randomized controlled trials (RCT) report inconsistent effects of prenatal DHA supplementation on offspring cardiometabolic health (CMH) outcomes (6, 15-18). While these inconsistencies may be attributable to differences in the dose, type, and timing of supplementation during pregnancy (19, 20), variants of fatty acid desaturase (*FADS*) genes that modulate the conversion of n–3 and n–6 fatty acids into LC-PUFAs may also contribute to this heterogeneity (5).

Tissue LC-PUFA concentrations are determined by both dietary intake of n-6 and n-3 LC-PUFAs and endogenous formation from dietary PUFA precursors, which occurs through a series of consecutive desaturation and elongation steps. The rate-limiting desaturase steps are mediated by Δ-6 and Δ-5 desaturase enzymes encoded in the *FADS* gene cluster (*FADS1, FADS2, FADS3*) (5). Multiple variants in *FADS* genes have been associated with lower LC-PUFA concentrations, indicating reduced conversion of dietary precursors (5, 21). Although a few observational studies suggest that maternal *FADS* genotype influences offspring LC-PUFA status and lipid profiles, to our knowledge, this association has not been investigated in the context of an intervention trial (22).

To address these research gaps, we leveraged data from a large prenatal DHA supplementation RCT in Mexico, in which pregnant females received either 400 mg algal DHA (treatment) or placebo daily from mid-pregnancy through delivery. We previously reported that maternal *FADS2* SNP rs174602 modified the effect of prenatal DHA supplementation on offspring birth weight (23), metabolome at age 3 months (24), and cognition at age 5 years (25). The objective of this study is to evaluate the effect of prenatal DHA supplementation on offspring CMH at age 11 years and assess whether it differed by variations in maternal *FADS* SNP rs174602.

5.3 Methods

5.3.1 Participants and Study Design

This study included children of pregnant females who participated in the Prenatal Omega-3 fatty acid Supplementation and Child Growth and Development (POSGRAD) trial in Cuernavaca, Mexico (NCT00646360). A detailed description of the trial design and protocol has been published previously (26). Briefly, pregnant females were recruited at 18-22 weeks gestation at the Mexican Social Security Institute (IMSS) and were eligible for inclusion if they were 18-35

years old, planned to deliver at the IMSS hospital, breastfeed for at least 3 months, and continue living in the area for ≥ 2 years following delivery. Exclusion criteria included high-risk pregnancies, lipid metabolism or absorption disorders, regular intake of fish oil or DHA supplements, or chronic use of certain medications. Once eligibility was confirmed, participants were contacted and provided with a thorough explanation of the study protocol, and written informed consent was obtained. Of 1,762 eligible pregnant females, 1,094 were randomized to receive 2 capsules containing either 200 mg algal DHA per capsule (treatment) or a corn/soy oil blend (placebo) daily through delivery; the fatty acid composition of the supplements has been previously published (24). Block randomization was used to randomize study participants into groups of equal sample size using a block size of eight. Assignment codes were placed in sealed envelopes at the beginning of the study and were held in a sealed location by a faculty member at Emory University who was not involved with the study. Enrollment took place from February 2005 to March 2007, and the last child was born in July 2007. All study participants and members of the study team were blinded to treatment allocations throughout the intervention period of the study. Data were unblinded for the analytical study team after the last baby was born and reached 6 mo. of age. The participants and fieldworkers remain blinded to the intervention, as the study is still ongoing for follow up. Due to budgetary limitations, venous blood samples were collected in a sub-sample of 485 children who were contacted and agreed to participate in the 11-year follow-up study from 2016-2018.

The study was conducted according to the guidelines of the Declaration of Helsinki. The Emory University Institutional Review Board and the Mexican National Public Health Institute (INSP) ethics committee approved all procedures involving human subjects. Informed consent was

obtained from all pregnant females at study enrollment. At the 11-year follow up visit, mothers provided written informed consent and children provided written assent.

5.3.2 Maternal Genotype Data

Fasting venous blood samples were obtained from all pregnant females at recruitment. Plasma, buffy coat, and red blood cells were separated and stored at INSP laboratories at −80◦C until buffy coats were transported to the Hemholtz Center, Munich, Germany. The genetic analysis was carried out during 2012–2013 for those who provided consent to genotyping (n=720), using methods that have been previously described (23). The resulting data sets containing information on 15 *FADS1, FADS2,* and *FADS3* SNPs, selected based on biological evidence of an effect on LC-PUFA metabolism (27-31), were sent to Emory University via encrypted files. Key SNPs were selected on basis of previous associations with cardiometabolic health in the literature (rs174548, rs174556, rs174570, rs174575, rs174576, rs174579, rs174602) (27, 28, 30, 31). Maternal *FADS2* SNP rs174602 was selected as the focus of this study based on previous evidence of effect modification on offspring birth weight, metabolome at 3 months, and cognition at 5y within the POSGRAD trial (23-25). Allele frequencies were calculated, and Hardy Weinberg Equilibrium (HWE) was tested with Fisher's exact test using the R 'genetics' package.

5.3.3 Follow-up study of children at 11 years

At age 11 years, body weight (kg) and height (cm) were collected in triplicate by trained personnel following standard procedures (32). Children were weighed wearing light clothing with a portable electronic pediatric scale (Tanita model 1582) with a precision of 100 g, which was calibrated daily with a known reference weight. Height was measured using a stadiometer with a precision of 0.1 cm. Average values of all three measurements were calculated. We calculated BMI-for-age z-scores according to the 2007 World Health Organization (WHO) Growth Reference

Standards using the 'zscorer' R package (33). Blood pressure (mmHg) was measured using a digital device (OMRON model HEM-711ACINT), which has been validated for use in children and adolescents. Blood pressure was taken when the child was at rest (> 5 minutes after the child arrived at the study visit). In each arm, four measurements were made with 2-minute intervals; the first measurement was discarded and the subsequent three were averaged (34).

5.3.4 Outcome Assessment: Cardiometabolic Markers

Venous blood samples were obtained from children after a 12-hour fasting period (verified by documenting the approximate time that food was last consumed) and centrifuged. Aliquots of serum were frozen in liquid nitrogen and stored at −80°C until further analysis at the National Institute of Medical Sciences and Nutrition Salvador Zubirán in Mexico. HDL cholesterol, triglyceride, and glucose concentrations were assessed using the Beckman Coulter SYNCHRON CX 5 Delta automated kit and expressed in mg/dL. Cardiometabolic risk factor thresholds were defined as follows: triglycerides ≥ 150 mg/dL, HDL < 40 mg/dL, Systolic Blood Pressure (SBP) ≥ 90^{th} percentile for sex and height, and fasting glucose ≥ 100 mg/dL (35, 36).

5.3.5 Derivation of Continuous MetS Score

To operationalize cardiometabolic health and reduce multiple testing, we used principal components analysis (PCA) to calculate a continuous MetS score (primary outcome) using systolic blood pressure, HDL, triglycerides, BMI-z, and glucose (37). We used BMI-z instead of waist circumference because it has greater reliability and has been used in other similar studies (38, 39). We log-transformed triglycerides and glucose and used the inverse of HDL when standardizing, so a higher factor loading score would have a similar interpretation to other measures in the model. Top principal components (PCs) were identified visually using scree plots and quantitatively using the Kaiser criterion (eigenvalues > 1). Subsequently, the score was calculated as the sum of the

top two components, weighted by variance explained. The score can be interpreted as a z-score, with higher scores representing increased cardiometabolic risk. PCA was performed using the 'FactoMineR' R package (40). Given the observed sex differences in cardiometabolic health measures, we performed a sensitivity analysis comparing sex-specific and overall MetS scores; their correlation coefficient was 0.97; so we use the overall score in subsequent analyses.

5.3.6 Covariates

Data on maternal, offspring, and household factors were available to further characterize the study population. Household SES at enrollment was calculated with the use of PCA on a list of assets collected through interview (26). Maternal BMI at enrollment was assessed based on weight and height measurements that were obtained using standard procedures. Maternal dietary intake at study enrollment was also assessed using a 110-item food-frequency questionnaire that was specifically designed to include important PUFA sources (41). Data on infant feeding practices at 3 months of age were obtained by maternal interview and used to categorize infant feeding practices according to the WHO classification (42, 43). Dietary intake of children at 11 years was assessed via multiple-pass 24-hour dietary recall developed for Mexican populations (44). Trained personnel administered the diet recall tool to the child's primary caregiver (in presence of the child). Nutrient and energy estimations were obtained using the 2012 and 2016 Mexican Food Database (BAM in Spanish): Compilation of the Frequently Consumed Foods in the Country, which are maintained by INSP (45). Sedentary time was estimated using a self-reported physical activity and inactivity questionnaire that was developed for and validated among children ages 10-14 years in Mexico (46). Sexual maturation was assessed by proxy using testosterone concentrations for males and age at attainment of menarche for females via self-reported questionnaire.

5.3.7 Statistical Analysis

Normality of data was assessed using histograms and quantile-quantile plots, and residual plots were used to check model assumptions. We calculated means and standard deviations for maternal and offspring characteristics at trial enrollment and birth and assessed differences by treatment group and maternal genotype using t-tests, ANOVA, and Wilcoxon rank-sum tests as appropriate. We compared these characteristics between those included in the analytic sample and the rest of the birth cohort, and variables that differed were considered for inclusion in models as covariates.

We used multivariable generalized linear models to assess the effect of prenatal DHA supplementation on offspring MetS scores and test interactions between maternal treatment group and genotype using five different models: 1) unadjusted model; 2) adjusted for household SES score, maternal age (years), parity (number of live births), BMI (kg/m^2), and offspring sex and age at measurement (days); 3) model 2 additionally adjusted for birth weight (g) and gestational age at birth (weeks); 4) model 3 additionally adjusted for energy intake (kcal/day) and omega-3 fatty acid intake (g/day); and 5) model 4 additionally adjusted for monounsaturated fat (MUFA) (g/day) and saturated fat intake (g/day). We used multiple imputation to account for missing values of covariates (diet (n = 85, 27.1%), sedentary time (n = 26, 8.3%), sexual maturation (testosterone concentrations in males: n = 101, 58.4%; attainment of menarche in females: n = 1, 0.7%), and infant feeding practices (n = 30, 9.6%) and duration (n = 2, 0.6%)). We used the R 'mice' package to generate 20 imputed datasets using fully conditional specification with 50 iterations, conducted GLMs for each of the 20 models, and pooled the estimates (47). Inclusion of covariates specified in models 3, 4, and 5 did not alter estimates; therefore, we report all findings adjusted for the covariates specified in model 2. All statistical analyses were performed using R version 4.0.4 (R

Foundation for Statistical Computing, Vienna, Austria). Statistical significance was held at $p < 0.05$. We tested six different outcomes (MetS score and five components included in MetS score) and used the Bonferroni correction to adjust for multiple testing ($p < 0.008$).

5.4 Results

The analytic sample included all children with complete data on maternal genotype and cardiometabolic risk factors (BMI-z, HDL, triglycerides, glucose, and SBP) at age 11 years (n = 314) (**Figure 5-1**). Maternal and offspring characteristics at trial enrollment and birth were balanced by treatment group and maternal genotype (**Table 5-1**). Mean maternal age and BMI at enrollment were 26.2 ± 4.7 years and 26.0 ± 4.2 kg/m^2, respectively. Median maternal dietary intake of DHA was very low (median (IQR): 56 (40-105) mg/d), combined with a dietary n-6:n-3 ratio of 12:1. Mothers of children in the analytic sample tended to be older, have a higher BMI, and higher SES score at trial enrollment relative to those lost to follow up or missing data (**Supplementary Table 5-1**). Lifestyle factors of children at age 11 years by treatment group and maternal genotype are presented in **Table 5-2**; children whose mothers received prenatal DHA tended to have higher intakes of polyunsaturated fatty acids, including total omega-3 and omega-6 intake, relative to children whose mothers received placebo ($p < 0.05$). Maternal and offspring characteristics at study enrollment and birth, and offspring lifestyle factors at age 11 years were similar when stratified by both prenatal treatment group and maternal genotype (**Supplementary Table 5-2).**

5.4.1 Genotype distribution of maternal SNP rs174602

Within this sample, the minor allele frequency for maternal SNP rs174602 was 0.37; 44 mothers (14%) were homozygous carriers of the minor T allele, 143 (46%) were heterozygous carriers (TC), and 127 (40%) were homozygous carriers of the major C allele. There were no

significant differences in genotype distribution by treatment group, and there were no HWE violations observed (p = 0.72).

5.4.2 Cardiometabolic health of children at age 11 years

At age 11 years, 42% of the children had BMI z-score > 1 SD, and 39% had ≥ 1 cardiometabolic risk factor (HDL ≤ 40 mg/dL, triglycerides ≥ 150 mg/dl, fasting glucose ≥ 100 mg/dL, SBP ≥ 90th percentile). The most frequently observed cardiometabolic risk factor was low HDL (30%), followed by high triglycerides (16%). Derivation of the MetS score via PCA showed that the top two PCs collectively explained 56% of the variance in the measured data (**Supplementary Figure 5-1a**). Triglycerides, BMI-z, and HDL contributed to the first PC (35.0% variance explained), while SBP and fasting glucose contributed to the second PC (20.7% variance explained). The distribution of the MetS score in the study population is shown in **Supplementary Figure 5-1b**.

5.4.3 Impact of prenatal DHA supplementation on offspring MetS score

Differences in MetS components and offspring MetS score by prenatal treatment group and maternal genotype are shown in **Table 5-3.** Intent to treat analysis showed no differences by treatment group for the MetS components (all p > 0.05) or MetS score at 11 years (Δ = 0.02, 95% CI: -0.09, 0.13). We observed evidence of effect modification by maternal SNP rs174602 (p = 0.001) (**Figure 5-2**). Offspring of homozygous minor T allele carriers who received prenatal DHA had lower MetS score relative to the placebo group (DHA (mean ± SEM): -0.21 ± 0.11, n=21; Placebo: 0.05 ± 0.11; Δ= -0.26 (95% CI: -0.55, 0.04), n = 23, p = 0.09). Among homozygous major C allele carriers, offspring of mothers who received prenatal DHA had higher MetS score (0.18 ± 0.06, n=62) relative to offspring whose mothers received placebo (-0.05 ± 0.06, n = 65, Δ=0.24 (0.06, 0.41), p < 0.01). Individual cardiometabolic risk factors, stratified by both maternal

genotype and treatment group, are shown in **Supplementary Table 5-3**. While we observed similar trends with individual MetS components, the results were not statistically significant after adjustment for multiple testing (**Figure 5-3**). Finally, we evaluated three-way *a priori* interactions with maternal BMI at study enrollment and offspring sex but did not find any evidence of further effect modification (G×BMI interaction p-value = 0.24, G×sex interaction p-value = 0.28).

5.5 Discussion

In this follow-up study of mother-offspring dyads from Mexico, we found no main effect of prenatal DHA supplementation on offspring cardiometabolic health at age 11 years, but there were differences by variants of maternal *FADS2* SNP rs174602. Offspring of homozygous minor allele (TT) carriers who received prenatal DHA had lower MetS scores relative to the placebo group, while offspring of homozygous major allele (CC) carriers who received DHA had higher MetS scores relative to offspring whose mothers received placebo. These exploratory findings suggest that prenatal DHA supplementation may have differential effects on a child's long-term cardiometabolic risk based on their mother's genotype.

The lack of main effects of the intervention are similar to the findings from the few studies that have evaluated the long-term effect of prenatal n-3 LC-PUFA supplementation on offspring metabolic health; Rytter et al. found no significant differences by treatment group in blood pressure or lipids in a Danish population at age 19 years, but the sample sizes were small (n = 180 for blood pressure outcomes, n = 243 for adiposity-related outcomes) (48, 49). When the analysis was restricted to mothers with low fish intake, however, children born to mothers who received fish oil trended towards better lipid profiles. A prenatal DHA RCT that was conducted in the US among 171 mothers and their offspring also did not find evidence of a main intervention effect but observed statistically significant interaction between prenatal DHA supplementation and child

weight status for SBP and DBP from 4 to 6 years of age and higher fat free mass at age 5 years (50). A previous study within the POSGRAD cohort also did not find any differences by treatment group in non-fasting serum lipid and glucose concentrations at age 4 years (51). Some evidence suggests that differences in cardiometabolic risk because of metabolic programming may become more apparent later in childhood, near the onset of puberty. One observational study reported associations of cord blood LC-PUFA composition with child BMI at 2 and 10 years, but not 6 years, highlighting the importance of age of outcome assessment (19). However, within our study, results were still null at age 11 years.

Our results provide additional support that the differences in the genetic makeup of individuals may partially explain null results observed across prenatal DHA supplementation RCTs. Here, we showed that the effect of the intervention on the offspring MetS score at 11 years differed by maternal *FADS2* SNP rs174602, located at an intron/exon boundary of the *FADS2* gene.. *FADS2* encodes for the Δ-5 desaturase enzyme that regulates the conversion of 20:3n-6 to 20:4n-6 (Arachidonic acid (AA)) and 20:4n-3 to 20:5n-3 (Eicosapentaenoic acid). Pregnancy is a complex period characterized by necessary metabolic adaptations, including alterations in lipid and glucose metabolism, to ensure an adequate supply of nutrients including DHA to the mother and growing fetus (52). During this critical period with heightened nutrient requirements, individuals with genotypes associated with lower endogenous conversion to DHA may be at greater risk for DHA deficiency and subsequently benefit more from supplementation with preformed DHA. One RCT in the United States showed that only among individuals with *FADS* SNPs associated with lower conversion of precursors (i.e., had minor alleles for *FADS* SNPs), prenatal DHA supplementation increased DHA concentrations and reduced AA:DHA ratios at delivery. These findings suggested a selective benefit of supplementation among carriers of variants for some *FADS* SNPs (53). A

recent birth cohort study in China also reported significant interaction between DHA supplementation and maternal SNP rs174602 on DHA concentrations in colostrum (54). Additionally, previous studies in European populations have shown that the CC genotype for SNP rs174602 is associated with lower Δ-5 desaturase activity (55).

We have also previously reported from the POSGRAD study that children born to TT carriers who received prenatal DHA had higher birthweight relative to those who received placebo, while no differences were observed among CC carriers (23). The fatty acid analysis performed in a subset of the study population (n = 140) showed that the minor T allele for SNP rs174602 was inversely associated with maternal plasma DHA concentrations at study enrollment. This suggests that these individuals were at greater risk of DHA deficiency, especially within the context of a diet high in n-6 fatty acids (23). To add further context to these differences in findings, it is important to consider ancestral variations in the distribution of *FADS* genotypes. Most studies assessing the role of maternal and offspring *FADS* genes in child health have been conducted in European populations (56, 57). However, Native American and Mexican populations have a greater proportion of carriers of alleles associated with slower conversion of precursors, along with diets high in n-6 PUFAs and low in n-3 LC-PUFAs (5, 58). Targeting provision of preformed LC-PUFAs to these populations may be particularly important. Overall, our findings reinforce the potential need for targeted interventions and inclusion of genotype information in the design and interpretation of supplementation trials to optimize benefit-risk ratios, particularly in the clinical nutrition setting. However, additional research is needed to reproduce these findings, confirm whether mother-offspring dyads with specific maternal genetic profiles and/or nutritional statuses benefit more from prenatal DHA supplementation, and determine whether it will ever be feasible to utilize genetic information in the design of public health interventions.

Several limitations should be acknowledged when interpreting our findings. First, selection bias may influence the generalizability of our results. The offspring in our study sample (32% of the birth cohort) tended to have mothers who were older and had higher BMI and household SES at study enrollment relative to those lost to follow up, but these values did not differ by prenatal treatment group or maternal genotype. Given the high loss to follow up, sample sizes were limited, which may have contributed to the non-significant findings in the TT group. Additionally, cardiometabolic health can be influenced by many factors over the life course, starting with prenatal and early life factors (e.g., maternal BMI during pregnancy, birth weight, infant feeding practices). However, lifestyle factors over childhood, including diet, physical activity, and maturation may be equally important in determining an individual's cardiometabolic risk (59). Although there were no differences in maternal characteristics at baseline and offspring characteristics at birth by treatment group or genotype, we did observe differences in diet at age 11 years. Total PUFA intake differed by treatment group, and MUFA and saturated fat intake differed by genotype. However, when interaction between treatment and genotype was considered, no differences in diet were observed. While these differences may be due to chance, we performed a sensitivity analysis additionally adjusting for PUFAs, MUFAs, and saturated fat, but effect estimates were not attenuated. There is potential bias related to the focus of this analysis on a SNP we have previously shown to be associated with birth weight; however, our findings remained significant even after adjusting for birth weight (i.e., the differences observed were not mediated by the effects of the interaction on birth weight).

Although PCA is a powerful dimensionality-reduction tool that allowed us to maximize power by reducing the number of tested outcomes, loading coefficients of individual cardiometabolic factors from PCA are only applicable to the population from which they are derived. Dietary data

were collected via single 24-hour recall and may be subject to recall bias. As the trial was not originally designed to assess offspring cardiometabolic health, sample sizes are small and there may be limited statistical power to detect differences by treatment group or genotype**.** Additionally, although plasma fatty acid concentrations were available in a small subset of the original birth cohort (n = 75), they were not included in the current manuscript due to small sample sizes. Future work should focus on identifying reliable markers of fatty acid status, which can potentially be used as a proxy for genotype. It is unclear whether the strong interaction observed with maternal genotype of SNP rs174602 is due to its high minor allele frequency in this sample (0.37), or because it is a functional SNP. To our knowledge, no biological function has been established for this SNP; it is therefore likely just a marker, not the causal variant. Further work is needed across larger, diverse datasets to reproduce these findings and investigate underlying biological mechanisms. Finally, while we show the possible importance of maternal *FADS* genotype in directing supplementation strategies, the role of offspring genotype remains unclear. Future work in adequately powered studies should incorporate offspring genotype information to elucidate this complex relationship.

