Infant growth trajectories and dyslipidemia in adolescence

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ABSTRACT

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Developmental Origins of Health and Disease (DOHaD) theory posits that elements of early life affect susceptibility to chronic disease during adulthood. Postnatal growth is one such element of early life that has been hypothesized as influencing chronic disease. Cardiovascular Disease (CVD) is responsible for one out of every three deaths in the United States with primary and modifiable risk factors including lipids. Researchers in the field of developmental origins hypothesize that abnormal postnatal growth can present an environmental factor that, when combined with activity on a molecular scale, can permanently program lipid metabolism. Most studies addressing the hypothesis of growth effects on dyslipidemia are limited in their characterization of growth and are homogeneous in racial and ethnic composition - being primarily European. We propose to investigate the association between postnatal anthropometric change and adverse lipid levels in adolescence in a contemporary Chilean birth cohort with extensive measures of weight and length in the first year of life accompanied by high quality clinical measures of CVD risk factors. Primary aims include a) characterization of predictors of infant growth, b) investigate the association between postnatal growth trajectories and dyslipidemia, and c) evaluate the capacity of postnatal growth trajectories to modify the association between functional genetic variants and dyslipidemia in adolescence. Results from this research can inform efforts to identify predictors of dyslipidemia and its accompanying risk of CVD as well as furthering support of postnatal growth as an environmental cue for lipid metabolism programming. In turn, this

information can further support the search for optimal postnatal growth and with it, the potential for modification of chronic disease risk factor development later in life.

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LIST OF ABBREVIATIONS

ALT Alanine Aminotransferase 30, 31

BMI Body Mass Index 27, 28

CGXE Candidate Gene-environment Interaction 38

CVD Cardiovascular Disease i, 1, 4, 5, 6, 7, 36, 51

DOHAD Developmental Origins Of Health And Disease i, 5, 37

GGT Gamma Glutamyltransferase 30, 31

GRS Genetic Risk Score 22, 48

GWAS Genome Wide Association Studies 22, 27, 28, 38

GXE Gene-environment Interaction 37, 38

HDL-C High Density Lipoprotein Cholesterol 1, 8, 9, 10, 12, 13, 14, 15, 16, 17, 20, 21, 22, 23, 24, 25

HL Hispanic/Latino 1, 7, 14, 19, 20, 21, 41

LDL-C Low Density Lipoprotein Cholesterol 1, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 23, 24

LGMM Latent Growth Mixture Modeling 36, 37

NAFLD Nonalcoholic Fatty Liver Disease 30

NON-HDL-C Non-High Density Lipoprotein Cholesterol 25

PAR Predictive Adaptive Response 29

POF Postnatal Overfeeding 23, 24

RNA Ribonucleic Acid 31

SITAR SuperImposition By Translation And Rotation 36

SLCS Santiago Longitudinal Cohort Study 1, 9, 11, 20, 36, 39, 40, 41, 42

SNP Single Nucleotide Polymorphism 47

TC Total Cholesterol 8, 9, 10, 13, 23, 25, 34

TG Triglycerides 8, 9, 10, 11, 13, 16, 17, 19, 21, 22, 23, 24, 25, 34

WFL Weight-for-length 30

CHAPTER 1

Specific Aims

1.1 Specific Aims

Cardiovascular disease (CVD) is rare in childhood, but risk factors for CVD are not. The prevalence of adverse levels of high density lipoprotein cholesterol (High Density Lipoprotein Cholesterol (HDL-C)) (<40 mg/dL), low-density lipoprotein cholesterol (Low Density Lipoprotein Cholesterol (LDL-C)) ($\geq$ 130 mg/dL) and triglycerides ($\geq$ 130 mg/dL) exceeded 5% in United States (U.S.) children from 2007 to 2010 [1–3]. Importantly, such dyslipidemia in childhood tracks into later life [4,5], predisposing children to a cumulative risk burden and subsequent CVD events in adulthood.

Strong associations exist between infant growth trajectories and the development of CVD in adulthood, supporting the Developmental Origins of Health and Disease concept [6–11]. Few empirical studies have captured the relationship between infant growth trajectories and risk factors for CVD such as dyslipidemia, and of seven recent studies on this topic [12–17], only one focuses on ancestrally diverse populations [12]. Furthermore, these characterizations of growth in infancy do not leverage advanced methodological approaches in assessing growth. The lack of ancestral diversity in samples and limited characterization of growth trajectories presents a knowledge gap and an important area for research in the etiologic origins of CVD. I propose to address this gap with a contemporary birth cohort of Hispanic/Latino (HL) origin – the Santiago Longitudinal Cohort Study (SLCS) [18,19]. Three specific aims are proposed as follows:

1.1.1 *Aim 1*

Characterize individual infant growth trajectories, identify predictors of such growth, and replicate predictors in a Chilean birth cohort.

1.1.1.1 ***Aim 1.1*** Characterize infant growth trajectories with nonlinear mixed effects models [20], which measure individual shifts relative to the population average curve in: (a) size (above or below on the outcome scale), (b) tempo (to the right or left on the age scale), and (c) velocity (steep or shallow slope). Assess the association between postnatal growth characteristics and maternal predictors of postnatal growth such as pre-pregnancy BMI, height, age and education.

Note: Subsequent references to 'growth trajectory' or 'postnatal growth' as nested within the hypotheses refer to change in weight-for-length (kg/cm), weight (kg) and length (cm) as a group.

1.1.1.1.1 ***Hypothesis 1a***

Findings will replicate **Growth and Obesity Chilean Cohort Study** (GOCS) results: positive associations will exist between maternal pre-pregnancy BMI, height, age and lower levels of education with infant trajectory size. A positive association will exist between maternal education and the trajectory velocity.

1.1.1.2 ***Aim 1.2*** Determine presence of child growth trajectory latent classes with latent growth mixture models (LGMM).

1.1.1.2.1 ***Hypothesis 1b***

Several classes of growth trajectories exist including one with a steeper weight change than other groups.

1.1.2 *Aim 2*

Examine the association between infant growth trajectory characteristics - size, tempo, and velocity - and lipid levels at 17 years of age.

1.1.2.1 ***Hypothesis 2a*** Infants at extreme levels of growth, i.e. fastest or slowest, are at greater risk of adverse lipid levels in adolescence than children with average growth.

1.1.2.2 ***Hypothesis 2b*** Sex differences exist with boys demonstrating a stronger effect than girls.

1.1.3 *Aim 3*

Assess gene-environment interaction between growth trajectory characteristics and genetic variants underlying lipid metabolism at 17 years of age.

1.1.3.1 Hypothesis 3 Infant growth influences the strength of association between selected genetic variants and adverse lipid levels for children. For example, in the context of extreme and less favorable growth characteristics, a stronger association will exist between genetic effects and adverse levels of low density lipoprotein cholesterol.

CHAPTER 2

Background and Significance

2.1 Background and Significance

2.1.1 Overview

2.1.1.1 Stages of development in childhood In this work, the term 'childhood' is meant to categorize all ages from birth to adulthood. Within that age group the stages will be categorized according to the Healthy People 2020 initiative. Guidelines from this organization define early childhood from birth to year 8, middle childhood from ages 6 to 12 years and adolescence from twelve to eighteen years [21], This definition of childhood growth stages is certainly not a worldwide standard, but can be considered as a starting point for age groups in the life course.

2.1.1.2 Dyslipidemia in children CVD prevention efforts targeting specific risk factors [22–24], are featured as a cornerstone of ideal cardiovascular health as outlined in the 2020 American Heart Association strategic plan [25]. Children may be free of CVD risk factors, but they certainly are not immune to their development and are prime candidates for a primordial prevention framework. Dyslipidemia is one such modifiable risk factor in a prevention framework that can accelerate the atherosclerotic process in children [26]. Also, this window of time deserves more research and scrutiny CVD as risk factors in children are stronger predictors of atherosclerosis than measurements at time of examination in adulthood [27].