Strengths of this study include the double-blind RCT design, high compliance to the prenatal intervention, extensive characterization of mothers and children throughout the trial and follow-up period, and availability of genetic information. Furthermore, our study participants were representative of a population with low dietary intakes of preformed DHA, high dietary intakes of n-6 fatty acids, and a high prevalence of alleles associated with lower conversion of precursor PUFAs into LC-PUFAs. Data collection and laboratory assays were standardized, validated, and conducted by trained personnel. The age at follow-up offers a stable time for lipid assessment, as

current guidelines promote universal screening of lipids in children aged 9-11 years to establish baseline cardiometabolic risk.

In summary, we showed that the effect of prenatal DHA supplementation on offspring MetS score at age 11 years differed by maternal *FADS2* SNP rs174602. Population differences in *FADS* genotypes and diet may partially explain mixed results observed across prenatal DHA supplementation trials. However, given the large variation in genotype distributions across populations, this work should be reproduced in larger, independent cohorts. These findings further support the need to incorporate genetic analysis of *FADS* polymorphisms in DHA supplementation trials and may ultimately help guide the development of targeted supplementation recommendations early in the life course to improve cardiometabolic health in the clinical setting.

5.6 References

1. Rinaudo P, Wang E. Fetal programming and metabolic syndrome. Annu Rev Physiol 2012;74:107-30. doi: 10.1146/annurev-physiol-020911-153245.
2. Koletzko B, Brands B, Poston L, Godfrey K, Demmelmair H. Early nutrition programming of long-term health. Proc Nutr Soc 2012;71(3):371-8. doi: 10.1017/s0029665112000596.
3. Guardamagna O, Abello F, Cagliero P, Lughetti L. Impact of nutrition since early life on cardiovascular prevention. Italian Journal of Pediatrics 2012;38(1):73. doi: 10.1186/1824-7288-38-73.
4. Innis SM. Metabolic programming of long-term outcomes due to fatty acid nutrition in early life. Matern Child Nutr 2011;7 Suppl 2(Suppl 2):112-23. doi: 10.1111/j.1740-8709.2011.00318.x.
5. Koletzko B, Reischl E, Tanjung C, Gonzalez-Casanova I, Ramakrishnan U, Meldrum S, et al. FADS1 and FADS2 Polymorphisms Modulate Fatty Acid Metabolism and Dietary Impact on Health. Annu Rev Nutr 2019;39:21-44. doi: 10.1146/annurev-nutr-082018-124250.
6. Voortman T, van den Hooven EH, Braun KV, van den Broek M, Bramer WM, Chowdhurry R, et al. Effects of polyunsaturated fatty acid intake and status during pregnancy, lactation, and early childhood on cardiometabolic health: A systematic review. Prog Lipid Res 2015;59:67-87. doi: 10.1016/j.plipres.2015.05.001.
7. Kabaran S, Besler HT. Do fatty acids affect fetal programming? J Health Popul Nutr 2015;33:14. doi: 10.1186/s41043-015-0018-9.
8. Sardinha FL, Fernandes FS, Tavares do Carmo MG, Herrera E. Sex-dependent nutritional programming: fish oil intake during early pregnancy in rats reduces age-dependent insulin

resistance in male, but not female, offspring. Am J Physiol Regul Integr Comp Physiol 2013;304(4):R313-20. doi: 10.1152/ajpregu.00392.2012.

9. D'Asti E, Long H, Tremblay-Mercier J, Grajzer M, Cunnane SC, Di Marzo V, et al. Maternal dietary fat determines metabolic profile and the magnitude of endocannabinoid inhibition of the stress response in neonatal rat offspring. Endocrinology 2010;151(4):1685-94. doi: 10.1210/en.2009-1092.

10. Hussain A, Nookaew I, Khoomrung S, Andersson L, Larsson I, Hulthén L, et al. A maternal diet of fatty fish reduces body fat of offspring compared with a maternal diet of beef and a post-weaning diet of fish improves insulin sensitivity and lipid profile in adult C57BL/6 male mice. Acta Physiol (Oxf) 2013;209(3):220-34. doi: 10.1111/apha.12130.

11. Siemelink M, Verhoef A, Dormans JA, Span PN, Piersma AH. Dietary fatty acid composition during pregnancy and lactation in the rat programs growth and glucose metabolism in the offspring. Diabetologia 2002;45(10):1397-403. doi: 10.1007/s00125-002-0918-2.

12. Voortman T, Tielemans MJ, Stroobant W, Schoufour JD, Kiefte-de Jong JC, Steenweg-de Graaff J, et al. Plasma fatty acid patterns during pregnancy and child's growth, body composition, and cardiometabolic health: The Generation R Study. Clin Nutr 2018;37(3):984-92. doi: 10.1016/j.clnu.2017.04.006.

13. Maslova E, Rifas-Shiman SL, Olsen SF, Gillman MW, Oken E. Prenatal n-3 long-chain fatty acid status and offspring metabolic health in early and mid-childhood: results from Project Viva. Nutr Diabetes 2018;8(1):29. doi: 10.1038/s41387-018-0040-2.

14. Satokar VV, Cutfield WS, Cameron-Smith D, Albert BB. Omega-3 fats in pregnancy: could a targeted approach lead to better metabolic health for children? Nutr Rev 2020. doi: 10.1093/nutrit/nuaa071.

15. Stratakis N, Gielen M, Chatzi L, Zeegers MP. Effect of maternal n-3 long-chain polyunsaturated fatty acid supplementation during pregnancy and/or lactation on adiposity in childhood: a systematic review and meta-analysis of randomized controlled trials. Eur J Clin Nutr 2014;68(12):1277-87. doi: 10.1038/ejcn.2014.158.

16. Middleton P, Gomersall JC, Gould JF, Shepherd E, Olsen SF, Makrides M. Omega-3 fatty acid addition during pregnancy. Cochrane Database Syst Rev 2018;11(11):Cd003402. doi: 10.1002/14651858.CD003402.pub3.

17. He S, Stein AD. Early-Life Nutrition Interventions and Associated Long-Term Cardiometabolic Outcomes: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Adv Nutr 2021;12(2):461-89. doi: 10.1093/advances/nmaa107.

18. Vahdaninia M, Mackenzie H, Dean T, Helps S. The effectiveness of ω-3 polyunsaturated fatty acid interventions during pregnancy on obesity measures in the offspring: an up-to-date systematic review and meta-analysis. Eur J Nutr 2019;58(7):2597-613. doi: 10.1007/s00394-018-1824-9.

19. Standl M, Thiering E, Demmelmair H, Koletzko B, Heinrich J. Age-dependent effects of cord blood long-chain PUFA composition on BMI during the first 10 years of life. British Journal of Nutrition 2014;111(11):2024-31. doi: 10.1017/S0007114514000105.

20. Weihrauch-Blüher S, Schwarz P, Klusmann JH. Childhood obesity: increased risk for cardiometabolic disease and cancer in adulthood. Metabolism 2019;92:147-52. doi: 10.1016/j.metabol.2018.12.001.

21. Schaeffer L, Gohlke H, Muller M, Heid IM, Palmer LJ, Kompauer I, et al. Common genetic variants of the FADS1 FADS2 gene cluster and their reconstructed haplotypes are associated with the fatty acid composition in phospholipids. Human molecular genetics 2006;15(11):1745-56. doi: 10.1093/hmg/ddl117.

22. Conway MC, McSorley EM, Mulhern MS, Strain JJ, van Wijngaarden E, Yeates AJ. Influence of fatty acid desaturase (FADS) genotype on maternal and child polyunsaturated fatty acids (PUFA) status and child health outcomes: a systematic review. Nutrition Reviews 2020;78(8):627-46. doi: 10.1093/nutrit/nuz086.

23. Molto-Puigmarti C, Jansen E, Heinrich J, Standl M, Mensink RP, Plat J, et al. Genetic variation in FADS genes and plasma cholesterol levels in 2-year-old infants: KOALA Birth Cohort Study. PLoS One 2013;8(5):e61671. doi: 10.1371/journal.pone.0061671.

24. Gonzalez-Casanova I, Rzehak P, Stein AD, Garcia Feregrino R, Rivera Dommarco JA, Barraza-Villarreal A, et al. Maternal single nucleotide polymorphisms in the fatty acid desaturase 1 and 2 coding regions modify the impact of prenatal supplementation with DHA on birth weight. Am J Clin Nutr 2016;103(4):1171-8. doi: 10.3945/ajcn.115.121244.

25. Tandon S, Gonzalez-Casanova I, Barraza-Villarreal A, Romieu I, Demmelmair H, Jones D, et al. Infant Metabolome in Relation to Prenatal DHA Supplementation and Maternal Single Nucleotide Polymorphism rs174602: Secondary Analysis of a Randomized Controlled Trial in Mexico. The Journal of Nutrition 2021(In press).

26. Gonzalez Casanova I, Schoen M, Tandon S, Stein AD, Barraza Villarreal A, DiGirolamo AM, et al. Maternal FADS2 single nucleotide polymorphism modified the impact of prenatal docosahexaenoic acid (DHA) supplementation on child neurodevelopment at 5

years: Follow-up of a randomized clinical trial. Clin Nutr 2021;40(10):5339-45. doi: 10.1016/j.clnu.2021.08.026.

27. Ramakrishnan U, Stein AD, Parra-Cabrera S, Wang M, Imhoff-Kunsch B, Juarez-Marquez S, et al. Effects of docosahexaenoic acid supplementation during pregnancy on gestational age and size at birth: randomized, double-blind, placebo-controlled trial in Mexico. Food and nutrition bulletin 2010;31(2 Suppl):S108-16. doi: 10.1177/15648265100312s203.

28. Koletzko B, Demmelmair H, Schaeffer L, Illig T, Heinrich J. Genetically determined variation in polyunsaturated fatty acid metabolism may result in different dietary requirements. Nestle Nutr Workshop Ser Pediatr Program 2008;62:35-44; discussion -9. doi: 10.1159/000146246.

29. Rzehak P, Thijs C, Standl M, Mommers M, Glaser C, Jansen E, et al. Variants of the FADS1 FADS2 gene cluster, blood levels of polyunsaturated fatty acids and eczema in children within the first 2 years of life. PLoS One 2010;5(10):e13261. doi: 10.1371/journal.pone.0013261.

30. Lattka E, Illig T, Koletzko B, Heinrich J. Genetic variants of the FADS1 FADS2 gene cluster as related to essential fatty acid metabolism. Curr Opin Lipidol 2010;21(1):64-9. doi: 10.1097/MOL.0b013e3283327ca8.

31. Lattka E, Koletzko B, Zeilinger S, Hibbeln JR, Klopp N, Ring SM, et al. Umbilical cord PUFA are determined by maternal and child fatty acid desaturase (FADS) genetic variants in the Avon Longitudinal Study of Parents and Children (ALSPAC). Br J Nutr 2013;109(7):1196-210. doi: 10.1017/s0007114512003108.

32. Lohman TG, Roche AF, Martorell R. Anthropometric standardization reference manual. Champaign, IL: Human Kinetics Books, 1988.

33. Myatt M, E G. Internet: https://nutriverse/zscorer/, https://github.com/nutriverse/zscorer.

34. Ramirez-Silva I, Rivera JA, Trejo-Valdivia B, Stein AD, Martorell R, Romieu I, et al. Relative Weight Gain Through Age 4 Years Is Associated with Increased Adiposity, and Higher Blood Pressure and Insulinemia at 4–5 Years of Age in Mexican Children. The Journal of Nutrition 2018;148(7):1135-43. doi: https://doi.org/10.1093/jn/nxy068.

35. Zimmet P, Alberti KGMM, Kaufman F, Tajima N, Silink M, Arslanian S, et al. The metabolic syndrome in children and adolescents – an IDF consensus report. Pediatric Diabetes 2007;8(5):299-306. doi: https://doi.org/10.1111/j.1399-5448.2007.00271.x.

36. Cossio-Bolaños M, Vidal-Espinoza R, de Campos FCC, Sulla-Torres J, Cossio-Bolaños W, Andruske CL, et al. Establishing percentiles for blood pressure based on absolute height for children and adolescents. BMC Pediatr 2021;21(1):26. doi: 10.1186/s12887-020-02489-9.

37. Lycett K, Juonala M, Magnussen CG, Norrish D, Mensah FK, Liu R, et al. Body Mass Index From Early to Late Childhood and Cardiometabolic Measurements at 11 to 12 Years. Pediatrics 2020;146(2):e20193666. doi: 10.1542/peds.2019-3666.

38. Magnussen CG, Cheriyan S, Sabin MA, Juonala M, Koskinen J, Thomson R, et al. Continuous and Dichotomous Metabolic Syndrome Definitions in Youth Predict Adult Type 2 Diabetes and Carotid Artery Intima Media Thickness: The Cardiovascular Risk in Young Finns Study. The Journal of Pediatrics 2016;171:97-103.e3. doi: 10.1016/j.jpeds.2015.10.093.

39. Lê S, Josse J, Husson F. FactoMineR: An R Package for Multivariate Analysis. Journal of Statistical Software 2008;25(1):1 - 18. doi: 10.18637/jss.v025.i01.

40. Parra-Cabrera S, Stein AD, Wang M, Martorell R, Rivera J, Ramakrishnan U. Dietary intakes of polyunsaturated fatty acids among pregnant Mexican women. Matern Child Nutr 2011;7(2):140-7. doi: 10.1111/j.1740-8709.2010.00254.x.

41. Ramirez-Silva I, Rivera JA, Trejo-Valdivia B, Martorell R, Stein AD, Romieu I, et al. Breastfeeding Status at Age 3 Months Is Associated with Adiposity and Cardiometabolic Markers at Age 4 Years in Mexican Children. The Journal of Nutrition 2015;145(6):1295-302. doi: 10.3945/jn.114.198366.

42. OMS/UNICEF/USA. Indicators for assessing infant and young child feeding practices. Geneva, Switzerland: World Health Organization, 2008.

43. Ramírez-Silva I, Rodríguez-Ramírez S, Barragán-Vázquez S, Castellanos-Gutiérrez A, Reyes-García A, Martínez-Piña A, et al. Prevalence of inadequate intake of vitamins and minerals in the Mexican population correcting by nutrient retention factors, Ensanut 2016. Salud Publica Mex 2020;62(5):521-31. doi: 10.21149/1109.

44. Ramírez Silva I B-VS, Rodríguez Ramírez S, Rivera Dommarco JA, Mejía-Rodríguez F, Barquera Cervera S, Tolentino Mayo L, Flores Aldana M, Villalpando Hernández S, Ancira Moreno M, et al. . Base de Alimentos de México (BAM): Compilación de la Composición de los Alimentos Frecuentemente Consumidos en el país, Version 18.1.1. 2021.

45. Hernández B, Gortmaker SL, Laird NM, Colditz GA, Parra-Cabrera S, Peterson KE. [Validity and reproducibility of a questionnaire on physical activity and non-activity for school children in Mexico City]. Salud Publica Mex 2000;42(4):315-23.

46. van Buuren S, Groothuis-Oudshoorn K. mice: Multivariate Imputation by Chained Equations in R. Journal of Statistical Software 2011;45(3):1 - 67. doi: 10.18637/jss.v045.i03.

47. Rytter D, Christensen JH, Bech BH, Schmidt EB, Henriksen TB, Olsen SF. The effect of maternal fish oil supplementation during the last trimester of pregnancy on blood pressure, heart rate and heart rate variability in the 19-year-old offspring. Br J Nutr 2012;108(8):1475-83. doi: 10.1017/s0007114511006799.

48. Rytter D, Schmidt EB, Bech BH, Christensen JH, Henriksen TB, Olsen SF. Fish Oil Supplementation During Late Pregnancy Does Not Influence Plasma Lipids or Lipoprotein Levels in Young Adult Offspring. Lipids 2011;46(12):1091-9. doi: 10.1007/s11745-011-3606-5.

49. Kerling EH, Hilton JM, Thodosoff JM, Wick J, Colombo J, Carlson SE. Effect of Prenatal Docosahexaenoic Acid Supplementation on Blood Pressure in Children With Overweight Condition or Obesity: A Secondary Analysis of a Randomized Clinical Trial. JAMA Netw Open 2019;2(2):e190088. doi: 10.1001/jamanetworkopen.2019.0088.

50. Gutierrez-Gomez Y, Stein AD, Ramakrishnan U, Barraza-Villarreal A, Moreno-Macias H, Aguilar-Salinas C, et al. Prenatal Docosahexaenoic Acid Supplementation Does Not Affect Nonfasting Serum Lipid and Glucose Concentrations of Offspring at 4 Years of Age in a Follow-Up of a Randomized Controlled Clinical Trial in Mexico. J Nutr 2017;147(2):242-7. doi: 10.3945/jn.116.238329.

51. Zeng Z, Liu F, Li S. Metabolic Adaptations in Pregnancy: A Review. Annals of Nutrition and Metabolism 2017;70(1):59-65. doi: 10.1159/000459633.

52. Scholtz SA, Kerling EH, Shaddy DJ, Li S, Thodosoff JM, Colombo J, et al. Docosahexaenoic acid (DHA) supplementation in pregnancy differentially modulates arachidonic acid and DHA status across FADS genotypes in pregnancy. Prostaglandins Leukot Essent Fatty Acids 2015;94:29-33. doi: 10.1016/j.plefa.2014.10.008.

53. Li P, Chen Y, Song J, Yan L, Tang T, Wang R, et al. Maternal DHA-rich n-3 PUFAs supplementation interacts with FADS genotypes to influence the profiles of PUFAs in the colostrum among Chinese Han population: a birth cohort study. Nutr Metab (Lond) 2022;19(1):48. doi: 10.1186/s12986-022-00683-3.

54. Bokor S, Dumont J, Spinneker A, Gonzalez-Gross M, Nova E, Widhalm K, et al. Single nucleotide polymorphisms in the FADS gene cluster are associated with delta-5 and delta-6 desaturase activities estimated by serum fatty acid ratios. J Lipid Res 2010;51(8):2325-33. doi: 10.1194/jlr.M006205.

55. Ameur A, Enroth S, Johansson A, Zaboli G, Igl W, Johansson AC, et al. Genetic adaptation of fatty-acid metabolism: a human-specific haplotype increasing the biosynthesis of long-chain omega-3 and omega-6 fatty acids. American journal of human genetics 2012;90(5):809-20. doi: 10.1016/j.ajhg.2012.03.014.

56. Mathias RA, Pani V, Chilton FH. Genetic Variants in the FADS Gene: Implications for Dietary Recommendations for Fatty Acid Intake. Curr Nutr Rep 2014;3(2):139-48. doi: 10.1007/s13668-014-0079-1.

57. Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, et al. A global reference for human genetic variation. Nature 2015;526(7571):68-74. doi: 10.1038/nature15393.

58. Drozdz D, Alvarez-Pitti J, Wójcik M, Borghi C, Gabbianelli R, Mazur A, et al. Obesity and Cardiometabolic Risk Factors: From Childhood to Adulthood. Nutrients 2021;13(11). doi: 10.3390/nu13114176.

5.7 Tables and Figures

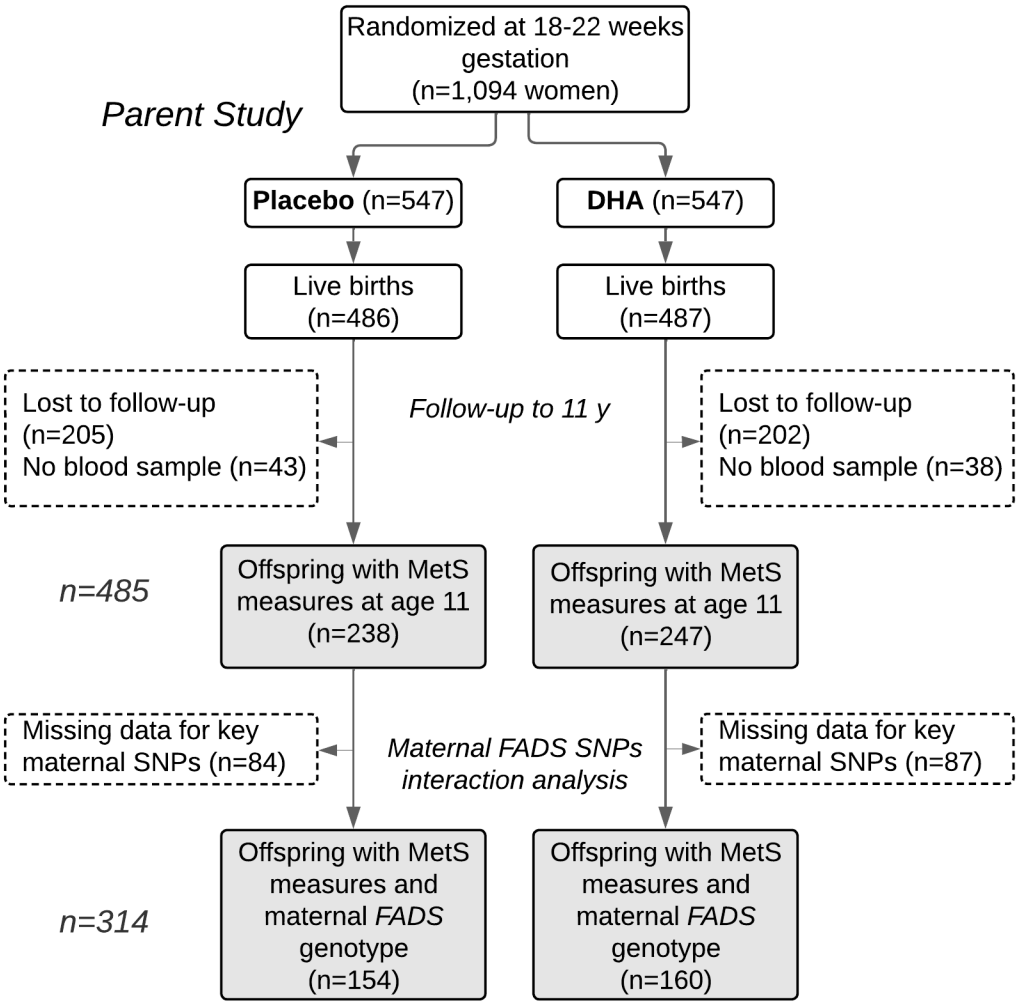


Figure 5-1. Procedures of study sample selection.
Abbreviations: DHA, Docosahexaenoic acid; MetS, Metabolic Syndrome; FADS, fatty acid desaturase

Table 5-1. Maternal baseline characteristics and offspring characteristics at birth, stratified by treatment group and maternal genotype of SNP rs174602.