Many biologic and demographic domains are unique to the age groups nested within childhood and can guide hypothesis formulation. Some of these domains include the definition

of dyslipidemia, tracking of lipid level to adulthood, known causal components of dyslipidemia, and patterns across age, gender, racial, and ethnic groups. The definition of dyslipidemia is one prominent difference between children and adults via lipid thresholds. Patterns in lipids vary across age within childhood and should be addressed when forming models of lipid variation as an outcome.

In adults, several large genetic consortia [28] have identified hundreds of loci influencing plasma lipid levels in humans. In particular, these studies have produced candidate genetic loci having a plausible function in lipid metabolism [29]. Although research is still ongoing, these genetic variants do appear to extend to populations of children [30–33]. Functional lipid variants offer a chance to test gene-environment interactions in a DOHaD context. In particular, the interactions would be formed by tests of lipid outcome changes across genetic variant values situated within different postnatal growth patterns.

2.1.1.3 Evidence for an association between postnatal growth and dyslipidemia later in life

Postnatal life presents a considerable area for intervention that can impact the health trajectory extending far past the immediate period. With this potential, there have been a variety of animal and human studies in the past ten years investigating the impact of postnatal growth on CVD risk factors later in life. These studies are motivated by the DOHaD paradigm, and they have offered evidence that what happens early in development can affect cardiovascular risk factors, including lipid levels, later in the life course.

Prenatal environment also plays a role in the formation of CVD outcomes, and the combination of prenatal and postnatal growth exposures can lead to different combinations with different effects on downstream lipid levels [34]. At the outset of DOHaD-motivated studies, the most common hypotheses and data supporting these hypotheses was intrauterine growth restriction and postnatal catch-up growth resulting in adverse lipid levels and CVD outcomes [8,9,35,36]. The frequency of research meant to support this paradigm reflected the availability of older European cohorts subject to food shortages. This scenario also

reflects what can currently occur in a large portion of developing countries [37], but with the increasing availability of energy dense foods and sedentary behavior in developed countries there is also a place to study the effects of faster than average growth on later CVD outcomes.

Studies of postnatal growth and associations with adverse health outcomes continue in human observational studies. More recent birth cohort studies include accelerated growth across a broad array of birth weights providing a proxy for prenatal growth schemes. These studies provide evidence of associations between growth patterns during infancy and altered lipid levels later in life [15,36,38,39]. In turn, these results have converged to a hypothesis that irregularities in fetal and postnatal growth influence liver growth and subsequent lipid metabolism [40–42].

Postnatal growth in the first year may provide a foundation for metabolic programming that permanently alters lipid metabolism later in life. Cues to alter lipid metabolism could lie in the size of the liver following abnormal growth patterns. [15,39,43]. Alternatively, and more likely given current research, it is possible that irregular infant growth could function as a cue to modify gene expression in tissues such as the liver [44], which in turn alters lipid levels. These explanations of potential biological mechanisms cover a broader concept of developmental plasticity in which a variety of phenotypes can arise from a specific genotype upon exposure to environmental cues [40].

Although studies with similar designs demonstrate different directions of effect, the consensus is that abnormal postnatal growth patterns, faster or slower than average, result in adverse CVD levels of risk factors. Animal models offer the most convincing evidence for postnatal growth as a causal effect for lipid metabolism disturbance through experimental manipulation of maternal and postnatal diet with growth change. Measurement of gene expression at the tissue level across these groups provides evidence that these metabolic disturbances are occurring at the molecular level.

2.1.1.4 Analytic Issues

Identification of non-optimal growth trajectories is available in the literature, and there are connections with adverse lipid profiles. However, sparse anthropometric measures early in life paired with CVD risk measures later in life have limited the ability to fully capture postnatal growth as an underlying environmental exposure. Alternative methodological approaches with numerous infant anthropometric measures can provide evidence to motivate prevention work such as the development of targets for intervention or therapy [45]. There are methods from evolving research that can summarize different components of infant growth such as velocity (slope) and size (birth weight), which are not necessarily independent of each other. Furthermore, intervention targets are not definitive as it relates to growth. The application of current methods paired with more abundant measures for growth can help fill research gaps by providing further evidence of relations between infant growth and CVD risk factors.

2.1.1.5 Summary

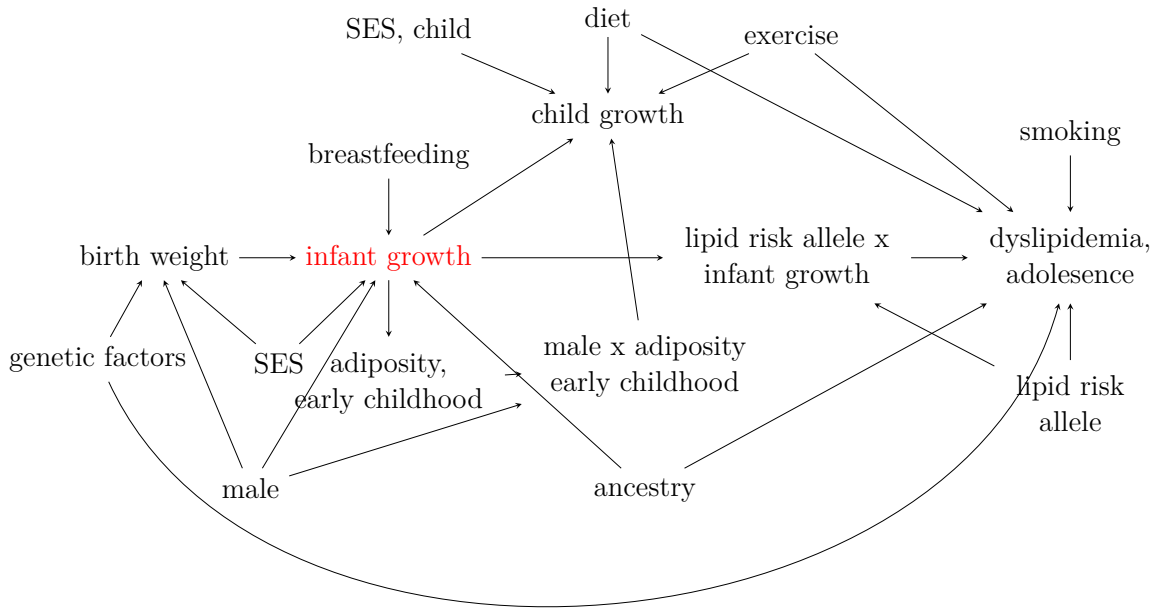
To summarize, prior evidence suggests postnatal growth may play a role in the conditioning of lipid metabolism. Continued study of infant growth and lipid levels with observational data as present in the proposed sample can 1) allow for the identification of predictors of postnatal growth, 2) provide a granular characterization of the association between infant growth and lipids, 3) assess gene-environment interactions in the context of postnatal growth, and 4) describe these relationships in a HL sample – the combination of which is previously lacking in prior studies on these topics. These four areas provide information that is needed to clarify the role of infant growth as a factor influencing adverse lipid levels and metabolism in diverse populations.

2.1.2 Dyslipidemia in children

2.1.2.1 Definition

Dyslipidemia is a common complex disease with origins in childhood. This disease carries the potential for sustained exposure to atherosclerosis making it an important area for identification and intervention. Adverse lipid levels characterize dyslipi-

Figure 2.1: Directed Acyclic Diagram (DAG) for research topic.



demia, and they signal higher risk of atherosclerosis that can be modified with causal factors including lifestyle choices. A conceptual diagram of the relationships judged relevant to the hypothesized disease process is included in Fig. 2.1.