	Treatment group[a]		Maternal Genotype of SNP rs174602[a]		
	Placebo N=154	DHA N=160	CC N=127	TC N=143	TT N=44
Maternal factors, enrollment					
Age, years	26.6 (4.61)	27.4 (5.18)	27.0 (4.81)	27.2 (5.01)	26.8 (5.02)
BMI, kg/m^2	26.3 (4.10)	26.6 (4.13)	26.3 (4.12)	26.4 (3.92)	27.1 (4.65)
Height, cm	156 (5.54)	155 (5.57)	155 (5.66)	155 (5.46)	157 (5.54)
First pregnancy, %	53 (34.4%)	45 (28.1%)	37 (29.1%)	50 (35.0%)	11 (25.0%)
SES Score[b]	0.13 (1.05)	0.13 (0.89)	0.01 (1.03)	0.13 (0.94)	0.46 (0.81)
Schooling, years	12.3 (3.39)	11.9 (3.45)	11.6 (3.56)	12.3 (3.40)	12.6 (2.96)
Dietary intake at enrollment, g/day					
ALA	1.76 (1.00)	1.79 (1.08)	1.76 (1.04)	1.80 (1.00)	1.73 (1.18)
DHA	0.08 (0.07)	0.08 (0.08)	0.08 (0.08)	0.08 (0.06)	0.10 (0.11)
LA	19.3 (7.76)	19.7 (8.64)	19.4 (7.78)	19.7 (7.79)	19.2 (10.6)
EPA	0.03 (0.04)	0.03 (0.04)	0.03 (0.04)	0.03 (0.03)	0.04 (0.06)
AA	0.15 (0.07)	0.16 (0.09)	0.15 (0.06)	0.16 (0.09)	0.15 (0.06)
Compliance to intervention, %	95.0 (5.00)	95.7 (4.99)	95.1 (5.55)	95.8 (4.53)	94.6 (4.76)
Offspring factors, birth					
Sex					
Male	81 (52.6%)	93 (58.1%)	69 (54.3%)	80 (55.9%)	25 (56.8%)
Female	73 (47.4%)	67 (41.9%)	58 (45.7%)	63 (44.1%)	19 (43.2%)
Birth weight, g	3220 (490)	3230 (449)	3229 (532)	3220 (440)	3231 (367)
Gestational age, weeks	39.0 (1.66)	39.1 (1.91)	38.8 (1.97)	39.1 (1.70)	39.4 (1.44)
Breastfeeding status, 3 mo, %					
Exclusively fed human milk	23 (16.2%)	17 (12.0%)	18 (15.8%)	15 (11.6%)	7 (17.1%)
Predominantly fed human milk	20 (14.1%)	15 (10.6%)	11 (9.65%)	17 (13.2%)	7 (17.1%)
Partially fed human milk	75 (52.8%)	80 (56.3%)	59 (51.8%)	78 (60.5%)	18 (43.9%)
Not fed human milk	24 (16.9%)	30 (21.1%)	26 (22.8%)	19 (14.7%)	9 (22.0%)
Duration of human milk feeding, mo	9.71 (7.96)	8.84 (7.65)	8.15 (6.86)	10.3 (8.56)	9.21 (7.53)

[a] Chi-square tests, t-tests, and ANOVA were used to test differences between groups. No differences by treatment group were observed. Values presented are mean (SD) unless otherwise stated.

[b] Differs by maternal genotype ($p < 0.05$)

Abbreviations: BMI, body mass index; SES, socioeconomic status score; ALA, Alpha-Linolenic Acid; DHA, Docosahexaenoic Acid; LA, Linoleic Acid; EPA, Eicosapentaenoic Acid; AA, Arachidonic Acid; mo, month.

Table 5-2. Offspring characteristics at 11 years follow up, stratified by treatment group and maternal genotype of SNP rs174602.

	Prenatal treatment group[a]		Maternal Genotype of SNP rs174602[a]		
	Placebo	DHA	CC	TC	TT
Dietary intake[b]					
Energy intake (kcal/d)	1904 (595)	2064 (746)	1999 (709)	2216 (660)	2216 (660)
Cholesterol (g/d)	246 (183)	300 (219)	276 (201)	328 (235)	328 (235)
Lipids (g/d)	70.2 (33.8)	78.3 (35.7)	75.3 (35.0)	85.2 (42.0)	85.2 (42.0)
Saturated fat (g/d)[c]	27.5 (14.2)	30.6 (17.7)	28.8 (15.3)	35.8 (18.4)	35.8 (18.4)
Monounsaturated fat (g/d)[c]	23.3 (12.8)	26.1 (12.9)	24.8 (12.7)	30.0 (16.9)	30.0 (16.9)
Polyunsaturated fat (g/d)[d]	13.6 (8.21)	16.2 (9.55)	14.9 (9.06)	16.3 (9.03)	16.3 (9.03)
Total omega-3 intake (g/day)[d]	0.71 (0.65)	1.09 (1.39)	0.88 (1.08)	0.80 (0.67)	0.80 (0.67)
Total omega-6 intake (g/day)[d]	5.32 (4.67)	7.34 (7.73)	6.71 (7.49)	6.12 (4.33)	6.12 (4.33)
Total sedentary time, hours/day	4.82 (2.21)	4.75 (2.06)	4.83 (2.05)	5.03 (2.31)	5.03 (2.31)
Maturation					
Females: attained menarche, n (%)	13 (8.5%)	11 (6.9%)	12 (8.39%)	3 (6.82%)	3 (6.82%)
Testosterone concentrations (pg/mL)	1.55 (0.84)	1.55 (0.72)	1.44 (0.77)	1.38 (0.65)	1.38 (0.65)
Age at examination, years	11.12 (0.20)	11.12 (0.17)	11.13 (0.18)	11.14 (0.16)	11.14 (0.16)

[a] Chi-square tests, t-tests, and ANOVA were used to test differences between groups. Values presented are mean (SD) unless otherwise stated.
[b] Dietary data only available for 229 individuals at age 11 years
[c] Differs by maternal genotype ($p < 0.05$)
[d] Differs by maternal prenatal treatment group ($p < 0.05$)

Table 5-3. MetS components by treatment allocation and maternal genotype of SNP rs174602 (n=314).

	Treatment group		Maternal genotype		
	Placebo N=154	DHA N=160	CC N = 127	TC N = 143	TT N = 44
Glucose (mg/dL)	88.2 (86.9, 89.5)	87.3 (86.0, 88.6)	86.6 (85.1, 88.1)	88.3 (87.0, 89.6)	89.0 (86.6, 91.4)
HDL (mg/dL)	46.9 (45.1, 48.6)	46.0 (44.3, 47.8)	45.8 (43.8, 47.7)	46.5 (44.7, 48.2)	48.4 (45.2, 51.6)
Triglycerides (mg/dL)	104 (94.3, 113)	111 (101.7, 120)	113 (102.7, 123)	104 (94.2, 113)	103 (85.5, 120)
SBP (mmHg)	103 (101, 104)	102 (101, 104)	103 (101.6, 105)	102 (100.5, 103)	102 (99.1, 104)
BMI z-score	0.64 (0.43, 0.85)	0.69 (0.49, 0.91)	0.88 (0.65, 1.12)	0.55 (0.22, 0.76)	0.48 (0.08, 0.87)
MetS Score	-0.01 (-0.10, 0.07)	0.01 (-0.08, 0.09)	0.06 (-0.03, 0.16)	-0.04 (-0.12, 0.05)	-0.07 (-0.22, 0.08)

Values presented are adjusted means (95% CI).
Abbreviations: HDL, high-density lipoprotein cholesterol; SBP, systolic blood pressure; BMI, body mass index; MetS, metabolic syndrome.

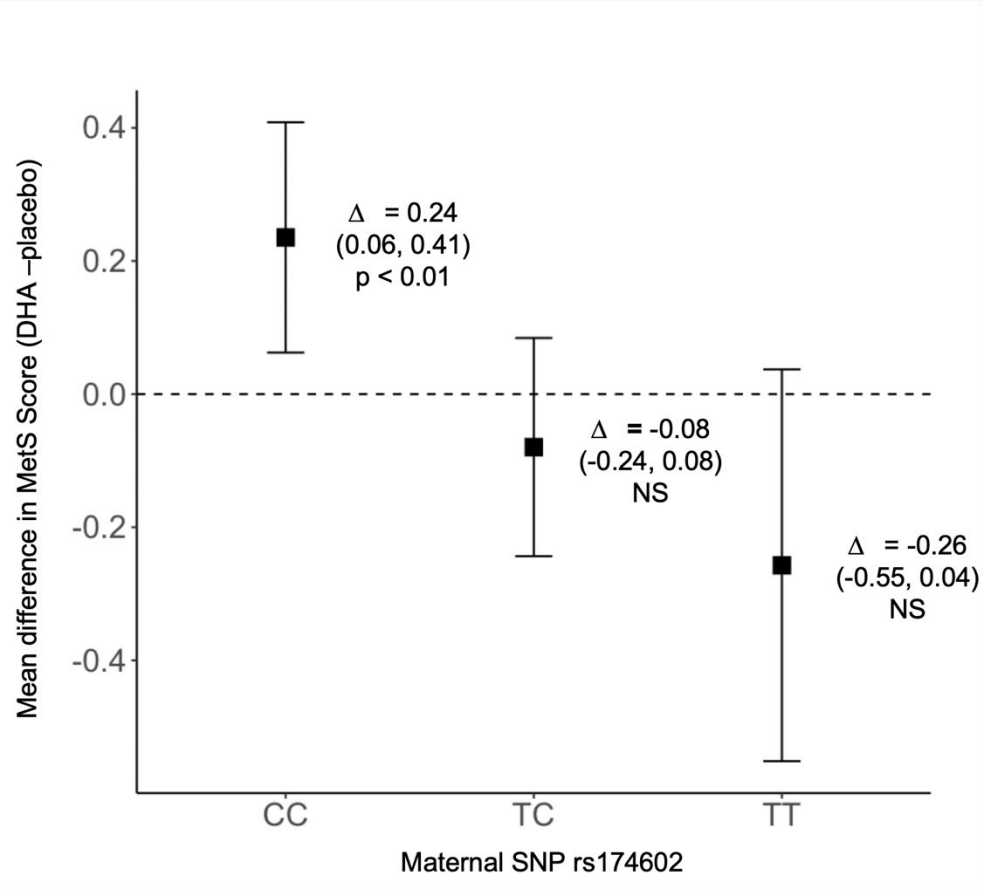


Figure 5-2. Effect modification by maternal SNP rs174602 on offspring MetS Score.
Values are contrast-specific mean differences (95% CI) between DHA and placebo groups from generalized linear models testing the interaction between *FADS2* single nucleotide polymorphism rs174602 and supplementation group on MetS score adjusted for child sex and age at measurement, maternal SES, BMI, parity and age at trial enrollment (p-interaction = 0.001).
Abbreviations: DHA, docosahexaenoic acid; MetS, metabolic syndrome; NS, not significant; SNP, single nucleotide polymorphism

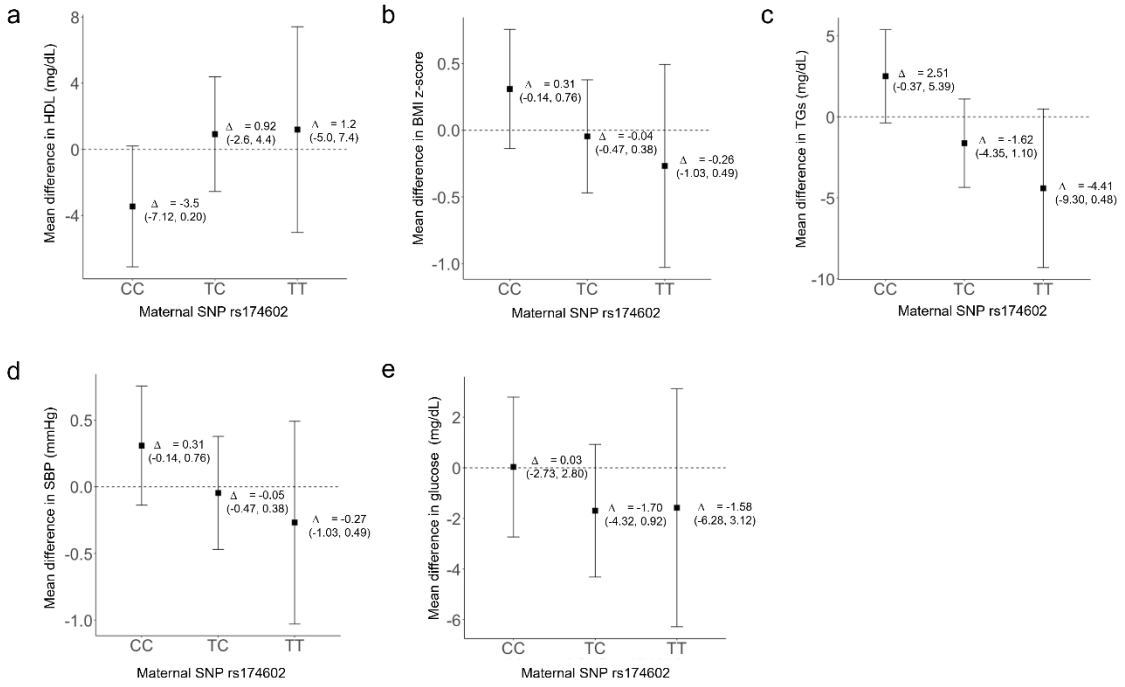


Figure 5-3. Effect modification by maternal SNP rs174602 on the impact of DHA supplementation on offspring MetS components.
Plots include a) HDL (p-interaction = 0.10); b) BMI z-score (p-interaction = 0.14); c) triglycerides (p-interaction = 0.03); d) systolic blood pressure (p-interaction = 0.01); and e) glucose (p-interaction = 0.43). Values are contrast-specific mean differences (95% CI) between DHA and placebo groups from generalized linear models testing the interaction between *FADS2* single nucleotide polymorphism rs174602 and supplementation group on MetS components adjusted for child sex and age at measurement, maternal SES, BMI, parity, and age at trial enrollment.
Abbreviations: SNP, single nucleotide polymorphism; HDL, High Density Lipoprotein cholesterol; BMI, body mass index; TGs, triglycerides; SBP, systolic blood pressure.

5.8 Supplementary Tables and Figures

Supplementary Table 5-1. Comparison of maternal characteristics at study enrollment and offspring characteristics in early life in analytic and excluded samples.

	Excluded sample N=659	Analytic sample N=314
Maternal factors at trial enrollment		
Age, year[a]	25.9 (4.57)	27.0 (4.91)
BMI, kg/m^{2}[a]	25.8 (4.27)	26.4 (4.11)
Height, cm	155 (5.76)	155 (5.57)
Parity	1.95 (1.05)	2.08 (1.00)
SES[a]	-0.02 (1.00)	0.13 (0.97)
Schooling, year	11.9 (3.59)	12.1 (3.42)
Dietary intake at enrollment (g/day)		
ALA	1.66 (0.95)	1.77 (1.04)
DHA	0.08 (0.09)	0.08 (0.08)
LA	19.0 (9.54)	19.5 (8.21)
EPA	0.03 (0.05)	0.03 (0.04)
AA	0.15 (0.09)	0.15 (0.08)
Received intervention	327 (49.6%)	160 (51.0%)
Compliance to intervention (%)	94.9 (5.95)	95.3 (5.00)
Offspring factors in early life		
Sex		
Male	339 (51.4%)	174 (55.4%)
Female	320 (48.6%)	140 (44.6%)
Birth weight, g	3195 (456)	3225 (469)
Gestational age at birth, weeks	39.1 (1.78)	39.1 (1.79)
Breastfeeding status, 3 months		
Exclusively fed human milk	64 (11.6%)	40 (14.1%)
Predominantly fed human milk	64 (11.6%)	35 (12.3%)
Partially fed human milk	332 (60.0%)	155 (54.6%)
Not fed human milk	93 (16.8%)	54 (19.0%)
Duration of BF, months	8.34 (7.62)	9.26 (7.81)

Values presented are mean (SD) unless otherwise stated.

[a] Values differ between analytic and excluded samples, as assessed by chi-square tests, t-tests, and/or ANOVA.

Abbreviations: BMI, body mass index; SES, socioeconomic status score; ALA, Alpha-Linolenic Acid; DHA, Docosahexaenoic Acid; LA, Linoleic Acid; EPA, Eicosapentaenoic Acid; AA, Arachidonic Acid; EBF, Exclusive Breastfeeding; PreBF, Predominantly Breastfeeding; PaBF, Partially Breastfeeding; NBF, No Breastfeeding

Supplementary Table 5-2. Maternal baseline characteristics and offspring characteristics at birth, stratified by treatment group and maternal genotype of SNP rs174602.

	CC		TC		TT	
	Placebo N=65	DHA N=62	Placebo N=66	DHA N=77	Placebo N=23	DHA N=21
Maternal factors, enrollment						
Age, year	26.6 (4.51)	27.4 (5.11)	26.8 (4.65)	27.4 (5.31)	26.1 (4.91)	27.6 (5.15)
BMI, kg/m^2	26.4 (4.30)	26.2 (3.96)	26.0 (3.49)	26.7 (4.26)	27.0 (5.11)	27.3 (4.20)
Height, cm	155 (4.96)	154 (6.32)	155 (5.67)	155 (5.30)	158 (6.17)	155 (4.19)
First pregnancy, %	19 (29.2%)	18 (29.0%)	28 (42.4%)	22 (28.6%)	6 (26.1%)	5 (23.8%)
SES Score [b]	0.00 (1.09)	0.02 (0.98)	0.14 (1.06)	0.13 (0.84)	0.50 (0.86)	0.42 (0.77)
Schooling, year	12.1 (3.40)	11.1 (3.67)	12.2 (3.60)	12.4 (3.24)	12.9 (2.75)	12.4 (3.22)
Dietary intake at enrollment, g/day						
ALA	1.79 (0.94)	1.73 (1.15)	1.70 (0.92)	1.88 (1.07)	1.85 (1.39)	1.61 (0.92)
DHA	0.08 (0.06)	0.08 (0.10)	0.08 (0.06)	0.08 (0.06)	0.09 (0.13)	0.10 (0.09)
LA	19.3 (6.71)	19.4 (8.83)	18.9 (6.84)	20.3 (8.51)	20.1 (12.2)	18.1 (8.75)
EPA	0.03 (0.03)	0.03 (0.05)	0.03 (0.03)	0.03 (0.03)	0.04 (0.07)	0.05 (0.05)
AA	0.16 (0.07)	0.15 (0.06)	0.15 (0.07)	0.16 (0.11)	0.16 (0.07)	0.15 (0.06)
Compliance to intervention, %	95.1 (4.86)	95.1 (6.25)	95.6 (4.89)	95.9 (4.23)	92.9 (5.42)	96.4 (3.11)
Offspring factors, birth						
Females	32 (49.2%)	26 (41.9%)	30 (45.5%)	33 (42.9%)	11 (47.8%)	8 (38.1%)
Birth weight, g	3309 (562)	3144 (489)	3159 (453)	3273 (424)	3145 (328)	3326 (392)
Gestational age, weeks	39.0 (1.74)	38.7 (2.19)	38.9 (1.71)	39.3 (1.69)	39.3 (1.28)	39.5 (1.62)
Breastfeeding status, 3 mo, %						
Exclusively fed human milk	12 (20.7%)	6 (10.7%)	8 (12.9%)	7 (10.4%)	3 (13.6%)	4 (21.1%)
Predominantly fed human milk	5 (8.62%)	6 (10.7%)	10 (16.1%)	7 (10.4%)	5 (22.7%)	2 (10.5%)
Partially fed human milk	31 (53.4%)	28 (50.0%)	34 (54.8%)	44 (65.7%)	10 (45.5%)	8 (42.1%)
Not fed human milk	10 (17.2%)	16 (28.6%)	10 (16.1%)	9 (13.4%)	4 (18.2%)	5 (26.3%)
Duration of BF, mo	8.56 (7.30)	7.73 (6.40)	10.9 (8.77)	9.74 (8.40)	9.63 (7.20)	8.75 (8.03)
Offspring factors, 11 years						
Dietary intake[a]						
Energy intake (kcal/d)	1809 (553)	1969 (720)	1945 (581)	2041 (796)	2031 (721)	2425 (530)
Cholesterol (g/d)	232 (155)	275 (226)	257 (200)	291 (202)	256 (210)	410 (241)
Lipids (g/d)	65.8 (30.3)	73.3 (32.3)	71.7 (30.1)	77.9 (38.3)	77.0 (48.5)	94.5 (32.2)
Saturated fat (g/d)	26.0 (14.1)	28.1 (17.5)	28.0 (13.3)	29.4 (16.8)	30.0 (16.6)	42.5 (18.4)
Monounsaturated fat (g/d)	21.0 (10.1)	24.3 (11.6)	23.6 (11.0)	25.7 (13.8)	27.8 (20.6)	32.5 (11.5)
Polyunsaturated fat (g/d)	12.8 (7.44)	16.1 (10.1)	13.7 (7.81)	15.8 (9.88)	15.0 (10.9)	17.8 (6.39)
Total omega-3 intake (g/day)	0.74 (0.69)	1.24 (1.66)	0.69 (0.62)	1.03 (1.32)	0.70 (0.69)	0.91 (0.65)
Total omega-6 intake (g/day)	4.89 (4.70)	7.32 (7.04)	5.53 (4.90)	7.60 (8.92)	5.84 (4.16)	6.44 (4.64)
Total sedentary time, hrs/day	4.74 (2.47)	4.56 (1.83)	4.88 (1.90)	4.80 (2.19)	4.90 (2.32)	5.19 (2.35)
Maturation						
Attained menarche (F), n (%)	4 (6.25%)	5 (8.06%)	7 (10.6%)	5 (6.49%)	2 (8.70%)	1 (4.76%)
Testosterone (M) (pg/mL)	1.76 (0.87)	1.68 (0.77)	1.44 (0.83)	1.43 (0.70)	1.28 (0.68)	1.51 (0.62)
Age at examination, years	11.12 (0.21)	11.08 (0.18)	11.12 (0.20)	11.14 (0.15)	11.11 (0.15)	11.18 (0.18)

Values presented are mean (SD) unless otherwise stated.

[a]Dietary data only available for 229 individuals at age 11 years

Abbreviations: BMI, body mass index; SES, socioeconomic status score; ALA, Alpha-Linolenic Acid; DHA, Docosahexaenoic Acid; LA, Linoleic Acid; EPA, Eicosapentaenoic Acid; AA, Arachidonic Acid; EBF, Exclusive Breastfeeding; PreBF, Predominantly Breastfeeding; PaBF, Partially Breastfeeding; NBF, No Breastfeeding

a

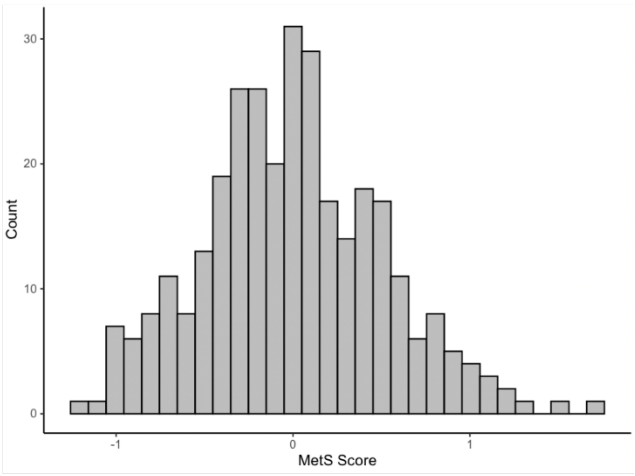
MetS Component	PC 1	PC 2
Fasting Glucose	4.24	3.51
Triglycerides	36.35	0.1
HDL	31.38	6.95
SBP	0.19	88.61
BMI z-score	27.83	0.83
Eigenvalue	1.74	1.03
% variance explained	35.0	20.7

Contributions of variables to each PC (%)

b

MetS Score = [% variance explained by PC 1(PC1)] + [% variance explained by PC 2(PC2)]

c



Supplementary Figure 5-1. Contribution of cardiometabolic factors to PCA-derived continuous MetS score.

a) Principal components analysis (PCA) was used to calculate the MetS score using five components used to define metabolic syndrome in pediatric populations. Top PCs were identified visually using scree plots and quantitatively using eigenvalues > 1. Scores were calculated as the sum of the top components, weighted by variance explained. The x axes represent the top PCs used to create the MetS score and percentage of variance in the data explained by each PC via PCA. The diameter of each bubble represents the magnitude of contributions (%) of individual MetS components to each PC. Cardiometabolic factors with the highest contributions represent the most important variables within each PC. The highest contributing components in the first PC contribute most to the derived scores, **b)** equation used to calculate MetS scores for participants in the study population, and **c)** distribution of continuous MetS score in study population

MetS, metabolic syndrome; PC, Principal Component

Supplementary Table 5-3. Offspring cardiometabolic health at 11 years follow up, stratified by treatment group and maternal genotype of SNP rs174602.

	CC		TC		TT	
	Placebo	DHA	Placebo	DHA	Placebo	DHA
	N=65	N=62	N=66	N=77	N=23	N=21
Cardiometabolic risk factors						
BMI z-score	0.69 (1.26)	0.98 (1.18)	0.52 (1.46)	0.51 (1.28)	0.65 (1.58)	0.40 (1.52)
Triglycerides	101 (45.3)	122 (74.1)	101 (53.8)	102 (55.2)	112 (51.8)	92.5 (47.0)
HDL	47.3 (10.0)	43.9 (11.5)	46.1 (9.78)	46.7 (10.4)	47.3 (10.1)	48.7 (11.4)
SBP	102 (7.32)	104 (8.94)	103 (7.55)	101 (8.63)	104 (10.3)	99.8 (7.25)
Glucose	86.9 (6.12)	87.2 (10.0)	89.3 (8.63)	88.0 (8.41)	90.3 (5.15)	88.9 (6.07)
MetS Score	-0.05 (0.44)	0.17 (0.54)	-0.01 (0.55)	-0.07 (0.53)	0.09 (0.38)	-0.17 (0.43)

Values presented are unadjusted means (SD).
BMI, Body Mass Index; DHA, Docosahexaenoic Acid; HDL, High Density Lipoprotein cholesterol; SBP, Systolic Blood Pressure; MetS, Metabolic Syndrome

Chapter 6 : Maternal and offspring fatty acid desaturase variants, prenatal DHA supplementation, and dietary n-6:n-3 fatty acid ratio in relation to cardiometabolic health in Mexican children

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6.1 Abstract

Objective: We evaluated the role of selected variants in maternal and offspring fatty acid desaturase (*FADS)* genes on offspring cardiometabolic health (CMH) at age 11y and assessed interactions of genotype with diet quality and prenatal docosahexaenoic acid (DHA) supplementation.

Methods: We used data from offspring (n=203) born to women who participated in a randomized controlled trial of prenatal DHA supplementation (400 mg/d) from mid-gestation through delivery in Mexico. We generated a continuous metabolic syndrome (MetS) score from body mass index, HDL cholesterol, triglycerides, systolic blood pressure, and fasting glucose measured at age 11 y and identified six distinct haplotypes from five offspring *FADS* SNPs (rs174578, rs2727271, rs174602, rs174605, rs174550). Dietary n-6:n-3 fatty acid ratios were derived from single 24-hour recalls (n=141). We used generalized linear models to test associations of offspring diet and *FADS* haplotypes with MetS score. We examined interactions of maternal and offspring *FADS* SNP rs174602 with prenatal treatment group and with dietary n-6:n-3 ratio on MetS score.

Results: Associations between all examined *FADS* haplotypes and MetS score were null. Offspring SNP rs174602 did not modify the association of prenatal DHA supplementation with MetS score. Among children with TT or TC genotype for SNP rs174602 (n = 88), those in the highest n-6:n-3 ratio tertile (> 8.61) had higher MetS score relative to those in the lowest tertile (< 6.67) (Δ= 0.36; 95% CI: 0.03, 0.69). Among children with CC genotype (n = 53), those in the highest n-6:n-3 ratio tertile had lower MetS score relative to those in the lowest tertile (Δ= -0.23; 95% CI: -0.61, 0.16).

Conclusions: In this population of Mexican children, there was evidence of interaction of offspring *FADS* SNP rs174602 with current dietary PUFA intake, but not with prenatal DHA

supplementation, on MetS score. Further studies are needed to validate these findings in other settings to determine the utility of targeted supplementation strategies and dietary recommendations based on genetic profile.

6.2 Introduction

Cardiometabolic diseases, which include cardiovascular disease, stroke, and type II diabetes mellitus, are the leading causes of mortality worldwide (1). Increasing evidence suggests that cardiometabolic risk factors emerge in childhood and persist through adulthood, highlighting the need to identify early and effective disease prevention strategies (2, 3). While cardiometabolic diseases have complex etiologies including genetic, behavioral, and environmental factors, nutritional status during critical periods of growth and development, such as gestation and early adolescence, may be a key modifiable determinant (4).

Dietary polyunsaturated fatty acids (PUFAs), which include n-6 linoleic acid (LA) and n-3 alpha-linolenic Acid (ALA), are important for cardiometabolic health (CMH) because they are precursors to the long-chain PUFAs (LC-PUFAs) n-6 arachidonic acid (AA), n-3 eicosapentaenoic acid (EPA) and n-3 docosahexaenoic acid (DHA), which have opposing roles in modulating inflammation (5). LC-PUFAs also contribute to cell membrane synthesis, gene expression, and appetite signaling, and have regulatory effects on enzymes involved in lipid and glucose metabolism (6). While much research has focused on the cardioprotective benefits of n-3 LC-PUFAs via improvements in insulin resistance, blood pressure, and triglycerides (7, 8), the relationship between n-6 fatty acids and cardiovascular health is less clear. In adults, some evidence suggests that high dietary n-6:n-3 ratios (~16:1) are associated with the pathogenesis of cardiovascular disease, diabetes, cancer, and inflammatory diseases (9, 10); however, other studies report that a combination of both n-6 and n-3 fatty acids is associated with lower inflammation,

and circulating concentrations of n-6 fatty acids are inversely associated with LDL cholesterol and cardiovascular risk (11-13). Overall, the role of both n-3 and n-6 LC-PUFAs in pediatric cardiometabolic health remains poorly characterized (14, 15).

Additionally, the influence of dietary PUFAs on CMH may differ by variations in fatty acid desaturase (*FADS*) genes (16, 17). Conversion of n-6 LA and n-3 ALA to their LC-PUFA forms is regulated by Δ5 and Δ6-desaturase enzymes encoded in the *FADS* gene cluster (*FADS1, FADS2, FADS3*) located in chromosome 11. Genome wide association studies (GWAS) have identified multiple variants in *FADS* genes that are associated with lower LC-PUFA concentrations, indicating reduced conversion of dietary precursors, along with alterations in lipid profiles and glucose homeostasis (18, 19). Studies show that multiple variants in *FADS* genes are associated with lower lipid profiles and inflammation in European children (20-22), but there is evidence of ancestral variation in the frequency of alleles associated with reduced conversion of PUFAs across populations. Compared to European populations, Mexican populations have a greater proportion of carriers of alleles associated with slower conversion of precursors, as well as diets high in n-6 and low in n-3 LC-PUFAs (18). Additional studies are needed across populations that vary in both genotype and diet quality to determine the utility of targeted dietary or supplementation recommendations based on genetic profile.

To our knowledge, no prior studies have characterized children spanning from the prenatal period through early adolescence with *FADS* genetic information for both the mother and child. In the present study, we address these gaps by using follow-up data from a large prenatal DHA supplementation randomized controlled trial (RCT) in Mexico, in which children have been followed prospectively since birth. We previously reported that maternal SNP rs174602 modified the effect of prenatal DHA supplementation on offspring birth weight (23), the infant metabolome

at 3 months (24), and offspring MetS scores at age 11 years (unpublished). However, the respective contributions of maternal and offspring *FADS* genetic profile towards CMH remain unclear. The objective of this study is to evaluate the role of selected variants in both maternal and offspring fatty acid desaturase (*FADS)* genes on CMH at age 11 years and assess interactions with 1) prenatal docosahexaenoic acid (DHA) supplementation and 2) offspring diet quality at age 11 years. We hypothesized that children who carry FADS alleles associated with more rapid conversion of precursors would have poorer CMH profiles relative to those with slower conversion. Secondarily, we hypothesized that maternal genotype would be more relevant than offspring genotype for modifying the effect of the prenatal DHA intervention whereas offspring genotype would be more relevant for modifying the effects of dietary intake on CMH.

6.3 Methods

6.3.1 Study Design and Participants

This study includes data from the Prenatal Omega-3 fatty acid Supplementation and Child Growth and Development (POSGRAD) trial in Cuernavaca, Mexico (NCT00646360). The original trial design, protocol, and inclusion/exclusion criteria has been published elsewhere (25). Briefly, from 2005-2007, 1,094 pregnant women were recruited at 18-22 weeks gestation at the Mexican Social Security Institute (IMSS) and randomized to receive either 400 mg algal DHA (treatment) or a corn/soy oil blend (placebo) daily through delivery. The fatty acid composition of the supplements has been previously published (24). Intervention compliance was measured as the percentage of capsules consumed. The 968 women who completed the study delivered 963 singleton live births and 5 pairs of twins (excluded from the present analysis). Children have been followed prospectively since birth and were most recently followed up at 11 years (2016-2018).

Of the 566 children who were successfully contacted and agreed to participate in the follow-up study, venous blood samples were collected in a sub-sample of 485 children.

The Emory University Institutional Review Board and the Ethics Board of the Mexican National Institute of Public Health (INSP) reviewed and approved all procedures involving human subjects. The study was conducted according to the guidelines of the Declaration of Helsinki. Written informed consent was obtained from mothers at trial enrollment. At the 11-year follow up visit, mothers provided written informed consent and children provided written assent.

6.3.2 Maternal Genotyping

Fasting venous blood samples were collected from all women at recruitment, and genomic information was extracted from the buffy coat using standard techniques. Buffy coat was separated and stored at INSP laboratories at −80°C until transport to the Helmholtz Center, Munich, Germany. The genetic analysis was carried out in 2012-13 for samples from women who provided consent to genotyping (n = 720) using methods that have been previously described (23, 26). The resulting dataset containing 15 SNPs across the *FADS* gene cluster was transferred to Emory University via encrypted files (23). For the purposes of this analysis, we only used data for maternal SNP rs174602.

6.3.3 Offspring Genotyping

Stored blood samples obtained from offspring at age 4 y were transported to LMU University of Munich and Helmholtz Center Munich for genotyping via Illumina microarray technology. DNA was extracted from stored buffy coat with the use of a High Pure PCR Template Preparation Kit (Roche). A total of 5 mL DNA was subjected to polymerase chain reaction amplification followed by the genotyping procedure with the use of the MassARRAY system and

iPLEX chemistry as suggested by the manufacturer (Sequenom) and previously described in detail (26).

Data quality was assessed using GenomeStudio version 2.0.4 and samples with call rate <99% were tested for potential contamination; data were cleaned locally and shared with Emory University. Quality control assessments were performed in PLINK version 1.9 to filter out samples and SNPs with minor allele frequency (MAF<0.1), SNP call rate < 95%, and sample call rate < 90%. Genetic data were subset to chromosome 11 and mapped to the human genome reference build GRCh37/hg19 for strand, id names, positions, alleles, and reference/alternative alleles assignment (27). The data were subset to available SNPs in the *FADS* gene region (rs174545 to rs1000778), resulting in 31 *FADS* variants for 626 children. Pairwise linkage disequilibrium (LD) among the 31 SNPs were calculated as both D' and r^2 using Haploview software, version 4.2 (28). Subsequently, 5 SNPs that captured all 31 SNPs at $r^2 \geq 0.3$ (rs174578, rs2727271, rs174602, rs174605, rs174450) were selected via Tagger for further analysis (**Supplementary Figure 6-1**).

6.3.4 Anthropometry and blood pressure measurements at age 11 y

Body weight (kg) and height (cm) were collected in triplicate by trained personnel using standard procedures, and the average of the three measurements was used (29). Children wore light clothing and were weighed using a portable electronic pediatric scale (Tanita model 1582) with a precision of 100 g, which was calibrated daily with a known reference weight. Height was measured with a stadiometer (precision of 0.1 cm). We used the 'zscorer' R package to calculate BMI-for-age z-scores according to the 2007 WHO Growth Reference Standards (30). Blood pressure (mmHg) was measured when the child was at rest using a digital device (OMRON model HEM-711ACINT), which has been validated for use in children and adolescents. In each arm, four

measurements were taken with 2-minute intervals; the first measurement was discarded and the last three were averaged (31).

6.3.5 Assessment of cardiometabolic markers and derivation of continuous MetS score

Venous blood samples were obtained from children at 11y after a 12-hour fasting period (verified by noting the approximate time that food/drink was last consumed) and centrifuged. Aliquots of serum were frozen in liquid nitrogen and stored at −80°C at the National Institute of Medical Sciences and Nutrition Salvador Zubirán in Mexico. HDL cholesterol, triglyceride, and glucose concentrations were assessed using the Beckman Coulter SYNCHRON CX 5 Delta automated kit and expressed in mg/dL. While there is heterogeneity in metabolic syndrome criteria in pediatric populations, cardiometabolic risk factor thresholds were defined according to criteria from the International Diabetes Federation for children ages 10-16 y as follows: triglycerides ≥ 150 mg/dL, HDL < 40 mg/dL, and fasting glucose ≥ 100 mg/dL, while high systolic blood pressure (SBP) was defined as ≥ 90^{th} percentile for sex and height (32, 33).

We used principal components analysis (PCA) to derive a continuous MetS score using systolic blood pressure, HDL, triglycerides, BMI z-score, and glucose. The methods have been described previously elsewhere (34). Briefly, principal components (PCs) were identified visually using scree plots and quantitatively using eigenvalues > 1, and the score was calculated as the sum of the first two components, weighted by variance explained. The score can be interpreted as a z-score, with higher scores representing increased cardiometabolic risk. PCA was performed using the 'FactoMineR' R package (35). The top two PCs collectively explained 56% of the variance in the measured data (**Supplementary Figure 6-2a**). Triglycerides, BMI-z, and HDL contributed to the first PC (34.8% variance explained), while SBP and fasting glucose contributed to the second

PC (20.9% variance explained). The distribution of the MetS score in the study population is shown in **Supplementary Figure 6-2b**.

6.3.6 Diet assessment and dietary n-6:n-3 ratio

A single multiple-pass 24-hour dietary recall developed for Mexican populations was used to assess dietary intake of the children at age 11 y (36). Trained personnel administered the diet recall tool to the child's primary caregiver in presence of the child. Energy and nutrient values were determined using the new fatty acid content update for Mexican Food Database (BAM, Spanish acronym) (37). The dietary n-6:n-3 ratio was calculated by dividing total daily n-6 (sum of 18:2 n-6 (LA), 18:3 n-6, 20:2 n-6, 20:3 n-6, 20:4 n-6 (AA), and 22:4 n-6) intake by total daily n-3 (sum of 18:3 n-3 (ALA), 20:5 n-3 (EPA), 22:5 n-3 (DPA), and 22:6 n-3 (DHA)) intake. Dietary n-6:n-3 ratios were classified into tertiles according to the distribution of the study population (low: <6.67, medium: 6.67-8.61, and high: > 8.61).

6.3.7 Statistical Analysis

We assessed normality of data using histograms and quantile-quantile plots. We calculated means and standard deviations for maternal and offspring characteristics at trial enrollment, birth, and 11 years and examined differences by offspring genotype and dietary n-6:n-3 ratio using Student's t tests, ANOVA, and chi-square tests, as appropriate. Baseline characteristics of the included subsample were compared with those of the rest of the birth cohort (participants lost to follow up or missing key data).

Allele frequencies were calculated, and Hardy Weinberg Equilibrium (HWE) was tested with Fisher's exact test using the R "genetic" package for both maternal and offspring genetic data. Offspring haplotype frequencies were estimated using the R "haplo.stats" package version 1.8.7 (https://cran.r-project.org/web/packages/haplo.stats/index.html), which uses an expectation

maximization (EM) algorithm to calculate maximum likelihood estimates of probabilities of haplotype pairs for each participant (38). Haplotypes with frequency <5% were grouped as rare haplotypes. We used the "haplo.glm" function to assess the association of offspring *FADS* haplotype with offspring MetS score (adjusted for sex), using the most common haplotype as the referent.

We used multivariable generalized linear models to test interaction between genotype for maternal and offspring SNP rs174602 and prenatal treatment group, adjusting for maternal SES, BMI, parity, and age at trial enrollment and offspring sex and age at examination. Due to the strong association between maternal and offspring genotype, we examined the associations separately for maternal and offspring genotype and adjusted one for the other. Finally, we used multivariable generalized linear models to a) examine the association of dietary n-6:n-3 ratio with MetS score and b) test interaction (specified *a priori*) between offspring genotype for SNP rs174602 and dietary n-6:n-3 ratio on MetS score. We assessed genotype-diet interaction using both an additive and dominant genetic model. Both approaches yielded similar conclusions, but we report the results for the dominant genetic model (i.e., carriers vs. non-carriers of the minor T allele for SNP rs174602) to conserve study power. Models were adjusted for sex, age at examination, and total energy intake. All statistical analyses were performed using R version 4.0.4 (R Foundation for Statistical Computing, Vienna, Austria). Power calculations performed using the 'genpwr' R package showed that we had ≥80% power to detect a genetic association of R^2=0.038 with a type I error rate of 0.05. P values < 0.05 were considered significant.