To assess dyslipidemia, four separate outcomes are commonly used: Total Cholesterol (TC), Triglycerides (TG), LDL-C, and HDL-C. Dyslipidemia occurs when at least one of the 'lipid, lipoprotein, or apolipoprotein levels are abnormal.' [46]. Thresholds for each of these four factors exist, beyond which there is a higher risk of 'initiation and progression of atherosclerotic lesions...' [47]. Values that are considered above or below acceptable thresholds are characterized in table 2.1, as reproduced from an expert panel in 2011 [47]. These values have evolved into a current pattern shaped by the rise in obesity defined by high triglycerides and LDL-C combined with lower HDL-C [48].

Although the standard for dyslipidemia in children started with the 1992 National Cholesterol Education Program report [49] and most recently ended with the 2011 guidelines

[47], there is room for change given the advances in research and understanding of the role for dyslipidemia in childhood negatively influencing the path of atherosclerosis [27,48].

Dyslipidemia in children is prevalent and widespread across countries. Over 20% of children ages 6 to 19 in the United States from 2011 to 2014 have at least one abnormal measure [50] relating to dyslipidemia. In the SLCS Chilean sample of adolescents aged 16-17 years, 79% of children had at least one abnormal measure of cardiometabolic risk including adverse levels of TG, HDL-C, blood pressure and fasting plasma glucose [51]. In particular, the mean HDL-C level (SD) in the Chilean sample was 40.1 (10.6).

Table 2.1: Recommended cut points for lipid and lipoprotein levels in young adults (adapted from table 9-1 in the 2011 NHLBI guidelines for lipid management in children and adolescents

Category	Low, mg/dL	Acceptable, mg/dL	Borderline-High, mg/dL	High, mg/dL
TC		<170	170-199	≥ 200
LDL		<110	110-129	≥ 130
Non-HDL		<120	120-144	≥ 145
Apolipoprotein B		<90	90-109	≥ 110
Triglycerides				
0-9 y		<75	75-99	≥ 100
10-19 y		<90	90-129	≥ 130
HDL cholesterol	<40	>45	40-45	
Apolipoprotein A-1	<115	>120	115-120	

2.1.2.1.1 Total cholesterol (TC)

Total cholesterol is a measure from a commonly conducted blood test and includes the total count of all cholesterol present in the blood sample, a combination of LDL-C, HDL-C and VLDL-C [52]. In evaluating trends in time from 1999 to 2012, the prevalence of TC [95% CI] has declined in the total U.S. population of children ages 8 to 17 years, from 10.6%

[8.3, 13.2] to 7.8% [5.7, 10.4] [2]. Among U.S. children ages 6 to 19 years the average TC levels [95% CI] have declined from 172 [169, 176] in 1988-1994 to 164 [161, 167] in 2007-2010. Among children ages 6-19 years in the United States between 2011-2014, 7.4% have high TC [50]. This prevalence statistic varies by age, sex and race. In comparison, estimates for Chilean children 5 to 18 years of age in the Chilean town of Concepión [**???**] was 9% and 12% for boys and girls, respectively. Another average TC estimate for children ages 11-14 from 2009 to 2011 is 159.2 (+/-28.3) [53]. The thresholds for high TC for these estimates are slightly larger compared to the prior two U.S. prevalence estimates.

2.1.2.1.2 Triglycerides (TG)

When combined with HDL-C and LDL-C, triglycerides provide unique information regarding cardiovascular disease risk. Yet the causal role for triglycerides is still a matter of current research in cardiovascular disease [54,55]. For children, there is evidence that triglyceride levels are an independent biomarker for subsequent cardiovascular disease [56]. A triglyceride measure at or above 150 mg/dL is considered abnormally high for children and corresponds to a percentile from a designated standard. For example, the value for triglycerides in table 2.1 corresponds to the 90th percentile for people ages 20-24 in the Lipid Research Clinics Prevalence Study [57]. Typically, triglyceride measures are collected after fasting, and are more difficult to collect as well as being less frequently done [2]. Interestingly, recent studies show that non-fasting levels are considered adequate for screening purposes making the fasting efforts less important [55]. However, in the United States fasting levels are still recommended and therefore they are not included in as many analyses as those with TC or HDL-C, in which fasting is not required. Also, triglyceride levels vary much more than HDL-C levels throughout the day.

Based on NHANES estimates from 1988 to 1994, the percent (95% CI) of adolescents aged 12-19 years with triglycerides $\geq$ 130 mg/dL was 17.3% (13.6-21.0) and this percentage declined to 12.4% (10.1-14.8) from 2007 to 2010 [1]. Also, over 10% of United States children

ages 12-19 (n=1,099) had triglycerides above 160 mg/dL [58]. In the same sample, when stratified by gender, average (SE) triglycerides were 91 (4.0) and 96 (3.9) mg/dL for males and females aged 12 to 19 years, respectively. In comparison, a study of Chilean youth ages 5 to 18 years old during the 1990s in Concepcion, Chile reported mean triglycerides of 80 (+/-35) and 87 (+/-38) mg/dL for males (n=1,286) and females (n=816), respectively [**???**]. A later sample from 2009 to 2011 for Chilean children ages 11 to 14 years reports an average triglyceride level at 93.2 (+/- 60) mg/dL [53]. Finally, the SLCS study population from Chile, ages 17 to 18 years reports an average (SD) triglyceride level of 88.3 (50.2) mg/dL [51]. Some of the reported triglyceride levels appear to be more favorable in the Chilean sample – lower – but it is worth noting that these samples are from one city as well as a different age range and the United States sample represents the country on the whole.

2.1.2.1.3 Low density lipoprotein cholesterol (LDL-C)

LDL-C levels are considered one of the strongest predictors of cardiovascular disease as well as playing a causal role in atherosclerosis fitting into the 'cholesterol hypothesis' [59–61]. Values are indirectly calculated with the most common method being the Friedewald equation: [LDL-cholesterol] = [total cholesterol] – [HDL-cholesterol] – [triglycerides/5]. This measure has not been without controversy [62]. The Friedwald equation was developed in a sample less than 500 people more than 40 years ago, and assumptions regarding the ratio of TG:VLDL-C equal to 5 may be inaccurate with the amount of variability. One alternative, proposed by Martin et al. [62] is to use an adjustable factor for the TG:VLDL-C ratio. However, the indirectly calculated values are very close to the directly measured LDL-C values [63,64].

In the United States, LDL-C levels in children ages 12-19 years were at a mean (95% CI) of 95 mg/dL (92-98) in 1998-1994 and dropping to 90 mg/dL (88-91) in 2007-2010 [1]. In terms of adverse levels of LDL-C, considered to be $\geq$ 130 mg/dL, across time in United States adolescents, the percentage (95% CI) has declined from 11.9% (8.3-15.5) in 1988-1994 to 7.4% (5.4-9.4) in 2007-2010 [1]. In contrast, mean LDL-C (+/- sd) levels for a sample of

Chilean males and females in the 1990s ages 5-18 years were 94 +/- 27 and 96 +/- 29 mg/dl with 10% and 11% $\geq$ 130 mg/dL, respectively [**???**]. For a Chilean sample of children ages 10 to 14 years from 2009 to 2011, the average LDL-C level was 89.0 (+/- 31.5) mg/dL [53]. Considering the time ranges, the estimated mean LDL-C levels appear similar across the two countries.

2.1.2.1.4 High density lipoprotein cholesterol (HDL-C)

HDL-C is an interesting component of dyslipidemia in that it is a well established biomarker for cardiovascular risk, but its causal role in atherosclerosis is yet clear [65]. The evolution of therapeutic treatment for LDL-C versus HDL-C levels is one salient example representing the paucity of evidence supporting HDL-C as a causal factor. No treatment has been found in which raising HDL-C leads to improved cardiovascular disease outcomes, unlike the case for LDL-C and statin use. In turn, the "cholesterol hypothesis" has thus far failed to apply to HDL-C. A plausible causal explanation, named the "HDL function" hypothesis [65], has shifted attention from the amount to the function of HDL-C. Following this hypothesis implies little is to be gained interpreting amounts of HDL-C in the bloodstream when evaluating HDL-C as a causal factor in atherosclerosis.