6.4 Results

The analytic sample included 203 children who had the complete set of CMH indicators and maternal and own genetic data (**Supplementary Figure 6-3**). Characteristics of mothers and

children in the study population are presented in **Table 6-1**. At study enrollment, mean maternal age and BMI were 27.1 ± 4.9 years and 26.3 ± 3.8 kg/m^2, respectively. Less than 30% of women were primiparous, and mothers breastfed their children for 9.7 ± 7.9 months. The intervention and placebo groups were balanced on maternal characteristics at enrollment and offspring birth characteristics (**Supplementary Table 6-1**). Children in the analytic sample were born to women who were older, had higher parity, and longer duration of breastfeeding relative to children lost to follow up or missing data (n=770) (**Supplementary Table 6-2**). Maternal characteristics at enrollment and offspring lifestyle characteristics at 11 y by offspring genotype and dietary n-6:n-3 ratio are presented in **Supplementary Table 6-3**.

At age 11 y, 85 children (42%) were overweight or obese, 65 (32%) had low HDL cholesterol, 35 (17.2%) had high triglycerides, and 9 (4%) had high fasting glucose concentrations. Glucose concentrations were significantly higher among males (90.1 ± 9.1 mg/dL) when compared to females (86.9 ± 6.5 mg/dL). The median dietary n-6:n-3 ratio among offspring at age 11 y was 7.4 (IQR: 5.8 – 10.3). Intakes of n-3 PUFAs were low (ALA: 528.2 (IQR: 231.2 – 1017.0) mg/day; DHA: 18.4 (IQR: 2.7 – 49.7) mg/day; EPA: 10.9 (IQR: 6.6 – 20.8) mg/day), along with high intakes of n-6 PUFAs (LA: 4,647 (IQR: 2,436 – 8,374) mg/day; AA: 16.8 (IQR: 8.3-31.0) mg/day). ALA contributed 87% of total n-3 PUFA intake and LA contributed 94% of total n-6 PUFA intake; therefore, the n-6:n-3 ratio in this study population primarily reflects the LA/ALA ratio (i.e., intake of dietary precursors, not LC-PUFAs).

Distribution of FADS SNPs in study population and association with MetS score

Within this sample, the minor allele (T) frequency for maternal SNP rs174602 was 0.37; 29 mothers (14%) were homozygous carriers of the minor T allele, 93 (46%) were heterozygous carriers (TC), and 81 (40%) were homozygous carriers of the major C allele. Genotype and allele

frequencies for the five selected offspring *FADS* SNPs are shown in **Table 6-2**. Minor allele frequencies ranged from 0.13 to 0.41, and there was no evidence of departure from HWE ($p > 0.05$). Haplotype frequencies are shown in **Supplementary Table 6-4**; 83% (n=167) of children were captured by the 6 identified haplotypes. In the single SNP analysis, there was no evidence of an association between offspring genotype for SNP rs174602 and MetS score [β=0.05 (95% CI: -0.13, 0.23)]. We also did not observe any association between *FADS* haplotypes and MetS score at 11 years relative to the reference haplotype [AATGT: β =-0.17 (95% CI: -0.63, 0.28); ATTGG: β = 0.38 (-0.24, 1.00); TTCGG: β = 0.05 (-0.33, 0.42); TTCGT: β = 0.60 (-0.07, 1.27); TTTTG: β = 0.16 (-0.43, 0.75)].

Contributions of maternal and offspring genotype to MetS score

For SNP rs174602, 57% of the mothers and offspring had the same genotype; among mothers who carried a minor T allele, 77% of offspring also carried a minor allele. We found that maternal genotype for SNP rs174602 modified the effect of the prenatal DHA supplementation on offspring MetS score (p-interaction = 0.02); among homozygous major C allele carriers (n = 81), offspring of mothers who received prenatal DHA had higher MetS score (0.19 ± 0.08) relative to offspring whose mothers received placebo (-0.05 ± 0.08, $\Delta = 0.23$ (0.01, 0.45), p = 0.04) (**Figure 6-1a**). However, offspring genotype for SNP rs174602 did not modify the association of prenatal DHA supplementation with MetS score (p-interaction = 0.11) (**Figure 6-1b**). The results for examining the effects of maternal genotype for SNP rs174602 stratified by offspring genotype for SNP rs174602 showed that mean differences in MetS scores between DHA and placebo groups were larger when maternal and offspring genotypes were matched relative to a mismatch (**Table 6-3**).

Interaction between offspring genotype for SNP rs174602 and dietary n-6:n-3 ratio

The results of the exploratory analysis examining the influence of gene-nutrient interactions on MetS scores at age 11 showed no overall association between the dietary n-6:n-3 ratio and MetS score ($\boldsymbol{\beta}$ = 0.003, 95% CI: -0.01, 0.02); however, there was heterogeneity in the association of SNP rs174602 and MetS score across tertiles of the dietary n-6:n-3 ratio (p = 0.05) (**Figure 6-2**). Among children with TT or TC genotype (n = 88), those in the highest n-6:n-3 ratio tertile (> 8.61) had higher MetS score relative to those in the lowest tertile (< 6.67) (Δ= 0.36; 95% CI: 0.03, 0.69); among children with CC genotype (n = 53), those in the highest n-6:n-3 ratio tertile had lower MetS score relative to those in the lowest tertile (Δ= -0.23; 95% CI: -0.61, 0.16). Mean MetS scores and individual MetS components stratified by offspring genotype and n-6:n-3 diet ratio are shown in **Supplementary Table 6-4**. Similar trends were observed with individual MetS components, although interaction was not statistically significant. We also found a positive association between n-6:n-3 ratio and MetS score among offspring of TT/TC genotype ($\boldsymbol{\beta}$ (95% CI) = 0.02 (-0.004, 0.04)), while there was an inverse association among offspring of CC genotype (-0.013 (-0.04, 0.01); p = 0.05 for interaction).

6.5 Discussion

In our earlier report, we identified an interaction between maternal genotype for SNP rs174602 and prenatal DHA supplementation on offspring MetS score at age 11 years, such that offspring of TT carriers who received prenatal DHA had lower MetS scores relative to the placebo group, and offspring of CC carriers who received DHA had higher MetS scores relative to the placebo group (unpublished). These findings suggested that prenatal DHA supplementation may have differential effects on offspring phenotypes based on maternal genotype. Given that maternal and offspring genetics are tightly linked, in this study, we first sought to 1) determine if this interaction also existed with offspring genotype and 2) examine whether offspring or maternal

genotype determines offspring phenotype to a greater extent. We found that, while effect estimates of offspring SNP-prenatal DHA supplementation interaction did align with results observed for maternal SNP-prenatal DHA supplementation interaction, after adjusting for maternal genotype, offspring genotype did not significantly modify the effect of the intervention on MetS score, although this may be partially attributable to limited study power.

Few studies have examined the role of maternal and offspring genetics in understanding effects of LC-PUFAs on offspring measures of health. Findings from the Avon Longitudinal Study, conducted in the United Kingdom, showed that both maternal and child *FADS* genotypes and haplotypes influenced cord plasma LC-PUFA concentrations (39). In contrast, in another study conducted in the United States, *FADS2* rs174575 genotyping was performed on a subset of mothers and their 16-month-old toddlers, and they found that the effect of maternal genotype on declarative memory task performance was larger than the child's own genotype (40). Although the examined SNPs and outcomes differ across these studies and ours, they collectively indicate the importance of maternal genotype in predicting offspring phenotypes.

Beyond the prenatal intervention, we also examined the independent contribution of offspring genetics towards cardiometabolic health measures using a haplotype-based approach to leverage additional genetic information and reduce multiple testing. All SNPs of interest were in the *FADS2* gene, and the MAF of the 5 included SNPs in this population aligned with allele frequencies for matching genetic ancestry documented in HapMap and the 1000 Genomes Browser (41). We did not observe evidence of an association between examined offspring *FADS* haplotypes and MetS score. Similarly, no evidence of an association was observed in the single SNP analysis using SNP rs174602. However, exploratory analyses revealed significant interaction between dietary n-6:n-3 ratio and offspring genotype for SNP rs174602 on MetS score. Individuals with

TT or TC genotype and high dietary n-6:n-3 ratios had higher MetS scores relative to those with low n-6:n-3 ratios, while individuals with CC genotype and high n-6:n-3 ratio ratios had lower MetS score relative to those in the lowest tertile. SNP rs174602 is located at an intron/exon boundary of the *FADS2* gene, which encodes for the Δ-6 desaturase enzyme that regulates the conversion of n-3 Eicosapentaenoic Acid (EPA) to DHA. Previous studies in European populations have shown that the CC genotype for SNP rs174602 is associated with lower Δ-5 desaturase activity, reflecting reduced conversion of dietary precursors, while TT and TC are associated with increased conversion of precursors (21). Although we were unable to assess Δ-5 desaturase activity in this study population, our results align with these previous findings, indicating that children who carried TT or TC alleles (i.e., higher conversion of PUFAs to LC-PUFAs) and had high n-6/n-3 intake had poorer cardiometabolic health, as defined by higher MetS scores. We also observed that children who carried CC alleles (i.e., lower conversion of PUFAs to LC-PUFAs) and higher n-6/n-3 ratios had lower MetS scores, although these results were not statistically significant (perhaps due to sample size). These findings contrast with an independent study in a large sample of Mexican adolescents, which showed that *FADS1* SNP rs174546 was a major contributor of plasma TG and VLDL concentrations; however, no significant interaction effects were detected between dietary intake and genotype (42). Another study in a population of 816 Taiwanese adults, however, reported significant interaction between ALA/LA ratio and rs2072114 and marginally significant interaction between ALA/LA ratios and rs174602 on LDL-c concentrations, although these SNPs are independent of each other (43). It is possible that n-3 LC-PUFA status may be more dependent on dietary intake than n-6 fatty acids, which rely more on endogenous synthesis (44). Overall, our findings suggest that based on their genotype, some individuals may have increased dietary requirements for omega-3 fatty acids, and offspring

genetics may only matter over and above maternal genotype in the context of poor diets (i.e., imbalance in n-6: n-3 ratio). However, this requires confirmation in independent and diverse populations.

To expand upon the clinical relevance of our results, we also explored the interaction of offspring genotype for SNP rs174602 and dietary n-6:n-3 ratio on individual MetS components. While we observed similar trends for each of the individual MetS components, interaction was most clearly demonstrated for HDL, BMI, and SBP, which aligns with the high prevalence of low HDL and high BMI in this study population. Studies have shown that PCA-derived MetS scores during early adolescence are associated with increased risk of adult cardiometabolic disease, with one study reporting that a 1 SD increase in a continuous MetS score during adolescence was associated with 30% increased risk of type II diabetes and 20% increased risk of high carotid intima-media thickness in adulthood (45). Whether these associations exist for this population will require follow-up through adulthood to see if differences in MetS scores at this time point have lasting implications for adult cardiometabolic health.

Strengths of this study include the double-blind RCT design of the original trial, high compliance to the prenatal intervention, extensive characterization of mothers and children from pregnancy through adolescence, and availability of *FADS* genetic information for both mothers and offspring. All data collection and laboratory assays were standardized, validated, and conducted by trained personnel. However, a few limitations of this study may influence the interpretability and generalizability of these findings, such as selection bias. The offspring in our study sample tended to have mothers who were older at study enrollment, had higher parity, and longer breastfeeding durations relative to those lost to follow up, although these values did not differ by treatment group or maternal/offspring genotype. Additionally, offspring diet at 11 y was

assessed using a single 24-hour recall and may be subject to recall bias. As the POSGRAD trial was not originally designed to explore this association, the analytic sample with complete genotype and phenotype information is small, and there may be limited statistical power to detect genetic associations. Further work is needed across larger, diverse datasets to reproduce these findings and investigate underlying biological mechanisms.

In summary, in this population of Mexican children, there was evidence of interaction between *FADS* SNP rs174602 and current dietary PUFA intake on MetS score, but not with prenatal DHA supplementation. A remaining question is whether the dose and type of supplementation matters (e.g., fish oil vs. DHA only). Future work could include a feeding trial among school-age children to examine further examine interaction between selected *FADS* genetic variants and dietary LC-PUFA intake. Replication of findings is critical for ensuring validity in genetic association studies. Analyses should be reproduced using similar study designs and study populations to help determine the utility of targeted supplementation strategies based on genetic profile.

6.6 References

1. Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, et al. Global, Regional, and National Burden of Cardiovascular Diseases for 10 Causes, 1990 to 2015. J Am Coll Cardiol 2017;70(1):1-25. doi: 10.1016/j.jacc.2017.04.052.
2. Weiss R, Bremer AA, Lustig RH. What is metabolic syndrome, and why are children getting it? Ann N Y Acad Sci 2013;1281(1):123-40. doi: 10.1111/nyas.12030.
3. Guardamagna O, Abello F, Cagliero P, Lughetti L. Impact of nutrition since early life on cardiovascular prevention. Italian Journal of Pediatrics 2012;38(1):73. doi: 10.1186/1824-7288-38-73.
4. Ralston J, Nugent R. Toward a broader response to cardiometabolic disease. Nature Medicine 2019;25(11):1644-6. doi: 10.1038/s41591-019-0642-9.
5. Samuelsson B. Prostaglandins, thromboxanes, and leukotrienes: formation and biological roles. Harvey Lect 1979;75:1-40.
6. Poudyal H, Panchal SK, Diwan V, Brown L. Omega-3 fatty acids and metabolic syndrome: Effects and emerging mechanisms of action. Progress in Lipid Research 2011;50(4):372-87.
7. Saravanan P, Davidson NC, Schmidt EB, Calder PC. Cardiovascular effects of marine omega-3 fatty acids. Lancet 2010;376(9740):540-50. doi: 10.1016/s0140-6736(10)60445-x.
8. Lorente-Cebrián S, Costa AG, Navas-Carretero S, Zabala M, Martínez JA, Moreno-Aliaga MJ. Role of omega-3 fatty acids in obesity, metabolic syndrome, and cardiovascular diseases: a review of the evidence. J Physiol Biochem 2013;69(3):633-51. doi: 10.1007/s13105-013-0265-4.

9. Eslick GD, Howe PR, Smith C, Priest R, Bensoussan A. Benefits of fish oil supplementation in hyperlipidemia: a systematic review and meta-analysis. Int J Cardiol 2009;136(1):4-16. doi: 10.1016/j.ijcard.2008.03.092.

10. Simopoulos AP. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp Biol Med (Maywood) 2008;233(6):674-88. doi: 10.3181/0711-mr-311.

11. Poli A, Agostoni C, Visioli F. Dietary Fatty Acids and Inflammation: Focus on the n-6 Series. International Journal of Molecular Sciences 2023;24(5):4567.

12. Kaikkonen JE, Kresanov P, Ahotupa M, Jula A, Mikkilä V, Viikari JSA, et al. High serum n6 fatty acid proportion is associated with lowered LDL oxidation and inflammation: The Cardiovascular Risk in Young Finns Study. Free Radical Research 2014;48(4):420-6. doi: 10.3109/10715762.2014.883071.

13. Pischon T, Hankinson SE, Hotamisligil GS, Rifai N, Willett WC, Rimm EB. Habitual Dietary Intake of n-3 and n-6 Fatty Acids in Relation to Inflammatory Markers Among US Men and Women. Circulation 2003;108(2):155-60. doi: 10.1161/01.CIR.0000079224.46084.C2.

14. Tureck C, Barboza BP, Bricarello LP, Retondario A, Alves MdA, de Moura Souza A, et al. Scientific evidence of the association between oral intake of OMEGA-3 and OMEGA-6 fatty acids and the metabolic syndrome in adolescents: A systematic review. Nutrition, Metabolism and Cardiovascular Diseases 2022;32(12):2689-704. doi: https://doi.org/10.1016/j.numecd.2022.08.020.

15. Crowe-White KM, Cardel MI, Burkhalter HH, Huo T, Fernández JR. Higher n–6:n–3 Fatty Acid Intake Is Associated with Decreased Cardiometabolic Risk Factors in a Racially

Diverse Sample of Children. Current Developments in Nutrition 2018;2(5):nzy014. doi: https://doi.org/10.1093/cdn/nzy014.

16. Gillingham LG, Harding SV, Rideout TC, Yurkova N, Cunnane SC, Eck PK, et al. Dietary oils and FADS1-FADS2 genetic variants modulate [13C]α-linolenic acid metabolism and plasma fatty acid composition. Am J Clin Nutr 2013;97(1):195-207. doi: 10.3945/ajcn.112.043117.

17. Roke K, Mutch DM. The role of FADS1/2 polymorphisms on cardiometabolic markers and fatty acid profiles in young adults consuming fish oil supplements. Nutrients 2014;6(6):2290-304. doi: 10.3390/nu6062290.

18. Koletzko B, Reischl E, Tanjung C, Gonzalez-Casanova I, Ramakrishnan U, Meldrum S, et al. FADS1 and FADS2 Polymorphisms Modulate Fatty Acid Metabolism and Dietary Impact on Health. Annu Rev Nutr 2019;39:21-44. doi: 10.1146/annurev-nutr-082018-124250.

19. Conway MC, McSorley EM, Mulhern MS, Strain JJ, van Wijngaarden E, Yeates AJ. Influence of fatty acid desaturase (FADS) genotype on maternal and child polyunsaturated fatty acids (PUFA) status and child health outcomes: a systematic review. Nutrition Reviews 2020;78(8):627-46. doi: 10.1093/nutrit/nuz086.

20. Standl M, Lattka E, Stach B, Koletzko S, Bauer CP, von Berg A, et al. FADS1 FADS2 gene cluster, PUFA intake and blood lipids in children: results from the GINIplus and LISAplus studies. PLoS One 2012;7(5):e37780. doi: 10.1371/journal.pone.0037780.

21. Bokor S, Dumont J, Spinneker A, Gonzalez-Gross M, Nova E, Widhalm K, et al. Single nucleotide polymorphisms in the FADS gene cluster are associated with delta-5 and delta-

6 desaturase activities estimated by serum fatty acid ratios. J Lipid Res 2010;51(8):2325-33. doi: 10.1194/jlr.M006205.

22. Wolters M, Dering C, Siani A, Russo P, Kaprio J, Risé P, et al. The role of a FADS1 polymorphism in the association of fatty acid blood levels, BMI and blood pressure in young children-Analyses based on path models. PLoS One 2017;12(7):e0181485. doi: 10.1371/journal.pone.0181485.

23. Gonzalez-Casanova I, Rzehak P, Stein AD, Garcia Feregrino R, Rivera Dommarco JA, Barraza-Villarreal A, et al. Maternal single nucleotide polymorphisms in the fatty acid desaturase 1 and 2 coding regions modify the impact of prenatal supplementation with DHA on birth weight. Am J Clin Nutr 2016;103(4):1171-8. doi: 10.3945/ajcn.115.121244.

24. Tandon S, Gonzalez-Casanova I, Barraza-Villarreal A, Romieu I, Demmelmair H, Jones DP, et al. Infant Metabolome in Relation to Prenatal DHA Supplementation and Maternal Single-Nucleotide Polymorphism rs174602: Secondary Analysis of a Randomized Controlled Trial in Mexico. J Nutr 2021;151(11):3339-49. doi: 10.1093/jn/nxab276.

25. Ramakrishnan U, Stein AD, Parra-Cabrera S, Wang M, Imhoff-Kunsch B, Juarez-Marquez S, et al. Effects of docosahexaenoic acid supplementation during pregnancy on gestational age and size at birth: randomized, double-blind, placebo-controlled trial in Mexico. Food and nutrition bulletin 2010;31(2 Suppl):S108-16. doi: 10.1177/15648265100312s203.

26. Schaeffer L, Gohlke H, Muller M, Heid IM, Palmer LJ, Kompauer I, et al. Common genetic variants of the FADS1 FADS2 gene cluster and their reconstructed haplotypes are associated with the fatty acid composition in phospholipids. Human molecular genetics 2006;15(11):1745-56. doi: 10.1093/hmg/ddl117.

27. McCarthy S, Das S, Kretzschmar W, Delaneau O, Wood AR, Teumer A, et al. A reference panel of 64,976 haplotypes for genotype imputation. Nat Genet 2016;48(10):1279-83. doi: 10.1038/ng.3643.

28. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21(2):263-5. doi: 10.1093/bioinformatics/bth457.

29. Lohman TG, Roche AF, Martorell R. Anthropometric standardization reference manual. Champaign, IL: Human Kinetics Books, 1988.

30. Myatt M, E G. Internet: https://nutriverse/zscorer/, https://github.com/nutriverse/zscorer.

31. Ramirez-Silva I, Rivera JA, Trejo-Valdivia B, Stein AD, Martorell R, Romieu I, et al. Relative Weight Gain Through Age 4 Years Is Associated with Increased Adiposity, and Higher Blood Pressure and Insulinemia at 4–5 Years of Age in Mexican Children. The Journal of Nutrition 2018;148(7):1135-43. doi: https://doi.org/10.1093/jn/nxy068.

32. Zimmet P, Alberti KGMM, Kaufman F, Tajima N, Silink M, Arslanian S, et al. The metabolic syndrome in children and adolescents – an IDF consensus report. Pediatric Diabetes 2007;8(5):299-306. doi: https://doi.org/10.1111/j.1399-5448.2007.00271.x.

33. Cossio-Bolaños M, Vidal-Espinoza R, de Campos FCC, Sulla-Torres J, Cossio-Bolaños W, Andruske CL, et al. Establishing percentiles for blood pressure based on absolute height for children and adolescents. BMC Pediatr 2021;21(1):26. doi: 10.1186/s12887-020-02489-9.

34. Wimalasena ST, Ramirez Silva CI, Sun YV, Stein AD, Rivera JA, Ramakrishnan U. Clustering of cardiometabolic risk factors in Mexican pre-adolescents. Diabetes Research and Clinical Practice 2023;202:110818. doi: https://doi.org/10.1016/j.diabres.2023.110818.

35. Lê S, Josse J, Husson F. FactoMineR: An R Package for Multivariate Analysis. Journal of Statistical Software 2008;25(1):1 - 18. doi: 10.18637/jss.v025.i01.

36. Ramírez-Silva I, Rodríguez-Ramírez S, Barragán-Vázquez S, Castellanos-Gutiérrez A, Reyes-García A, Martínez-Piña A, et al. Prevalence of inadequate intake of vitamins and minerals in the Mexican population correcting by nutrient retention factors, Ensanut 2016. Salud Publica Mex 2020;62(5):521-31. doi: 10.21149/1109.

37. Ramírez Silva I B-VS, Rodríguez Ramírez S, Rivera Dommarco JA, Mejía-Rodríguez F, Barquera Cervera S, Tolentino Mayo L, Flores Aldana M, Villalpando Hernández S, Ancira Moreno M, et al. . Base de Alimentos de México (BAM): Compilación de la Composición de los Alimentos Frecuentemente Consumidos en el país, Version 18.1.1. 2021.

38. Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA. Score tests for association between traits and haplotypes when linkage phase is ambiguous. American journal of human genetics 2002;70(2):425-34. doi: 10.1086/338688.

39. Koletzko B, Lattka E, Zeilinger S, Illig T, Steer C. Genetic variants of the fatty acid desaturase gene cluster predict amounts of red blood cell docosahexaenoic and other polyunsaturated fatty acids in pregnant women: findings from the Avon Longitudinal Study of Parents and Children. Am J Clin Nutr 2011;93(1):211-9. doi: 10.3945/ajcn.110.006189.

40. Cheatham CL, Lupu DS, Niculescu MD. Genetic and epigenetic transgenerational implications related to omega-3 fatty acids. Part II: maternal FADS2 rs174575 genotype and DNA methylation predict toddler cognitive performance. Nutr Res 2015;35(11):948-55. doi: 10.1016/j.nutres.2015.09.005.

41. Auton A, Abecasis GR, Altshuler DM, Durbin RM, Abecasis GR, Bentley DR, et al. A global reference for human genetic variation. Nature 2015;526(7571):68-74. doi: 10.1038/nature15393.

42. Vazquez-Vidal I, Voruganti VS, Hannon BA, Andrade FCD, Aradillas-García C, Nakamura MT, et al. Serum Lipid Concentrations and FADS Genetic Variants in Young Mexican College Students: The UP-AMIGOS Cohort Study. Lifestyle Genom 2018;11(1):40-8. doi: 10.1159/000488085.

43. Huang PC, Cheng H, Su YT, Huang MC, Hsu CC, Hwang SJ, et al. Interaction among dietary n-3 and n-6 polyunsaturated fatty acid intake, fatty acid desaturase 2 genetic variants, and low-density lipoprotein cholesterol levels in type 2 diabetes patients. J Diabetes Investig 2023;14(2):297-308. doi: 10.1111/jdi.13944.

44. Lattka E, Koletzko B, Zeilinger S, Hibbeln JR, Klopp N, Ring SM, et al. Umbilical cord PUFA are determined by maternal and child fatty acid desaturase (FADS) genetic variants in the Avon Longitudinal Study of Parents and Children (ALSPAC). Br J Nutr 2013;109(7):1196-210. doi: 10.1017/s0007114512003108.

45. Magnussen CG, Cheriyan S, Sabin MA, Juonala M, Koskinen J, Thomson R, et al. Continuous and Dichotomous Metabolic Syndrome Definitions in Youth Predict Adult Type 2 Diabetes and Carotid Artery Intima Media Thickness: The Cardiovascular Risk in Young Finns Study. The Journal of Pediatrics 2016;171:97-103.e3. doi: 10.1016/j.jpeds.2015.10.093.

6.7 Tables and Figures

Table 6-1. Characteristics of mothers and children in study population (n = 203).

Maternal factors at enrollment into POSGRAD	
Age, years	27.1 (4.9)
BMI, kg/m^2	26.3 (3.8)
Height, cm	155.0 (5.2)
Primiparous (n, %)	59 (29.1%)
Schooling, years	12.1 (3.4)
Received prenatal DHA intervention	103 (50.7%)
Offspring factors	
Female (n, %)	96 (47.3%)
Early life characteristics	
Birth weight, g	3260 (475)
Gestational age at birth, weeks	39.2 (1.8)
Duration of BF, mo.	9.7 (7.9)
At age 11 years	
Dietary intake	
Energy intake (kcal/day)	2052 (707)
Lipids (g/day)	76.2 (36.4)
Saturated fat (g/day)	30.3 (16.9)
Monounsaturated fat (g/day)	25.2 (13.6)
Polyunsaturated fat (g/day)	15.0 (8.8)
Cholesterol (g/day)	265 (184)
Omega-3 fatty acid intake (g/day)	0.9 (1.1)
Omega-6 fatty acid intake (g/day)	6.5 (7.1)
n-6:n-3 fatty acid ratio	8.9 (5.7)
Sedentary time per day, min	4.9 (2.1)
Cardiometabolic factors	
BMI, kg/m^2	19.2 (3.6)
HDL-c, mg/dL	46.5 (10.7)
Glucose, mg/dL	88.6 (8.1)
TG, mg/dL	108.0 (60.4)
TC, mg/dL	162.0 (27.8)
LDL-c, mg/dL	95.3 (23.4)
MetS Score	0.0 (0.5)

Values presented are mean (SD) unless otherwise stated.
BMI, body mass index; SES, socioeconomic status score; EBF, Exclusive Breastfeeding; PreBF, Predominantly Breastfeeding; PaBF, Partially Breastfeeding; NBF, No Breastfeeding; HDL, High Density Lipoprotein Cholesterol; TG, Triglycerides; TC, Total Cholesterol; LDL-c, Low Density Lipoprotein Cholesterol; SBP, Systolic Blood Pressure; MetS, Metabolic Syndrome; CMH, Cardiometabolic Health

Table 6-2. Genotype and allele frequencies of maternal and offspring FADS2 SNPs.

	Position (base pair)	M/m	MAF	MM genotype	Mm genotype	Mm genotype	HWE p-value
Maternal							
rs174602	61624414	C/T	0.37	81 (40)	93 (46)	29 (14)	1
Offspring							
rs174578	61605499	T/A	0.2	130 (65)	59 (30)	10 (5)	1
rs2727271	61603358	A/T	0.41	71 (35)	98 (49)	33 (16)	0.57
rs174602	61624414	C/T	0.37	78 (38)	101 (50)	24 (12)	1
rs174605	61626921	G/T	0.13	150 (75)	48 (24)	3 (1)	0.45
rs174450	61641542	G/T	0.24	116 (57)	78 (38)	9 (4)	0.96

Data are n (%). M/m, major/minor alleles; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium
Genetic data were subset to chromosome 11 and mapped to the human genome reference build GRCh37/hg19.

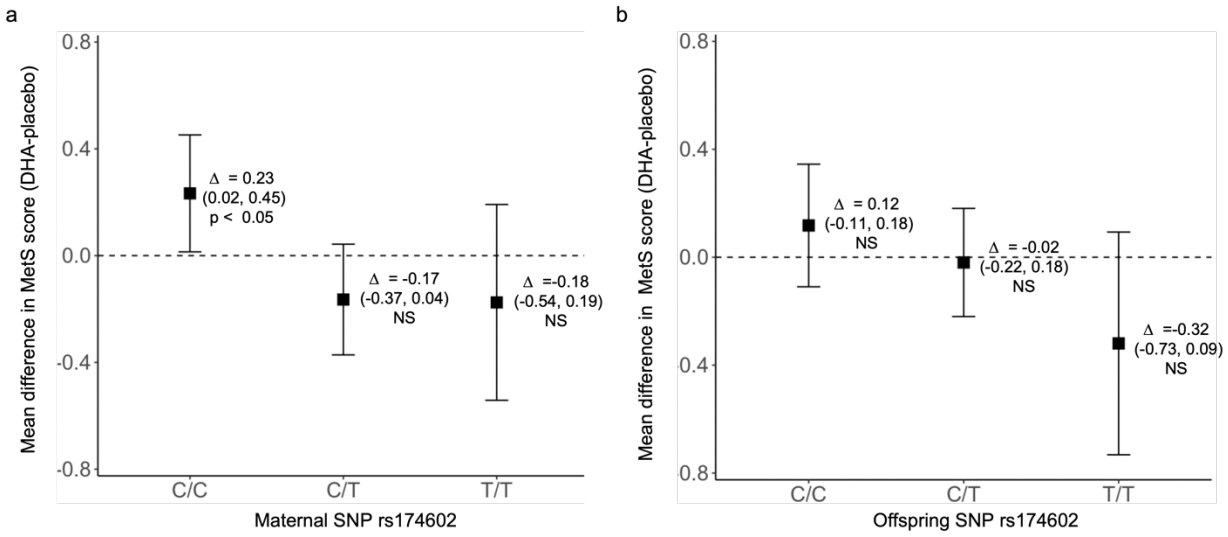


Figure 6-1. Differences in MetS scores attributable to prenatal DHA supplementation (DHA – placebo) by maternal and offspring genotype for SNP rs174602.
Graphs depict differences in MetS scores by category of a) maternal genotype for SNP rs174602 (p-interaction = 0.02) and b) offspring genotype for SNP rs174602 (p-interaction = 0.11) on offspring MetS Score. Values are contrast-specific mean differences (95% CI) between DHA and placebo groups from generalized linear models testing the interaction between *FADS2* single nucleotide polymorphism rs174602 and supplementation group on MetS score adjusted for child sex and age at measurement, maternal SES, BMI, parity and age at trial enrollment.

Table 6-3. Mean differences in MetS Scores attributable to DHA supplementation (DHA – placebo), by category of maternal and offspring genotype of SNP rs174602 (n = 203).

	Offspring CC Carriers N = 78	Offspring TC Carriers N= 101	Offspring TT Carriers N = 24
Maternal CC Carriers	0.34 (0.07, 0.62) N = 51	-0.01 (-0.38, 0.35) N = 30	--
Maternal TC Carriers	-0.28 (-0.72, 0.15) N = 27	-0.1 (-0.40, 0.21) N = 53	-0.27 (-0.88, 0.34) N = 13
Maternal TT Carriers	--	-0.06 (-0.44, 0.32) N = 18	-0.6 (-1.1, -0.1) N = 11

Values are contrast-specific mean differences (95% CI) between DHA and placebo groups from generalized linear models testing the interaction between *FADS2* single nucleotide polymorphism rs174602 and supplementation group on MetS score adjusted for child sex and age at measurement, maternal SES, BMI, parity and age at trial enrollment.

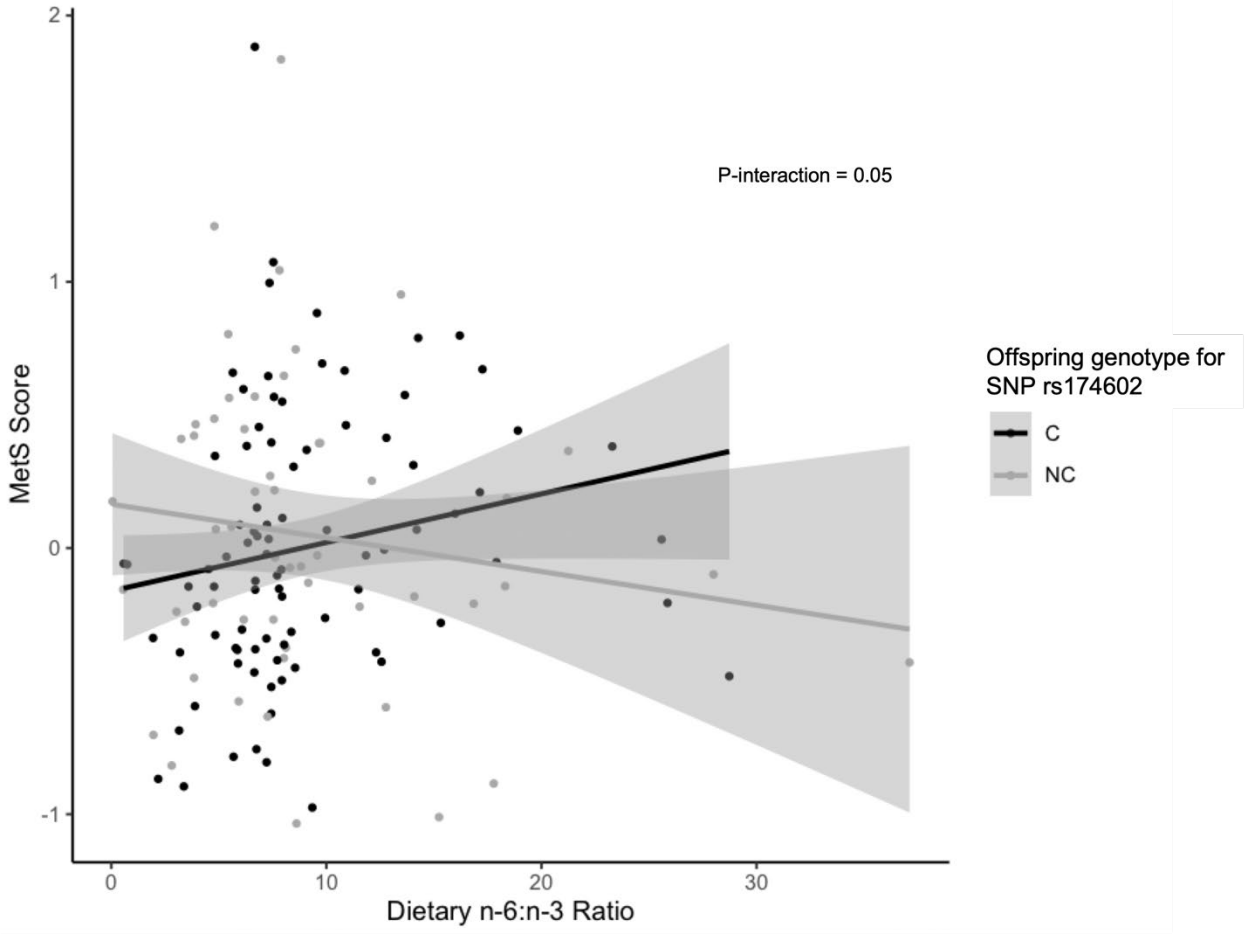
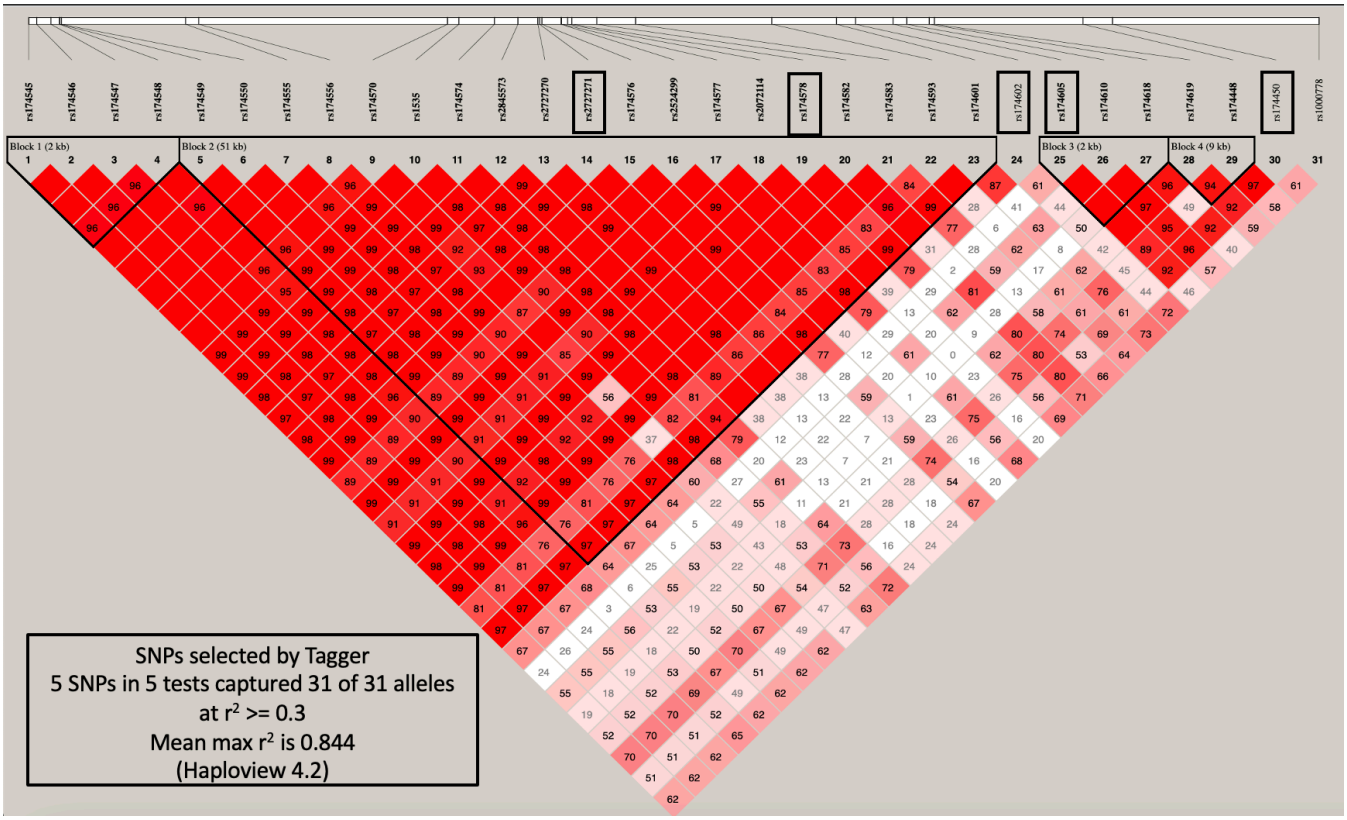
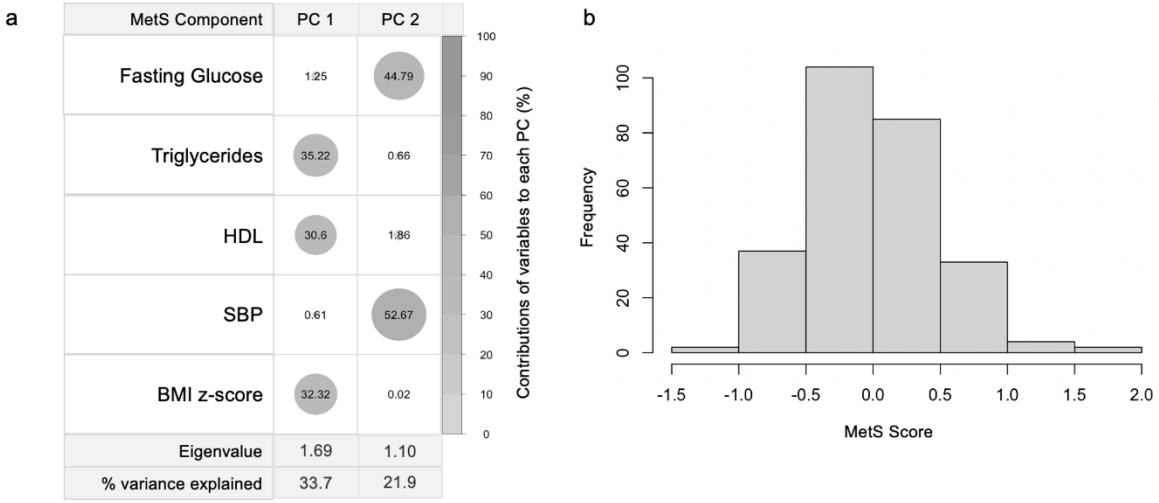


Figure 6-2. Scatter plot of Mean MetS scores and dietary n-6:n-3 ratio, by categories of offspring genotype for SNP rs174602.
Fitted lines represent linear regression estimates and shaded areas are 95% confidence bounds.
Abbreviations: C, Carriers of minor T allele (TT, TC), n=88; NC, Non-carriers of minor allele (CC), n= 53.