Unlike LDL-C, direct measurement of HDL-C is common and occurs via an assay. For example, NHANES has used the direct immunoassay method since 2003 [2]. Low HDL-C is considered a risk factor and the threshold is 40 mg/dL. In the United States the percent (95% CI) of children aged 8 to 17 years with low HDL-C has gone from 17.9% (15.0, 21.0) in 1999-2000 to 12.8% (9.8-16.2) in 2011-2012 [2]. The mean HDL-C in children aged 6 to 19 years in 2007-2010 was 52.2 (51.6, 52.8) mg/dL [1]. In contrast, from 2009 to 2011, the mean HDL-C (SD) for children ages 10 to 14 in Chile are 51.9 (12.1) mg/dL [53]. The proposed study sample of Chilean children aged 17-18 had a mean (SD) HDL-C level of 40.1 (10.6). Values for HDL-C appear lower for the study sample in Chile compared to the U.S. sample.

2.1.2.1.5 Other combinations of lipid measures [HDL-C / TG ratio, ApoB/ApoA ratio, non-HDL, etc..]

HDL-C/TG ratio One measure that has been classified as a good marker for insulin resistance is the ratio of HDL-C to TG. This association has been shown to be present in a diverse range of racial/ethnic groups such as obese American youth from a range of ethnic/racial backgrounds [**???**–hdl_2011], indigenous Argentinean children [66], Chinese adults with newly diagnosed diabetes [67], and obese/overweight Italian children [68]. In the study of Italian children, a positive association was found between carotid intima-media thickness (cIMT) and concentric left ventricular hypertrophy (cLVH) and the HDL-C to TG ratio. A caveat for this relationship is that this association between HDL/TG ratio and insulin resistance and organ damage has been mostly in subsets of general populations such as obese youth, whose lipid profile is characterized by high TG/low HD [69]. One recent study in another group of obese and overweight children in rural Appalachia, United States, indicated no advantage of a TG/HDL-C marker to identify insulin resistance [70]. Further research is needed to confirm the utility of this marker.

Non-HDL-C

Also associated with the TG/HDL-C ratio, non-HDL-C is another measure incorporating a composite of lipid measures. Calculation of non-HDL-C is simply equal to TC - HDL-C. Unlike a measure with directly measured TG, non-HDL-C does not require any fasting to obtain meaningful estimates and is a stronger marker for CAD than LDL-C alone [69,71]. An advantage of this measures is the inclusion of 'triglyceride-rich lipoproteins whereas LDL cholesterol does not.' [72] This particular measure has been shown in Mendelian randomization studies to have an additional association beyond that of LDL with coronary artery disease (CAD) risk whereas there was no effect when examining TG and CAD [72]. Remnant cholesterol, with a strong association with CAD [71,73] may figure more prominently in non-HDL-C and support the strength of association with CAD.

2.1.2.2 Age patterns in lipid level variation Average lipid levels in U.S. children follow a distinct pattern from age 2 to 18 years. The National Health and Examination Study (NHANES) is one of the few studies allowing examination of lipid levels in children across a broad age range. In the United States mean non-HDL-C levels remain between 100 and 120 mg/dL between 6 and 18 years of age between 2005-2010 for three racial/ethnic groups: Non-Hispanic Whites, Non-Hispanic Blacks and Mexican-Americans. Within that range the levels rise from age 6 years peaking at age 10 then decline in early teens to rise again. In the same NHANES sample with some overlap in sampling years, this prevalence pattern across age occurs when examining separate measures of nonfasting LDL-C and triglycerides, total cholesterol and HDL-C [74].

Notably, the pattern described above does not apply to Mexican-American females showing little change in average levels from 6 to 12 years. In a study of more than 38,000 Brazilian adolescents aged 12-14 and 15-17 years from 2013-2014 [75], evidence of gender differences also occurs. Age was positively associated with LDL-C levels in males but not in females, which is similar to the NHANES pattern for Mexican-American females mentioned above. These published studies provide clues for lipid variation across different ages in childhood in different HL samples, which may not match the average United States patterns for children.

Given these fluctuations in lipid levels across childhood, universal lipid level screening is now recommended between ages 9 to 11 given a decline in cholesterol levels during adolescence [46,47]. The next age at which screening is recommended is 17 to 21 years. Despite these recommendations no more than 11 percent of U.S. children in a sample exceeding 200,000 were screened in 2012 [76].

2.1.2.3 Tracking into adulthood Once a child achieves an adverse lipid level leading to a diagnosis of dyslipidemia, the chances are high that this child will have dyslipidemia

as an adult [5,77]. Thus far the four largest cohorts with information to provide estimates tracking lipids from childhood to adulthood are: 1) the Young Finns study, 2) the American Bogalusa study, 3) the Australian Childhood Determinants of Adult Health study, and 4) the Muscatine study [78]. These studies have built strong evidence over time that adverse lipid levels in childhood is associated with various measures of subclinical atherosclerosis in adulthood such as carotid intima-media thickness (IMT) and coronary artery calcification (CAC) [79–87].

One example representative of findings from these four studies is LDL-C levels in Finnish adolescents predict CAC in adults aged 40 to 46 years independent of LDL-C changes between the two age periods [79]. For a one standard deviation increase in adolescent LDL-C measure (mg/dL) the odds ratio was 1.34. Also, high LDL-C levels during childhood were found to track given the development of adverse lifestyle changes between adolescence and measures taken during adulthood [88]. If children developed higher adiposity and and smoking behaviors after the adolescent measures these people were found to maintain adverse LDL-C levels in adulthood. This evidence signals the importance of intermediary lifestyle factors such as overweight and obesity along with smoking in maintaining dyslipidemia.

The most recently published study outlining risk of atherosclerosis in adults after exposure to abnormal lipid levels in childhood is from Finland [89]. In this study more than 2,200 boys and girls followed for more than 27 years have a linear association of 0.43 ($p < 0.0001$) for cholesterol levels during childhood and adulthood. Also, the ages of 12 to 18 years was considered as having the best sensitivity and specificity for abnormal LDL-C and HDL-C levels, ranging from 75% to 95% and 20% to 57%, respectively. The evidence supports a strong link between exposure to adverse lipid levels during adolescence and continuing link to this factor as an adult.

Research on tracking of abnormal lipid values is limited in racial diversity with the majority of study populations being of European descent. However, in the Bogalusa study

in which over 25% of the sample are black, there were racial differences discovered for a linear association between childhood lipid levels and carotid intima–media thickness (IMT) in adulthood [83]. LDL-C was identified as being linearly associated across the lifecourse in regression analyses for both black and white females, but restricted to whites for males. Also, triglycerides appeared to predict IMT for white males only. Given these findings it is important to note that selecting one measure for the largest studies may not extend to other populations, including the Chilean study sample identified for this project thus it is important to evaluate all measures contributing to a diagnosis of dyslipidemia.

2.1.2.4 Gender differences In terms of lipid metabolism, males and females are not identical given different hormonal characteristics [90]. However, gender differences in nationally representative studies indicate little gender differences in prevalence of dyslipidemia in childhood. In the most recent evaluation of NHANES U.S. data from 2011-2012 for children aged 8 to 17 years, the prevalence of dyslipidemia mg/dL (95% CI) was 21.0 (148.8-28.5) and 19.3 (16.2-22.8) for girls and boys, respectively [2]. Similarly, the values for non-HDL-C, often considered a good indicator in childhood for later preclinical atherosclerotic change [82], is similar in U.S. children ages 6 to 19 years across three different time periods: 1988-1994, 1999-2002, and 2007-2010 [1]. Average non-HDL-C (95% CI) for males was 113 (111-115), 114 (112-116) and 107 (105-109) across the three time periods and for females it was 116 (114-118), 116 (114-118) and 108 (106-110).

Similarities across gender are also similar in countries outside of the U.S. In the proposed study sample of Chilean children the mean HDL-C levels (SD) mg/dL for males and females aged 16-17 years was 38.0 (10.1) and 42.5 (10.7), respectively [51]. Mean TG (mg/dL) were 88.4 (52.4) and 88.2 (47.6) for males and females, respectively. In a recent group of urban Mexican children aged 6-15 years (n=383) [91], the average HDL-C (SD) mg/dL was 45.2 (12.7) and 42.7 (13.1). Mean triglyceride levels (SD) mg/dL for participants aged 10-15 years were 99.3 (52.6) and 101.8 (50.3). U.S. estimated mean HDL-C mg/dL [1] are also

similar across gender, but higher and at a more favorable level with 52.2 (51.6-52.8) and 52.9 (52.2-53.7) for males and females, respectively. In both of these samples outside of the U.S., the differences in HDL-C and TG were not substantial between gender groups.

Dyslipidemia prevalence estimates are similar in males and females, but there appears to be effect modification occurring by gender when examining the association between dyslipidemia and obesity. With obesity being a strong comorbidity with dyslipidemia outside of primary genetic causes in children, presence of this effect modification is an important reason to stratify the analyses by gender. Although little is published on this topic, a recent study of 161 Portuguese children indicates that males bear the brunt of adverse risk compared to females [92]]. In particular, there are no associations between waist circumference and measures of dyslipidemia including total cholesterol, LDL-C, and triglycerides.

2.1.2.5 Primary genetic causes of dyslipidemia

2.1.2.5.1 Monogenic lipid disorders

Monogenic lipid disorders are distinguished by lipid levels that cannot be altered by lifestyle choices. Familial hypercholesterolemia (FH) is an autosomal dominant lipid disorder characterized by very high LDL levels. Marked by disruptions in the lipid metabolic pathway, these primary disorders are also rare. FH occurs in about 1 in 500 children in the United States population [[93]; peterson_review_2012] and '50% of untreated male heterozygotes and 25% of untreated female heterozygotes will develop CAD by 50 years of age.' [93] Another example of an inherited disorder is familial combined hyperlipidemia (FCHL), a disorder characterized by overproduction of very low density lipoproteins (VLDL). FCHL is considered to be the most common genetic dyslipidemia in people of European ancestry [94].

Given the rarity of these genetic disorders in the populations we do not expect to find many if anyone at at with this disease. However, it is worthwhile further investigating any outliers in the analyses as a rare condition such as this may serve as a confounder for infant

growth and dyslipidemia outcomes [95].

2.1.2.6 Secondary causes of dyslipidemia

Several high-level risk factors worth noting as secondary causes of dyslipidemia in children include severe hypertension exceeding the 99th percentile, cigarette smoking, oral contraceptive use, type 1 and type 2 diabetes mellitis (DM), and BMI exceeding the 97th percentile [46,47]. Type I and 2 DM could be potential confounders if these factors were on a pathway between dyslipidemia during adolescence and childhood. One such pathway could be a genetic variant associated with both T1DM or T2DM and infant growth. However, no such factors have been explained in the literature. Also, childhood weight following infant growth can be considered a mediator and as such should not be considered in the set of adjustment factors. Although these conditions or risk factors can lead to dyslipidemia in adolescence they are considered confounders and will not be considered in the analyses.

Other secondary special high risk conditions include chronic kidney disease, children with heart transplants and Kawasaki disease [47]. The infrequency of these conditions also disallows any further consideration of these factors in proposed analyses.

2.1.2.7 Environmental factors for infants

2.1.2.7.1 Feeding

During the first four to six months of infancy, breastfeeding, formula feeding or some combination thereof is the primary food source for infants. There is potential for the type of feeding to be associated with both dyslipidemia in adolescence and weight change, thus creating a confounding factor between the outcome and exposure of interest in this proposal. Many studies, exceeding 30 as of 2012 [96–98], present weak evidence supporting a persistence of association between infant feeding and dyslipidemia once a child achieves adolescence. Meta-analyses present evidence supporting a small reduction in total and LDL cholesterol in

adulthood, but no biological basis for a programming effect for adult lipid profiles exists [99].

A recent study examining 'lipidomic profiles' indicates there are distinct profiles according to breastfeeding status at 3 months of age, which disappear by 12 months of age. These results, relying on metabolic phenotyping previously unavailable in research alongside a cohort starting after 2000, further supports the prior findings of no persistence of breastfeeding association with lipid profiles later in age [100]. Interestingly, there were specific lipids detected at three months found to predict weight gain at 12 months. Despite the association between earlier lipid profile and growth, a connection between breastfeeding as an environmental exposure in childhood and dyslipidemia in adolescence is weak if not existent, eliminating it as a potential confounder between infant growth and later dyslipidemia.

2.1.2.8 Racial and ethnic disparities Racial and ethnic disparities in dyslipidemia exist and research in this topic is growing to counter the existing gap in knowledge. Relative to the non-Hispanic White (NHW) population, the HL population on average has a higher prevalence of dyslipidemia. Addressing these disparities is important because higher cardiovascular disease burden accompanies a higher prevalence of risk factors including dyslipidemia.

Among the first to examine subtypes of dyslipidemia patterns across major racial/ethnic groups in the United States [101], the authors found that the adult HL population is characterized by a pattern of raised triglycerides (TG) and lower HDL, increasing the risk of atherosclerosis. The raised TG levels in HL groups has been observed elsewhere, including a systematic review of literature representing North American countries, including Mexico, from 2000 to 2014 [102]. This particular literature review determined no difference in low HDL levels between the HL and NHW groups, but this finding is contradicted in other studies, including ones using NHANES data.

National Health and Nutrition Examination Survey (NHANES) is one of the most commonly used data sources to document health disparities on a national level [103]. In this

survey from 1999 to 2006, the levels of hypercholesteremia (total cholesterol >= 5.2 mmol/L = 201 mg/dL) for the NHW group were lower than the HL group, for either gender group [104]. When considering the HL subgroups with potential for considerable heterogeneity, the cholesterol levels were higher than the NHANES sample, but were lower even when considering six different sub groups within the Hispanic Community Health Study/Study of Latinos sample [105].

Subtypes of dyslipidemia patterns by race and ethnicity exist in an adult population [101], and it is not clear if the same patterns exist in children either due to cohort differences or selection bias with fewer people surviving from childhood to the adulthood. No study currently exists for children examining subtypes of dyslipidemia patterns, but there are estimates of lipid profiles by their individual measure stratified by race and ethnic groups [1,2].

This differential in which the HL group has higher proportion with low HDL-C mirrors findings for adults. In a sample representative of the United States, 15.7% of the Hispanic group aged 8 to 17 years had low HDL-C compared to 13.9% of NHW group [2] in 2011-2012. Also, in the SLCS sample, the primary sample in these analyses, the prevalence (95% CI) of low HDL-C is 69.9% (66.4%, 73.4%) [51]. In this Chilean sample, the prevalence of low HDL-C is over five times the U.S. representative NHW sample. The U.S. sample is more Mexican Americans and a different age group indicating heterogeneity in these estimates.

Mean triglyceride levels in samples of children also show an atherogenic pattern in which the HL group is more likely to have adverse levels than the NHW group. In the U.S. a geometric mean of triglycerides in NHW children aged 12-19 years is 79 (73-86) versus 83 (73-94) for Mexican American group. In the Chilean sample of children aged 17-19 the overall sample mean (SD) was 88.3 mg/dL (50.2). As discussed previously in the section addressing triglycerides, the average level in a sample of children from Chile could exceed 90 mg/dL [53].

Deviations from this pattern emerge across racial/ethnic groups when examining pro-

portions with hypertriglyceridemia. In the United States, a recent estimate taken from U.S. NHANES data estimates 12% of children have TG levels > 150 mg/dL from 2001 to 2008 [106]. A similar study, stratifying estimates by race/ethnicity had similar findings with 12.1% (95% CI: 9.5, 15.2) of NHW and 9.3% (95% CI: 7.7-11.2) with high triglycerides exceeding 150 mg/dL [107]. In comparison, 9.4% of a sample of 2,900 Chilean school children with an average age of 11 years from 2009-2011 tested positive for triglyceridemia [108]. In contrast, a study of a U.S. population with subgroups including NHW and HL (Mexicans) demonstrated the HL group more likely to ever have high TG [101]. For men and women participants in this study comparing NHW versus HL groups, the prevalence was 27.6% versus 45.4% and 42.5% versus 55.9%, respectively.