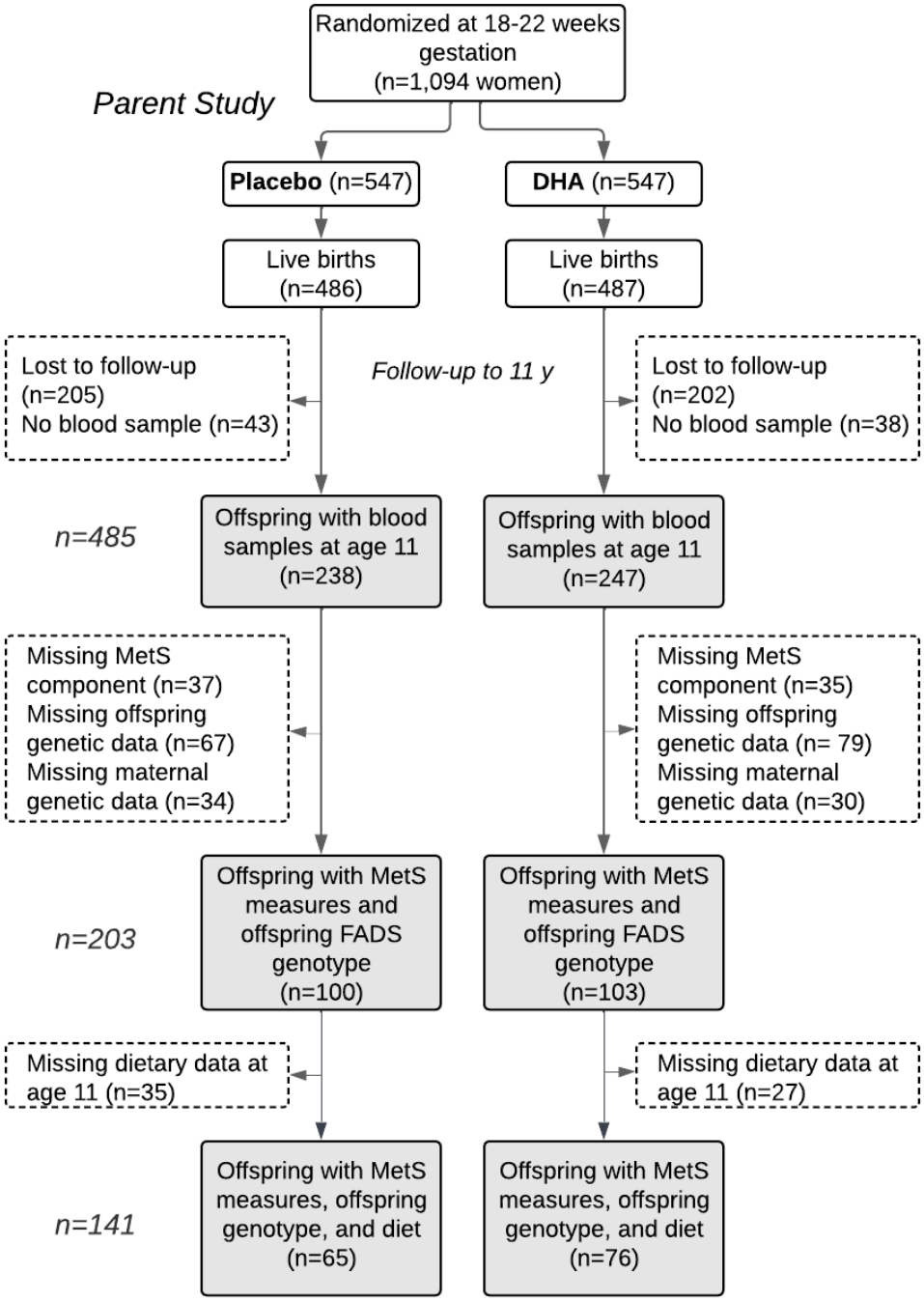
6.8 Supplementary Tables and Figures



Supplementary Figure 6-1. Linkage disequilibrium (LD) plot of single nucleotide polymorphisms (SNPs) the FADS region.
LD calculations were based on the coefficient of LD (D') between SNPs; pairwise D' values are displayed as a percentage in each box. The red color scheme represents varying degrees of LD; darker shades indicate stronger LD. The SNPs outlined in black boxes were selected by Tagger in Haploview 4.2.



Supplementary Figure 6-2. Contribution of cardiometabolic factors to PCA-derived continuous MetS score.
a) Principal components analysis (PCA) was used to calculate the MetS score using five components used to define metabolic syndrome in pediatric populations. Top PCs were identified visually using scree plots and quantitatively using eigenvalues > 1. Scores were calculated as the sum of the top components, weighted by variance explained. The x axes represent the top PCs used to create the MetS score and percentage of variance in the data explained by each PC via PCA. The diameter of each bubble represents the magnitude of contributions (%) of individual MetS components to each PC. Cardiometabolic factors with the highest contributions represent the most important variables within each PC. The highest contributing components in the first PC contribute most to the derived scores and **b)** distribution of continuous MetS score in study population.
Abbreviations: MetS, metabolic syndrome; PC, Principal Component.



Supplementary Figure 6-3. Flowchart for the offspring FADS2 single nucleotide polymorphism effect modification analysis of the POSGRAD study.

Supplementary Table 6-1. Maternal baseline characteristics and offspring characteristics at birth, stratified by treatment group.

	Treatment group[a]	
	Placebo N=100	DHA N=103
Maternal factors, enrollment		
Age, years	26.9 (4.62)	27.3 (5.11)
BMI, kg/m^2	26.2 (3.74)	26.4 (3.89)
Height, cm	156 (5.00)	155 (5.31)
First pregnancy, %	29 (29.0%)	30 (29.1%)
SES Score	0.12 (1.05)	0.16 (0.86)
Schooling, years	12.3 (3.51)	11.8 (3.37)
Dietary intake at enrollment, g/day		
ALA	1.80 (1.05)	1.80 (1.11)
DHA	0.08 (0.06)	0.09 (0.09)
LA	19.6 (8.08)	20.1 (9.48)
EPA	0.03 (0.03)	0.04 (0.05)
AA	0.15 (0.07)	0.16 (0.10)
Compliance to intervention, %	94.9 (5.05)	95.9 (4.67)
Offspring factors, birth		
Sex		
Male	47 (47.0%)	60 (58.3%)
Female	53 (53.0%)	43 (41.7%)
Birth weight, g	3245 (512)	3274 (437)
Gestational age, weeks	39.1 (1.65)	39.3 (1.87)
Breastfeeding status, 3 mo, %		
EBF	16 (17.4%)	11 (11.8%)
PreBF	18 (19.6%)	11 (11.8%)
PaBF	44 (47.8%)	50 (53.8%)
NBF	14 (15.2%)	21 (22.6%)
Duration of BF, mo	10.7 (8.24)	8.65 (7.41)

[a] Chi-square tests, t-tests, and ANOVA were used to test differences between groups. No differences by treatment group were observed. Values presented are mean (SD) unless otherwise stated.

BMI, body mass index; SES, socioeconomic status score; ALA, Alpha-Linolenic Acid; DHA, Docosahexaenoic Acid; LA, Linoleic Acid; EPA, Eicosapentaenoic Acid; AA, Arachidonic Acid; EBF, Exclusive Breastfeeding; PreBF, Predominantly Breastfeeding; PaBF, Partially Breastfeeding; NBF, No Breastfeeding; mo, month.

Supplementary Table 6-2. Characteristics of mothers at study enrollment and POSGRAD offspring at 11 years of age in analytic and excluded samples (n = 973).

	Analytic sample n = 203	Excluded sample n = 770
Maternal factors		
Age, years[a]	27.1 (4.9)	26.0 (4.6)
BMI, kg/m^2	26.3 (3.8)	26.0 (4.3)
Height, cm	155 (5.2)	155 (5.8)
Primiparous[a]	59 (29.1%)	311 (40.4%)
SES Score	0.00 (1.00)	0.00 (1.01)
Schooling, years	11.9 (3.6)	11.9 (3.6)
Dietary intake at enrollment		
ALA, mg/d	1.80 (1.08)	1.67 (0.96)
DHA, mg/day	0.08 (0.07)	0.08 (0.08)
LA, mg/day	19.8 (8.80)	19.0 (9.21)
EPA, mg/day	0.03 (0.04)	0.03 (0.05)
AA, mg/day	0.16 (0.09)	0.15 (0.09)
Received prenatal DHA intervention	103 (50.7%)	384 (49.9%)
Compliance to intervention, %	95.4 (4.9)	94.9 (5.9)
Offspring factors		
Females	96 (47.3%)	364 (47.3%)
Birth weight, g	3260 (475)	3190 (456)
Gestational age at birth, weeks	39.2 (1.8)	39.1 (1.8)
Breastfeeding status, 3 mo		
EBF	27 (14.6%)	77 (11.8%)
PreBF	29 (15.7%)	70 (10.7%)
PaBF	94 (50.8%)	393 (60.3%)
NBF	35 (18.9%)	112 (17.2%)
Breastfeeding duration, mo[a]	9.67 (7.9)	8.36 (7.6)

Values presented are mean (SD) unless otherwise stated.
[a] Values differ between analytic and excluded samples, as assessed by chi-square tests, t-tests, and/or ANOVA.
BMI, body mass index; SES, socioeconomic status score; ALA, Alpha-Linolenic Acid; DHA, Docosahexaenoic Acid; LA, Linoleic Acid; EPA, Eicosapentaenoic Acid; AA, Arachidonic Acid; EBF, Exclusive Breastfeeding; PreBF, Predominantly Breastfeeding; PaBF, Partially Breastfeeding; NBF, No Breastfeeding

Supplementary Table 6-3. Maternal and offspring characteristics at study enrollment, birth, and 11 years follow up, stratified by offspring genotype of SNP rs174602 and dietary n-6:n-3 ratio.

		Offspring FADS2 SNP rs174602			Offspring n-6:n-3 fatty acid ratio		
		CC	CT	TT	Low (<6.67)	Medium (6.67-8.61)	High (>8.61)
	N = 203	N=78	N=101	N=24	N=50	N=49	N=49
Maternal factors							
Age, years	27.1 (4.9)	26.7 (4.5)	27.7 (5.0)	26.1 (5.24)	28.0 (4.3)	26.8 (5.1)	26.6 (4.4)
BMI, kg/m^2	26.3 (3.8)	25.9 (3.4)	26.6 (4.1)	26.5 (3.7)	26.9 (3.4)	26.0 (3.8)	26.1 (3.4)
Height, cm	155 (5.2)	155 (5.8)	156 (4.9)	156 (3.7)	155 (6.1)	155 (4.6)	156 (4.6)
First pregnancy, %	59 (29.1%)	23 (29.5%)	28 (27.7%)	8 (33.3%)	9 (18.0%)	14 (28.6%)	15 (30.6%)
SES Score	0.14 (0.96)	0.03 (0.98)	0.14 (0.93)	0.54 (0.93)	0.00 (0.95)	0.36 (1.01)	0.12 (0.93)
Schooling, years	12.1 (3.4)	11.6 (3.7)	12.2 (3.3)	13.0 (2.9)	12.3 (3.6)	12.3 (3.2)	12.1 (3.5)
Dietary intake at enrollment, g/day							
ALA	1.80 (1.08)	1.82 (1.01)	1.76 (1.13)	1.92 (1.08)	1.73 (0.98)	1.68 (1.03)	1.82 (1.10)
DHA	0.08 (0.07)	0.09 (0.09)	0.08 (0.06)	0.08 (0.07)	0.08 (0.06)	0.09 (0.07)	0.07 (0.05)
LA	19.8 (8.80)	20.3 (7.69)	19.2 (9.14)	20.7 (10.7)	19.9 (9.44)	19.4 (9.06)	19.8 (9.00)
EPA	0.03 (0.04)	0.03 (0.05)	0.03 (0.04)	0.03 (0.03)	0.03 (0.04)	0.03 (0.04)	0.03 (0.03)
AA	0.16 (0.09)	0.17 (0.08)	0.15 (0.06)	0.18 (0.16)	0.15 (0.05)	0.18 (0.12)	0.15 (0.07)
Received prenatal DHA intervention, %	103 (50.7%)	42 (53.8%)	49 (48.5%)	12 (50.0%)	28 (56.0%)	30 (61.2%)	23 (46.9%)
Compliance to intervention, %	95.4 (4.9)	95.2 (5.5)	95.4 (4.3)	95.8 (5.0)	94.6 (5.8)	96.0 (4.9)	96.5 (3.6)
Offspring factors							
Sex							
Male	107 (52.7%)	41 (52.6%)	54 (53.5%)	12 (50.0%)	25 (50.0%)	30 (61.2%)	23 (46.9%)
Female	96 (47.3%)	37 (47.4%)	47 (46.5%)	12 (50.0%)	25 (50.0%)	19 (38.8%)	26 (53.1%)
Birth weight, g	3259 (475)	3248 (563)	3254 (425)	3320 (356)	3204 (440)	3266 (502)	3274 (460)
Gestational age at birth, weeks	39.2 (1.8)	39.2 (1.9)	39.1 (1.70)	39.4 (1.36)	39.1 (1.91)	39.1 (2.02)	39.0 (1.71)
Duration of BF, mo.	9.7 (7.9)	9.2 (7.5)	10.3 (8.3)	8.6 (7.5)	9.5 (7.00)	9.9 (8.8)	8.7 (7.7)
Total sedentary time at age 11 years, hrs/day	4.91 (2.10)	4.66 (2.18)	5.05 (2.15)	5.10 (1.74)	4.78 (2.64)	5.32 (1.80)	4.88 (2.00)
Dietary intake at age 11 years[b]							
Energy intake (kcal/day)	2052 (707)	2032 (734)	2073 (671)	2030 (789)	2135 (715)	2048 (717)	1970 (695)
Lipids (g/day)	76.2 (36.4)	73.9 (36.7)	78.8 (31.6)	73.5 (50.0)	78.3 (35.8)	75.3 (33.4)	74.9 (40.2)
Saturated fat (g/day)	30.3 (16.9)	29.8 (18.9)	31.5 (15.3)	27.1 (17.3)	34.1 (18.8)	26.7 (13.5)	29.8 (17.5)
Monounsaturated fat (g/day)	25.2 (13.6)	24.9 (13.5)	25.9 (11.7)	24.0 (19.5)	26.7 (12.9)	23.8 (10.6)	25.1 (16.7)
Polyunsaturated fat (g/day)[b]	15.0 (8.8)	14.2 (7.6)	15.7 (8.1)	14.9 (13.1)	14.8 (7.6)	17.7 (10.4)	12.5 (7.4)
Cholesterol (g/day)	265 (184)	265 (197)	268 (179)	255 (176)	294 (200)	236 (136)	266 (207)
Omega-3 fatty acid intake[b]	0.90 (1.07)	0.82 (0.82)	0.96 (0.92)	0.94 (1.88)	1.01 (0.98)	1.25 (1.43)	0.45 (0.37)
Omega-6 fatty acid intake[b]	6.52 (7.15)	5.54 (4.76)	6.85 (5.93)	7.92 (13.6)	4.39 (4.43)	9.31 (10.3)	5.89 (4.11)
N-6:n-3 fatty acid ratio[b]	8.94 (5.74)	8.97 (6.56)	8.43 (4.99)	10.6 (5.79)	4.41 (1.74)	7.52 (0.53)	15.0 (6.06)

Values presented are mean (SD) unless otherwise stated.

[a] Dietary data only available for 141 individuals at age 11 years

[b] Values differ by offspring dietary n-6:n-3 ratio ($p < 0.05$) as assessed by chi-square tests, t-tests, or ANOVA.

Abbreviations: BMI, body mass index; SES, socioeconomic status score; ALA, Alpha-Linolenic Acid; DHA, Docosahexaenoic Acid; LA, Linoleic Acid; EPA, Eicosapentaenoic Acid; AA, Arachidonic Acid; BF, Breastfeeding; mo, month.

Supplementary Table 6-4. Frequencies of offspring FADS haplotypes in study population (n = 203).

	rs174578	rs2727271	rs174602	rs174605	rs174450	Frequency
Haplotype 1	A	A	T	G	T	0.13
Haplotype 2	A	T	T	G	G	0.07
Haplotype 3	T	T	C	G	G	0.25
Haplotype 4	T	T	C	G	T	0.05
Haplotype 5	T	T	T	T	G	0.06
Haplotype 6	A	T	C	G	G	0.26
Rare haplotype	*	*	*	*	*	0.17

Rare haplotypes defined as any haplotypes with frequency <5%.

Supplementary Table 6-5. Mean (95% CI) of MetS components and MetS score stratified by offspring genotype for SNP rs174602 and n-6:n-3 diet ratio.

	CC N = 53	TT or TC N = 88
BMI-z		
Low n-6:n-3 ratio (<6.67)	0.96 (0.38, 1.53)	0.18 (-0.33, 0.69)
Medium n-6:n-3 ratio (6.67-8.61)	1.02 (0.26, 1.77)	0.58 (0.11, 1.04)
High n-6:n-3 ratio (>8.61)	0.27 (-0.33, 0.87)	0.72 (0.23, 1.21)
HDL		
Low n-6:n-3 ratio (<6.67)	43.5 (38.5, 48.4)	49.4 (45.0, 53.7)
Medium n-6:n-3 ratio (6.67-8.61)	44.1 (37.6, 50.6)	46.1 (42.1, 50.1)
High n-6:n-3 ratio (>8.61)	50.5 (45.3, 55.7)	45.5 (41.3, 49.8)
Triglycerides		
Low n-6:n-3 ratio (<6.67)	130.1 (101.8, 159)	96.4 (71.2, 122)
Medium n-6:n-3 ratio (6.67-8.61)	115.9 (78.5, 153)	105.4 (82.3, 129)
High n-6:n-3 ratio (>8.61)	116.2 (86.3, 146)	124.5 (100.2, 149)
Systolic Blood Pressure		
Low n-6:n-3 ratio (<6.67)	102.0 (98.5, 105)	99.9 (96.8, 103)
Medium n-6:n-3 ratio (6.67-8.61)	105.8 (101.2, 110)	102.6 (99.8, 105)
High n-6:n-3 ratio (>8.61)	99.7 (96.0, 103)	104.8 (101.8, 108)
Glucose		
Low n-6:n-3 ratio (<6.67)	86.4 (83.2, 89.7)	88.5 (85.6, 91.3)
Medium n-6:n-3 ratio (6.67-8.61)	91.9 (87.7, 96.2)	87.9 (85.3, 90.6)
High n-6:n-3 ratio (>8.61)	87.3 (83.9, 90.7)	88.6 (85.8, 91.4)
MetS Score		
Low n-6:n-3 ratio (<6.67)	0.08 (-0.15, 0.30)	-0.19 (-0.39, 0.007)
Medium n-6:n-3 ratio (6.67-8.61)	0.31 (0.02, 0.61)	0.02 (-0.16, 0.21)
High n-6:n-3 ratio (>8.61)	-0.15 (-0.39, 0.09)	0.17 (-0.03, 0.36)

Data are expressed as mean MetS scores adjusted for sex, age, and energy intake

Chapter 7 : Discussion and Conclusions

Since the first documentation of the potential cardioprotective benefits of n-3 LC-PUFAs among Inuit populations in the 1970s, there has been substantial progress made investigating the relationship between dietary LC-PUFAs and cardiometabolic health (1, 2). However, important research gaps remain, especially during important periods of growth and development such as gestation and early adolescence. First, additional research is needed to better characterize cardiometabolic risk during childhood and adolescence and examine how risk factors cluster (3, 4). Second, evidence for the long-term effects of prenatal DHA supplementation on offspring CMH are inconsistent and limited by heterogeneity across studies with respect to population characteristics, supplement dose and duration, follow-up of offspring, and variations in *FADS* genes (5-8). Additionally, most studies incorporating genetic information have typically been conducted in European populations (9). Third, the respective contributions of maternal and offspring *FADS* genetic profiles towards CMH are poorly understood. This dissertation addressed these key gaps in the literature by examining the relationship between prenatal DHA supplementation, maternal and offspring *FADS* polymorphisms, and cardiometabolic health in a population of Mexican children. This discussion will first provide an overview of the key findings from each of the three original research studies. Next, I will outline the strengths and limitations of the methodological approaches used. Third, I will discuss the clinical and public health implications of this work. Finally, I will offer recommendations for future research needed to improve understanding of gene-nutrient interactions and ultimately guide the development of targeted dietary and supplementation recommendations for mothers and children.

7.1 Key Findings

In our first study, we examined clustering of cardiometabolic markers in a population of Mexican children at age 11 years and compared a MetS score to an exploratory CMH score, which additionally included adipokines, lipids, inflammatory markers, and adiposity, to assess cardiometabolic profiles and better define cardiometabolic risk. We found that measures of adiposity and lipids explained the most variation for both MetS and CMH scores, and the MetS score captured nearly as much variation across measures of CMH as the exploratory CMH score. Furthermore, children with overweight or obesity had higher MetS and CMH scores relative to children with normal weight, reflecting increased risk. Our results aligned with previous research reporting that low HDL and high triglycerides, along with increased BMI, are the most observed cardiometabolic risk factors among Mexican populations (10-12). Additionally, this work informed our decision to use the MetS score as the primary outcome for specific aims 2 and 3.

Next, we investigated the interactions between maternal and offspring *FADS* polymorphisms, dietary LC-PUFA intake, and prenatal DHA supplementation in relation to offspring measures of CMH at age 11 years. In our second study, we examined the effect of prenatal DHA supplementation on offspring MetS scores and investigated effect modification by variations in maternal *FADS2* SNP rs174602. We focused on this SNP because, within the POSGRAD study, we previously observed that this SNP modified the effect of the prenatal intervention on offspring birth weight (13), metabolome at 3 mo. (14), and cognition at age 5 years (15). Consistent with prior research, we did not find a main effect of prenatal DHA supplementation on offspring MetS scores at 11 y (16-18); however, we observed evidence of effect modification by variants of maternal SNP rs174602. Offspring of homozygous minor allele (TT) carriers who received prenatal DHA had lower MetS scores relative to the placebo group, while offspring of homozygous major allele (CC) carriers who received DHA had higher MetS

scores relative to offspring whose mothers received placebo. These findings remained significant even after adjusting for birth weight (i.e., the differences observed were not mediated by the effects of the interaction on birth weight). Overall, our results suggested that prenatal DHA supplementation may have differential effects on a child's long-term cardiometabolic risk based on their mother's genotype.