In sum, disparities between HL and NWH groups of children appear to follow those for adults when examining HDL-C but does not track as closely for TG. Differences in the distributions of triglycerides in racial ethnic groups across the lifecourse may account for this difference, emphasizing the importance of examining children separately from adults. Other explanations for divergence across age groups in racial/ethnic lipid differences may also lie in the changing heterogeneity of HL subtypes present in U.S. populations, which may not match the heterogeneity present in the samples with children.

2.1.2.9 Polygenic factors As previously noted, there are distinct monogenic lipid disorders, which point to mutations in one specific gene as the cause of extreme lipid levels starting in childhood. Genetic causes of dyslipidemia also include polygenic factors in which many genetic factors contribute to a susceptibility to dyslipidemia when combined with environmental factors such as body weight, activity and poor diet [109]. Polygenic dyslipidemias are the most common type, occupying the remainder of dyslipidemias after accounting for monogenic dyslipidemias, the most prevalent is familial hypercholesterolemia with 1 in 500 people being affected. Interestingly, distinguishing between the two based on phenotypes alone is not straightforward [110,111].

Current thought includes the concept that small variants with a cumulative effect influence lipid levels in the population to create a polygenic effect acting in concert with modifiable environmental factors to trigger dyslipidemia [109]. In the past 10 years an ascendence of Genome Wide Association Studies (GWAS)[112] has revealed many genetic variants associated with lipid levels. As of 2013 the largest GWAS identifies 157 loci associated with lipid levels [29]. Given this large sample size, this particular study was able to identify 62 new loci with smaller effects than the 95 previously identified loci [113] (Appendix A. Table A.1). The individual loci were often associated with more than one lipid trait. For example, there are 16 loci that associated with both HDL-C and TG levels. Taking this information together, the field is rich for further exploration of genetic variants associated with lipid levels.

A diverse set of genetic factors also contributes to dyslipidemia in children in addition to primary, secondary and environmental factors such as obesity. Research has mostly focused on adults in determining associations between genetic variants and lipids, but with recent analyses of child cohorts information on children is growing [30–33,114–117]. Variants characterized as being associated with lipids in adults have also been implicated in children. In particular, work has been done with Genetic Risk Score (GRS)'s showing the same scores associate with lipids for children and adults [32,33]. Of note, this work replicating associations in children pertains to people of European descent. Analyses with smaller samples have been conducted for Turkish [114] and Chinese samples [30], but further work is needed in other racial and ethnic groups.

2.1.3 Evidence for an association between postnatal growth and distal cardiovascular disease risk factors

2.1.3.1 Animal models In the past ten years, evidence has grown linking infant growth trajectories with cardiovascular risk factors, including lipid levels, later in life. Animal studies provide one avenue to explore risk factors such as lipid levels in later life following postnatal interventions not ethically possible with human beings, including starvation and

organ harvesting.

There are many animal models that demonstrate a link between postnatal growth and lipid outcomes. Slower growth during the postnatal period leads to altered plasma lipid concentrations. One experimental animal study demonstrated that food restriction during the postnatal period resulted in lower TC, TG and HDL-C in the offspring levels at the end of the lactation period [118]. In another study, a food restricted prenatal diet accompanied by a normal maternal diet in rats during lactation resulted in higher plasma TG levels [119]. In contrast, another animal study with rats, displayed evidence that offspring of rats fed a normal diet during gestation but food restricted during lactation had higher HDL-C, LDL-C, TC but lower TG [120] than normal fed control groups. Conversely, a different study in rats with postnatal food restriction following a normal prenatal diet presented evidence of a decline in HDL-C levels and TG levels one year later [34] relative to a normal diet. This model of restricted prenatal growth followed by normal or catch-up postnatal growth has dominated the literature, and substantial animal models have been published on this topic.

Although receiving less attention but arguably more relevant in developed countries with Western diets, postnatal overfeeding Postnatal Overfeeding (POF) and the accompanying increased growth (Fig. 2.2 [121]) occupies a deserved space in the developmental origins of disease concept. Importantly, postnatal growth can also play a role independent of gestational status [122]. This particular set of conditions can lead to impaired lipid metabolism. In particular, POF has been generally noted in rodent models to lead to higher TG, TC and HDL-C levels [122]. Study-specific results indicate POF leads to higher triglyceride levels [123,124] and higher HDL levels [124]. Mice that were overfed, and with faster growth, after either a deprived prenatal environment or control/standard regime had 15% higher levels of plasma TC at age 7 months, considered adulthood [122]. Similar to this experimental setup, another animal study offers evidence of a positive association between a normal prenatal/high fat postnatal diet, known to cause faster growth than normal [97], and higher liver weight and

TG concentrations at one year of age in Wistar rats [34]. An earlier study examining both rats and mice [125] provides evidence for higher cholesterol levels after postnatal overfeeding across species. However, these associations are not consistently observed in all animal models of POF [122]. Evidence is mixed in these models, but all point towards lipid levels as a function different growth via altered postnatal food intake in offspring.

Figure 2.2: Diagram of postnatal overfeeding.

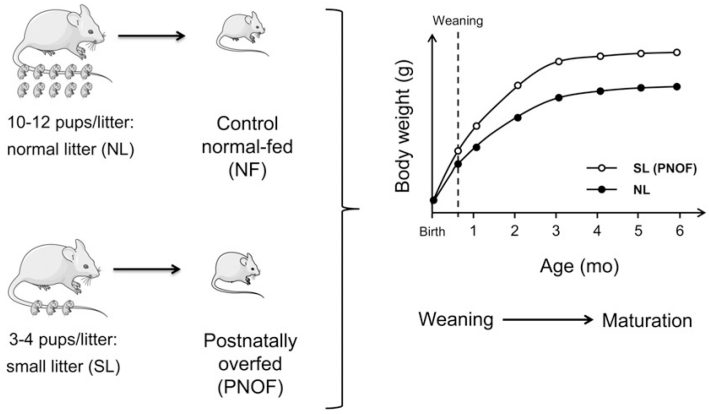


FIGURE 1 Schematic diagram representing the model of PNOF by litter-size reduction in rodents (rats and mice) and its consequences on body weight. Control litters of 10–12 pups/dam were considered NF, whereas SL of 3–4 pups induced PNOF. At weaning, depending on strains, PNOF Wistar and Sprague Dawley rats weighed 10–56% more than their NF counterparts, whereas the PNOF mice were nearly 30% heavier than their control NF littermates. In the postweaning period, PNOF rodents maintained their body weight gain throughout life. Depending on the experimental study, the increase in body weight may vary, the vast majority reporting a 10–25% increase in body weight during adulthood in rats, whereas PNOF mice were 20–30% heavier than control litters at maturity. NF, normally fed; PNOF, postnatal(ly) overfeeding; SL, small litter. Figures were produced using Servier Medical Art (www.servier.com).

2.1.3.2 Human Studies Observational human studies also demonstrate associations between postnatal growth and cholesterol levels. Findings from a prospective cohort study (ALSPAC, Avon Longitudinal Study of Parents and Children) in Britain starting in 1991 indicate positive associations between ponderal index (kg/m^3) change during infancy and LDL-C and TG levels at 15 years of age [14] with a negative association for HDL-C. Another prospective birth cohort, the Amsterdam Born Children and their Development (ABCD) Study [16] found a negative association for HDL-C and a positive association for TG at 5-6 years when considering weight or weight-for-length change between one and three months as an exposure.

Unlike the two prior birth cohort studies, the Stockholm Weight and Pregnancy Development Study, a contemporary prospective birth cohort, reported a positive association between infancy weight gain from zero to six months with HDL-C and TG levels of participants at

age 17 years [13]. In the Helsinki Birth Cohort, evidence suggests that a larger than expected increase in BMI in the first six months of life was associated with higher HDL-C and lower non-High Density Lipoprotein Cholesterol (non-HDL-C) and lower TC for those aged 57-70 years in 1999 [15]. Lastly, the Northern Finland Birth Cohort 1966 Study provides evidence towards an inverse association between peak weight velocity (kg/year) in the first six months of life and HDL-C and TGlevels for participants with average age of 31 years [17].

Contrary to the prior five observational studies, the Growth and Obesity Cohort Study (GOCS) reported no evidence of a statistically significant association between weight change from birth to six months and plasma lipids at 4 years of age [12] in a Chilean cohort study starting in 2006 when the participants were ages 2 to 4 years of age. Although these studies in humans do not reveals a consistent direction of association between postnatal growth and lipid profiles as a person ages, the majority of these seven observational studies in humans support the hypothesis that postnatal growth influences lipid metabolism, with different directions of association, analytic approaches and age ranges (Table 2.2).

Table 2.2: Postnatal growth studies and association with later plasma lipid levels.

#	First Author	Year	Country	Sample size	Age at growth measurements	Growth measurement	Outcome measures	Mean age at outcome measures (years (SD))
1	Ekelund	2007	Sweden	128	6 months, 1 year, 2 year	change in weight Z-score between 0 to 6 months	HDL (+), TG (+)	16.8 (0.4) male; 16.7 (0.4) female
2	Kajantie	2008	Finland	1999	0, 3, 6, 9, 12, 15, 18, 21, 24 months	BMI SD scores at 6 months adj. for birthweight	TC (-), HDL (+), non-HDL (-), TG (-) at 6 months	61.5 (3.0) women; 61.5 (sd=2.8)
3	Leunissen	2009	Netherland	200	0, 3, 6, 9, 12 months	SD score of weight gain (Z-score?) per 3 month period in first year of life.	HDL-C (-), LDL-C, TG(+), TC (at 0-3 months only)	20.8 (1.67)
4	Corvalan	2009	Chile	323	0, 6, 12, 18, 24 and 36 months	WHO Z-scores difference 0 to 6 months	HDL-C (-), LDL-C (+), TG (+), TC [exclusive bf]	4
5	Tzoulaki	2010	Finland	3778	not specified: during infancy	peak weight velocity (kg/year)	HDL-C (-), TG (-)	31
6	Howe	2010	Great Britain	4601	0, 2, 4 months	SD score of ponderal index (PI), kg/m3. change from 0 to 2 months (boys) and 0 to 4 months (girls)	LDL (+), HDL (-), TG (+)	15
7	Oostvogels	2014	Netherland	1459		change in internal SD score for weight-for-length from 1 to 3 months	HDL-C (-), TG (+)	5-6

2.1.3.3 Factors affecting postnatal growth

Factors affecting postnatal growth may function as confounders if they serve as a common cause of dyslipidemia in later ages. However, any such factors remain unidentified at this point (Fig. 2.1). This lack of relevant confounders rules out use of confounders related to prenatal status in subsequent models aimed at investigating the association between prenatal growth and lipid outcomes.

2.1.3.3.1 Environmental factors

Size at birth is a common measure in studies and associated with growth during the postnatal period and adverse cardiovascular outcome, but if included as part of a postnatal growth trajectory then this analytic framework would not be subject to confounding bias. Other factors determine postnatal growth, including gestational age at delivery, gestational weight gain, parental anthropometrics, socioeconomic status and breastfeeding [[126]; pizzi_prenatal_2014; fuemmeler_association_2016]. With the exception of socioeconomic status, commonly associated with adverse health outcomes across the lifespan, these factors are not implicated in lipid levels later in the lifespan and as such do not represent potential confounders when assessing the relationship between postnatal growth and dyslipidemia in adolescence.

2.1.3.3.2 Genetics

Postnatal growth varies across populations, and these differences have roots in both genetic and environmental causes [127]. From GWAS a total of 15 variants were found that relate to childhood body mass index (BMI) between ages 2 and 10 years explaining 2% of the variance [128], and it is safe to assume more variants will be discovered in the future. These 15 variants (rs13130484, rs11676272, rs4854349, rs543874, rs713208, rs1421085, rs12429545, rs987237, rs12041852, rs6567160, rs13253111, rs8092503, rs3829849, rs13387838, rs7550711), are near or in the following genes that are related to adult BMI: ADCY3, GNPDA2, TMEM18, SEC16B, FAIM2, FTO, TFAP2B, TNNI3K, MC4R, GPR61, LMX1B and OLFM4. Higher

childhood BMI is linked to faster postnatal weight gain so it could be considered as a crude proxy for postnatal weight gain and as such an indicator for genetic determinants. However, variants associated with infant growth thus far do not exist given the difficulty in collecting this phenotype on a scale large enough to justify GWAS. These variants, and their potential relationships with these listed genes, have not shown any functionality related to lipids discounting any case for confounding. However, childhood BMI variants are noted as being associated with TG variants and we cannot rule out any residual confounding [129].

Functioning as an upstream link to postnatal growth, a birthweight phenotype can provide clues to the genetics of postnatal growth. A large GWAS exceeding 150,000 participants has revealed 60 genetic variants that are associated with birthweight [130]. In this comprehensive study there is evidence that lipids as a cardiometabolic measure later in life correspond directly to genetic variants associated with birthweight at TRIB1 and MAFB loci. When assessing the relationship between postnatal growth and dyslipidemia these pleiotropic variants may serve as confounders 2.1. In sum, with this and many other cardiometabolic associations under investigation including blood pressure and coronary artery disease, the authors suggest a complementary hypothesis in the life course associations with chronic disease implicating genetic determinants of later metabolic disease.

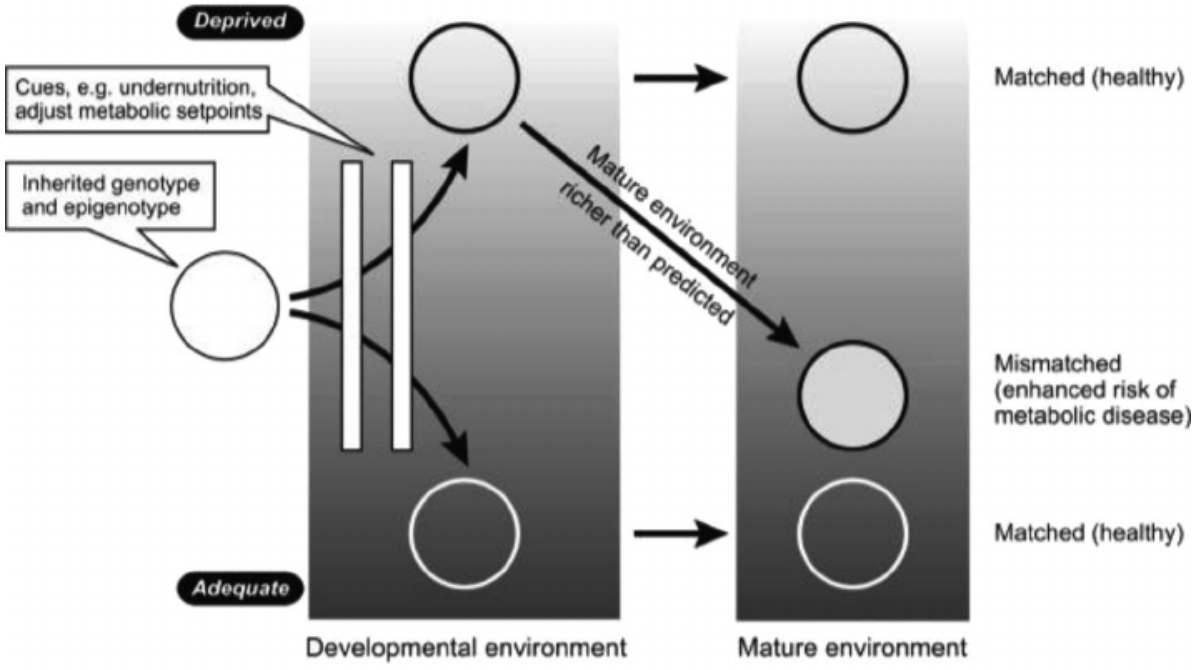
[add paragraph on population stratification and confounding between variants linked to postnatal growth and adolescent dyslipidema. [131,132] ### Biological pathways

Explaining biological pathways that underpin the association between postnatal growth environment and later life cardiovascular disease phenotypes is a crucial step in determining the validity of any causal effect. Despite active research in molecular mechanisms underlying postnatal programming of chronic adult disease, explanations do not yet clearly define these pathways [133,134]. However, there have been studies in the past 10 years that leverage new molecular techniques and enable a better understanding of these processes.