Although maternal and offspring genetics are tightly linked, there is limited information available on the relative contributions of offspring genotype towards CMH. To improve understanding of the relationship between offspring *FADS* genetic profiles and CMH, in our third study, we examined the role of selected variants in both maternal and offspring fatty acid desaturase (*FADS)* genes on CMH at age 11 years and assessed interactions with prenatal docosahexaenoic acid (DHA) supplementation and offspring diet quality at age 11 years. First, to leverage the wealth of genetic information we had available for offspring and to reduce multiple testing, we derived six *FADS* haplotypes from five offspring SNPs. We examined the association of offspring *FADS* haplotypes with MetS score and did not find any significant associations relative to the reference haplotype. Next, we tested interaction between maternal treatment group and maternal and offspring genotypes for SNP rs174602 on the offspring MetS score. While offspring genotype did not independently modify the effect of the intervention on MetS score, when we examined the effects of maternal genotype for SNP rs174602 stratified by offspring genotype for SNP rs174602, mean differences in MetS scores between DHA and placebo groups were larger when maternal and offspring genotypes were matched relative to a mismatch. Additionally, we found evidence of interaction of offspring *FADS* SNP rs174602 with current dietary PUFA intake. Individuals with TT or TC genotype and high dietary n-6:n-3 ratios had higher MetS scores relative to those with low n-6:n-3 ratios, while individuals with CC genotype

and high n-6:n-3 ratio ratios had lower MetS score relative to those in the lowest tertile. As we hypothesized, these findings suggested that maternal genotype was more relevant than offspring genotype for modifying the effect of the prenatal DHA intervention whereas offspring genotype was more relevant for modifying the effects of dietary intake. However, contrary to our hypothesis, among non-carriers, higher n-6:n-3 ratios were associated with lower MetS scores. These findings align with recent research suggesting that n-6 fatty acids may be beneficial for cardiovascular health (19, 20). Our aim 2 and 3 findings corroborate this, suggesting that based on genotype, different diets may be advantageous for subsets of the population; however, further research is needed to confirm these findings.

7.2 Strengths and Limitations

There are several important strengths to this dissertation, including the randomized design of the original trial, standardized data collection methods, and the application of innovative statistical methods to address the research aims. First, this dissertation leverages data from a large, double-blind, placebo-controlled randomized controlled trial of prenatal DHA supplementation during pregnancy. The intervention included a dose of 400 mg/day of algal DHA; use of DHA alone instead of fish oil (DHA + EPA) enabled these analyses to isolate the effect of a sole nutrient on the specified outcomes. Compliance to the prenatal intervention, measured as the percentage of distributed treatment capsules consumed, was monitored weekly by fieldworkers and was high in our study (> 90%).

Following the intervention, offspring born to POSGRAD mothers have been followed since birth. The randomized design of the trial, along with extensive characterization of these children over the life course, is one of the key strengths of this study. Block randomization at study enrollment helps maintain the balance of known and unknown confounders between the

comparison groups, offering a valuable opportunity to assess the effects of the intervention on long-term outcomes. To our knowledge, no prior studies have characterized children spanning from the prenatal period through early adolescence with *FADS* genetic information for mother-child pairs within the context of a prenatal DHA supplementation trial. At age 11 years, multiple measures of cardiometabolic health were measured simultaneously, including traditional MetS risk factors, adiposity, adipokines, and measures of inflammation and insulin resistance. Additionally, the age period of 11 years old offers a stable time for lipid assessment in children prior to the onset of puberty for most of the study population. Furthermore, data collection and laboratory assays were standardized, validated, and conducted by trained personnel within a clinical setting.

Another strength of our study was that it was conducted in Mexico, a region with populations that typically have low dietary intakes of preformed DHA, high dietary intakes of n-6 fatty acids, and a high prevalence of alleles associated with lower conversion of precursor PUFAs into LC-PUFAs. Most studies investigating the effects of n-3 DHA supplementation during pregnancy, and especially those incorporating *FADS* genetic information, have been conducted in populations of European descent (9); thus, this work is needed and timely to inform the development of personalized dietary and supplement recommendations across diverse populations.

Along with these strengths, a few relevant limitations should be considered when interpreting the findings from this dissertation. We used secondary data from a previously conducted RCT that was originally designed to assess outcomes of child growth and development early in the life course. Although the original size of the birth cohort was large (n=973 live births) (21), loss to follow-up was high and the final analytical sample with data through age 11 years was only 58% of the birth cohort; thus, selection bias may influence our findings. However, we evaluated and addressed selection bias by adjusting for factors that differed between individuals

in the cohort and those lost to follow-up in all multivariable regression models. While mothers of children in our analytic samples tended to be older, have a higher BMI, and have a higher socioeconomic status at trial enrollment relative to those lost to follow up, all maternal characteristics were balanced by treatment group in both the analytic samples and missing samples, and loss to follow up did not differ by treatment group. Additionally, there may be limited statistical power to detect differences by treatment group or genotype**.** However, we maximized power by leveraging linkage disequilibrium between genetic variants to estimate haplotypes and reducing the number of tested CMH outcomes by deriving a continuous MetS score via PCA. Although PCA is a powerful dimensionality-reduction tool, it should be noted that loading coefficients of individual cardiometabolic factors from PCA are only applicable to the population from which they are derived. Maternal dietary intake at study enrollment was assessed using a food-frequency questionnaire that was specifically designed to include important PUFA sources (22); however, LC-PUFA status of mothers was only available in a subset of the population. Offspring dietary data at age 11 y were collected via single 24-hour recall and may be subject to recall bias. Pubertal status was assessed by documenting attainment of menarche in females and testosterone concentrations among a subset of males, as compared to more robust methods such as assessing Tanner stages (23). Finally, although we were able to account for total sedentary time (including screen time), we did not have access to a gold-standard measure of physical activity. Overall, generalizability of results may be limited; this work should be reproduced using large, nationally representative datasets in Mexico with consistent sets of cardiometabolic measures, as well as across populations with diverse ethnic backgrounds.

7.3 Clinical and Public Health Implications

Findings from this dissertation have several relevant implications for improving characterization of cardiometabolic risk in pediatric populations and the development of targeted dietary and supplementation recommendations for mothers and children. First, as it becomes less expensive to collect and test blood samples, it is becoming increasingly feasible to collect more comprehensive cardiometabolic data. However, it is important to understand if there is added value in utilizing additional biomarkers to characterize overall cardiometabolic health during this life stage, especially in low-resource environments. Results from our first study showed that MetS scores capture a similar amount of variation compared to CMH scores that incorporated additional biological measures, and across both scores, adiposity accounted for most of the variation in CMH at age 11 years. These results suggested that, particularly within resource-limited settings, it may be most efficient and cost-effective to screen individuals at elevated risk (those with at least one cardiometabolic risk factor, including overweight/obesity) for more intense disease prevention strategies, which may include additional testing of cardiometabolic markers.

Results from our second and third studies have important implications for the design and interpretation of supplementation trials. We showed that diet quality and/or supplementation may have differential effects on cardiometabolic risk based on maternal or offspring genotype. Although additional research is needed to confirm these findings, this work suggests that, in the future, it may be beneficial to account for a mother's *FADS* genetic profile when providing prenatal supplementation recommendations, while offspring *FADS* genetic profile can be used to inform diet quality and, if needed, use of supplements during childhood and adolescence. Overall, our findings reinforce the need for inclusion of genotype information in nutrition supplementation randomized controlled trials. This also has relevance beyond the observed interaction between LC-

PUFAs and *FADS* genes; several other nutrient-gene interactions have been identified, such as interactions between the *MTHFR* gene and folate intake and the *PEMT* gene and choline (24, 25).

Our findings also provide further support that some women may benefit more from n-3 LC-PUFA supplementation based on baseline LC-PUFA status, habitual diet, and genetic variation. We performed this study in a population of Mexican women with low dietary intakes of DHA, along with diets high in n-6 fatty acids (22). While there are currently no dietary reference intake recommendations for LC-PUFAs established by the Institute of Medicine, several groups recommend that women of reproductive age consume at least 200 mg DHA per day, which is attainable by consuming two servings of fish per week (26). For example, one 3 oz serving of Atlantic salmon provides 1.22 g of DHA; two servings per week would provide nearly 2.5 g DHA/week, or 350 mg/day. Therefore, the dose of DHA used in this intervention trial and associated findings align with an amount of DHA that can be obtained through diet alone. Additionally, demonstrating these effects on CMH using an algal form of DHA has important implications, especially as vegetarian and vegan diets become increasingly popular due to religious, ethical, and other reasons, such as reducing greenhouse gas emissions due to animal agriculture. Using an algal form of DHA in this trial further adds to the evidence base, especially for those who do not consume fish oil or seafood. Currently, the Dietary Guidelines for Americans recommend that pregnant women suggest eating two servings of seafood per week, rather than emphasizing the importance of nutrients (e.g., folate, iron). It will be important to engage with healthcare providers and improve knowledge translation in the healthcare setting by better connecting food to nutrients. This will help ensure that patients are able to easily practice dietary recommendations once established, no matter their personal diet.

7.4 Recommendations for Future Research

This dissertation utilized data from a randomized controlled trial of DHA supplementation, in which children have been followed since birth. The current study focused on cardiometabolic health of the children at age 11 years; however, there are several important research questions, among others, that can be answered using the POSGRAD study framework. It will be useful to continue to follow the POSGRAD children through adulthood to: 1) consistently collect a comprehensive set of cardiometabolic factors at multiple time points during adolescence and early adulthood to determine the temporality of risk factor development and disease progression; 2) compare MetS and CMH scores at multiple time points and determine which of these scores offer more useful predictions of adult cardiometabolic risk; and 3) determine if differences in MetS scores at age 11 years have lasting implications for adult cardiometabolic health.

In the current study, we assessed dietary intake of children using a single 24-hour recall. However, future observational research should consider more accurate methods of assessing dietary intake, such as collecting repeated measures of diet, along with objective biomarkers of PUFA and LC-PUFA status. For example, plasma phospholipid DHA, erythrocyte DHA, and platelet DHA have all been identified as effective and robust markers of DHA status (27). By quantifying fatty acid concentrations, Δ5 and Δ6-desaturase activity can be calculated (by dividing parent PUFA by LC-PUFA concentrations for the n-6 and n-3 series, respectively) and correlated with *FADS* SNPs. Additionally, the utility of dietary n-6:n-3 ratios remains controversial, with some evidence suggesting that absolute intake of n-6 and n-3 PUFAs is more relevant than the n-6:n-3 ratio, but this merits further examination. Within the POSGRAD study, a dietary intervention trial could be conducted to further examine interaction between selected *FADS* genetic variants and dietary LC-PUFA intake to assess whether an optimal n-6:n-3 ratio for cardiometabolic health exists.

Historically, randomized controlled trials have been designed with a focus on generalizability (i.e., understanding whether supplementing the general population with a nutrient will yield a health benefit). However, for most nutrients, a one-size-fits-all approach is suboptimal. It is now known that individuals can respond differently to the same foods, nutrients, and supplements consumed due to a variety of factors including, but not limited to diet, environmental exposures, microbiome, intrauterine exposures, and genetics. As the world moves towards using personalized approaches to solve nutrition challenges, trials should be designed such that they better capture background exposures, including dietary intake, supplement use, baseline nutrient status, and genetic information. For example, a multi-center, double-blind, randomized, superiority trial recently showed that women with low baseline DHA status benefit more from a high-dose DHA supplement (1000 mg) during pregnancy relative to a low dose supplement (200 mg) to prevent preterm birth (28). Likewise, future work could investigate whether women with low baseline DHA status and certain *FADS* genotypes benefit more from high dose DHA supplementation during pregnancy. In a similar vein, it is also important to consider systems biology approaches for assessing effects of nutrition supplementation. As high-dimensional data and advanced analytic methods to probe gene-environment interactions become increasingly available, it is becoming more apparent that nutrients do not work in isolation on one organ system. Future work should focus on understanding how the entire biological system responds to DHA supplementation using various "omics" technologies (i.e., genomics, transcriptomics, proteomics, metabolomics) to better guide the development of personalized dietary recommendations (29).

Overall, this work emphasizes the value of a life course approach towards studying prevention of cardiometabolic disease. While the DOHaD theory generally reflects the importance of maternal nutritional status for long-term offspring health, there is increasing recognition of the

paternal role as well; newly designed studies and trials should also consider including paternal nutritional exposures and genetic information (30). Finally, replication of findings is critical for ensuring validity, especially in studies incorporating genetic information. Given the large variation in habitual diet, LC-PUFA status, and genotype distributions across populations, this work should be reproduced in larger, independent cohorts.

7.5 References

1. Bang HO, Dyerberg J, Hjøorne N. The composition of food consumed by Greenland Eskimos. Acta Med Scand 1976;200(1-2):69-73. doi: 10.1111/j.0954-6820.1976.tb08198.x.
2. Leaf A. Historical overview of n-3 fatty acids and coronary heart disease. Am J Clin Nutr 2008;87(6):1978s-80s. doi: 10.1093/ajcn/87.6.1978S.
3. Magge SN, Goodman E, Armstrong SC. The Metabolic Syndrome in Children and Adolescents: Shifting the Focus to Cardiometabolic Risk Factor Clustering. Pediatrics 2017;140(2):e20171603. doi: 10.1542/peds.2017-1603.
4. Ford ES, Li C. Defining the metabolic syndrome in children and adolescents: will the real definition please stand up? J Pediatr 2008;152(2):160-4. doi: 10.1016/j.jpeds.2007.07.056.
5. Stratakis N, Gielen M, Chatzi L, Zeegers MP. Effect of maternal n-3 long-chain polyunsaturated fatty acid supplementation during pregnancy and/or lactation on adiposity in childhood: a systematic review and meta-analysis of randomized controlled trials. Eur J Clin Nutr 2014;68(12):1277-87. doi: 10.1038/ejcn.2014.158.
6. Voortman T, van den Hooven EH, Braun KV, van den Broek M, Bramer WM, Chowdhurry R, et al. Effects of polyunsaturated fatty acid intake and status during pregnancy, lactation, and early childhood on cardiometabolic health: A systematic review. Prog Lipid Res 2015;59:67-87. doi: 10.1016/j.plipres.2015.05.001.
7. Middleton P, Gomersall JC, Gould JF, Shepherd E, Olsen SF, Makrides M. Omega-3 fatty acid addition during pregnancy. Cochrane Database Syst Rev 2018;11(11):Cd003402. doi: 10.1002/14651858.CD003402.pub3.

8. Vahdaninia M, Mackenzie H, Dean T, Helps S. The effectiveness of ω-3 polyunsaturated fatty acid interventions during pregnancy on obesity measures in the offspring: an up-to-date systematic review and meta-analysis. Eur J Nutr 2019;58(7):2597-613. doi: 10.1007/s00394-018-1824-9.

9. Koletzko B, Reischl E, Tanjung C, Gonzalez-Casanova I, Ramakrishnan U, Meldrum S, et al. FADS1 and FADS2 Polymorphisms Modulate Fatty Acid Metabolism and Dietary Impact on Health. Annu Rev Nutr 2019;39:21-44. doi: 10.1146/annurev-nutr-082018-124250.

10. Cárdenas-Villareal VM, López Alvarenga JC, Bastarrachea RA, Rizo-Baeza MM, Cortés-Castell E. [Metabolic syndrome prevalence in teenagers of Monterrey, Nuevo Leon]. Arch Cardiol Mex 2010;80(1):19-26.

11. Cardoso-Saldaña GC, Yamamoto-Kimura L, Medina-Urrutia A, Posadas-Sánchez R, Caracas-Portilla NA, Posadas-Romero C. [Obesity or overweight and metabolic syndrome in Mexico City teenagers]. Arch Cardiol Mex 2010;80(1):12-8.

12. Evia-Viscarra ML, Rodea-Montero ER, Apolinar-Jiménez E, Quintana-Vargas S. Metabolic syndrome and its components among obese (BMI >=95th) Mexican adolescents. Endocr Connect 2013;2(4):208-15. doi: 10.1530/ec-13-0057.

13. Gonzalez-Casanova I, Rzehak P, Stein AD, Garcia Feregrino R, Rivera Dommarco JA, Barraza-Villarreal A, et al. Maternal single nucleotide polymorphisms in the fatty acid desaturase 1 and 2 coding regions modify the impact of prenatal supplementation with DHA on birth weight. Am J Clin Nutr 2016;103(4):1171-8. doi: 10.3945/ajcn.115.121244.

14. Tandon S, Gonzalez-Casanova I, Barraza-Villarreal A, Romieu I, Demmelmair H, Jones DP, et al. Infant Metabolome in Relation to Prenatal DHA Supplementation and Maternal

Single-Nucleotide Polymorphism rs174602: Secondary Analysis of a Randomized Controlled Trial in Mexico. J Nutr 2021;151(11):3339-49. doi: 10.1093/jn/nxab276.

15. Gonzalez Casanova I, Schoen M, Tandon S, Stein AD, Barraza Villarreal A, DiGirolamo AM, et al. Maternal FADS2 single nucleotide polymorphism modified the impact of prenatal docosahexaenoic acid (DHA) supplementation on child neurodevelopment at 5 years: Follow-up of a randomized clinical trial. Clin Nutr 2021;40(10):5339-45. doi: 10.1016/j.clnu.2021.08.026.

16. Rytter D, Schmidt EB, Bech BH, Christensen JH, Henriksen TB, Olsen SF. Fish Oil Supplementation During Late Pregnancy Does Not Influence Plasma Lipids or Lipoprotein Levels in Young Adult Offspring. Lipids 2011;46(12):1091-9. doi: 10.1007/s11745-011-3606-5.

17. Rytter D, Christensen JH, Bech BH, Schmidt EB, Henriksen TB, Olsen SF. The effect of maternal fish oil supplementation during the last trimester of pregnancy on blood pressure, heart rate and heart rate variability in the 19-year-old offspring. Br J Nutr 2012;108(8):1475-83. doi: 10.1017/s0007114511006799.

18. Kerling EH, Hilton JM, Thodosoff JM, Wick J, Colombo J, Carlson SE. Effect of Prenatal Docosahexaenoic Acid Supplementation on Blood Pressure in Children With Overweight Condition or Obesity: A Secondary Analysis of a Randomized Clinical Trial. JAMA Netw Open 2019;2(2):e190088. doi: 10.1001/jamanetworkopen.2019.0088.

19. Poli A, Agostoni C, Visioli F. Dietary Fatty Acids and Inflammation: Focus on the n-6 Series. International Journal of Molecular Sciences 2023;24(5):4567.

20. Kaikkonen JE, Kresanov P, Ahotupa M, Jula A, Mikkilä V, Viikari JSA, et al. High serum n6 fatty acid proportion is associated with lowered LDL oxidation and inflammation: The

Cardiovascular Risk in Young Finns Study. Free Radical Research 2014;48(4):420-6. doi: 10.3109/10715762.2014.883071.

21. Ramakrishnan U, Stein AD, Parra-Cabrera S, Wang M, Imhoff-Kunsch B, Juarez-Marquez S, et al. Effects of docosahexaenoic acid supplementation during pregnancy on gestational age and size at birth: randomized, double-blind, placebo-controlled trial in Mexico. Food and nutrition bulletin 2010;31(2 Suppl):S108-16. doi: 10.1177/15648265100312s203.

22. Parra-Cabrera S, Stein AD, Wang M, Martorell R, Rivera J, Ramakrishnan U. Dietary intakes of polyunsaturated fatty acids among pregnant Mexican women. Matern Child Nutr 2011;7(2):140-7. doi: 10.1111/j.1740-8709.2010.00254.x.

23. Emmanuel M, Bokor BR. Tanner Stages. Edtion ed. StatPearls. Treasure Island (FL): StatPearls Publishing

24. Lisboa JVC, Ribeiro MR, Luna RCP, Lima RPA, Nascimento R, Monteiro M, et al. Food Intervention with Folate Reduces TNF-α and Interleukin Levels in Overweight and Obese Women with the MTHFR C677T Polymorphism: A Randomized Trial. Nutrients 2020;12(2). doi: 10.3390/nu12020361.

25. Smallwood T, Allayee H, Bennett BJ. Choline metabolites: gene by diet interactions. Curr Opin Lipidol 2016;27(1):33-9. doi: 10.1097/mol.0000000000000259.

26. Koletzko B, Lien E, Agostoni C, Böhles H, Campoy C, Cetin I, et al. The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations. J Perinat Med 2008;36(1):5-14. doi: 10.1515/jpm.2008.001.

27. Serra-Majem L, Nissensohn M, Øverby NC, Fekete K. Dietary methods and biomarkers of omega 3 fatty acids: a systematic review. Br J Nutr 2012;107 Suppl 2:S64-76. doi: 10.1017/s000711451200147x.

28. Carlson SE, Gajewski BJ, Valentine CJ, Kerling EH, Weiner CP, Cackovic M, et al. Higher dose docosahexaenoic acid supplementation during pregnancy and early preterm birth: A randomised, double-blind, adaptive-design superiority trial. eClinicalMedicine 2021;36. doi: 10.1016/j.eclinm.2021.100905.

29. Badimon L, Vilahur G, Padro T. Systems biology approaches to understand the effects of nutrition and promote health. Br J Clin Pharmacol 2017;83(1):38-45. doi: 10.1111/bcp.12965.

30. Soubry A. POHaD: why we should study future fathers. Environ Epigenet 2018;4(2):dvy007. doi: 10.1093/eep/dvy007.