Thus far, the overarching theme into which the hypothesized biological pathways fit

is the 'predictive adaptive response' Predictive Adaptive Response (PAR) described by Gluckman and colleagues [135]. In this theory, metabolic disturbance in adulthood is the result of varying epigenetic responses to environmental exposures early in life. The ability of a genotype to produce different phenotypes given particular environments is termed plasticity [136]. These responses to particular environments can result in permanent change in genetic expression to adapt the body for anticipated environments later in life. When these anticipated environments do not occur there is a mismatch and metabolic disturbances occur (Fig. 2.3), including dyslipidemia. One of the most prominent explanations for altered lipid levels during childhood is the fetal/postnatal dietary mismatch [40,137].

Figure 2.3: Diagram of Match-mismatch paradigm of metabolic disease from Hochberg et al. 2011.



2.1.3.3.3 Liver function

During postnatal development, liver development can respond to environmental exposures with varying phenotypic responses [133]. Altered postnatal weight change and accompanying impaired liver growth during postnatal development, a crucial developmental windows

[41], is hypothesized as contributing to adverse lipid metabolism. Impaired postnatal liver growth affecting cholesterol metabolism was a concept was first introduced by Barker when discussing the dyslipidemic outcomes of children and abdominal circumference at birth [138] and continues in the literature [15,39]. However, experimental evidence linking liver growth per se to impaired metabolism is sparse. Emphasis in developmental programming of lipid metabolism has evolved from this explanation of liver size change altering metabolic programming to one of epigenetics as a causal factor [139].

Research on postnatal programming of nonalcoholic fatty liver disease (NAFLD), a liver disorder, offers clues on changing liver function in the face of infant growth change, within the context of nutritional manipulation. Non-optimal gestational liver growth was associated with biomarkers for liver damage [140], Alanine aminotransferase (ALT) and Gamma glutamyltransferase (GGT). In a similar, corroborative, and more recent study in a Chinese population, a positive association existed between famine exposure during either gestation and/or childhood with ALT and NAFLD [141]. To explain this association between undernutrition exposure and liver impairment the authors offer a hypothesis that undernutrition affects 'hepatic processing power on lipid metabolism' [141]. Specifically, the authors mention an experimental study pointing towards increased hepatic sirtuin 1 activity reprogramming hepatic nutrient sensors misinterpreting postnatal availability and inducing hepatic lipid storage [142]. In these cases nutrient deficiency not only slows overall growth but changes the composition of the liver, an organ essential for lipid synthesis.

Although undernutrition functions as the exposure, a similar biological mechanism may occur with nutrient excess and above average growth. A study in Europeans demonstrated that accelerated infant weight-for-length (WFL) gain from months 0 to 3 led to a higher risk of NAFLD during early adulthood, independent of birth weight, but no increase in ALT at that age [143]. Another study in Europeans examined WFL change from birth to age one year and found no relationship with liver outcomes [144]. However, this particular study did find a positive association between BMI change after year one and blood-based liver outcomes

around 18 years, including ALT and GGT. Postnatal growth does affect liver composition and alters liver composition upon exposure to infant growth. This biological mechanism to explain non-optimal growth as an exposure leading to dyslipidemia is one that exists in the literature, but is not as well formed as the argument made for differences in genetic expression.

2.1.3.3.4 Gene expression timing

Genetic expression in rat liver tissue [145] is enriched during the neonatal period and suppressed during the prenatal period. During the postnatal period a thorough study of gene expression levels during prenatal and postnatal development in the rat shows stronger expression of genes linked with lipid metabolism occurring exclusively during early postnatal life [145] (Fig. 2.4). The timing of maximum gene expression during neonatal liver development is also confirmed for long noncoding Ribonucleic Acid (RNA) [44]. Peng et al [44] note that this increased activity can relate to the 'functional transition during postnatal liver maturation'.

Figure 2.4: Function analysis of expressed or inhibited lipid metabolic genes in mice by prenatal and postnatal time periods (from Li et al. 2009)

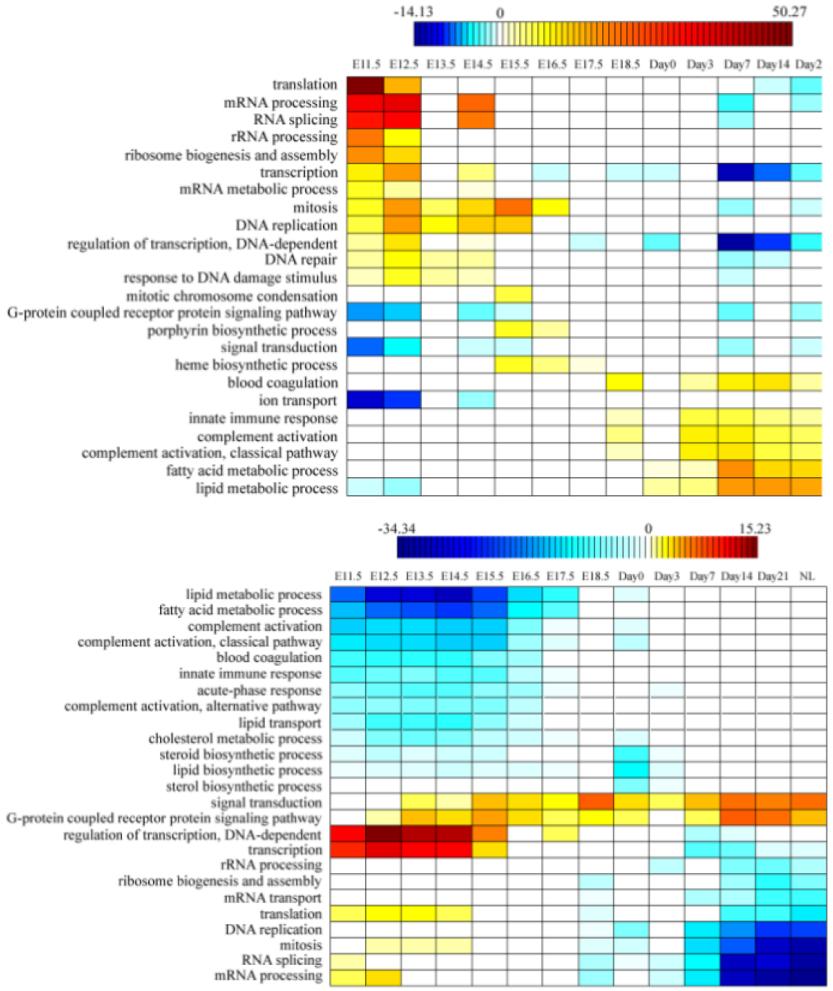


Fig. 2. Function analysis of elevated and inhibited genes at each time point
(A) The functional profile for up-regulated genes. Enriched (red) and depleted (blue) functions of activated genes at each time point are listed in the table, with shade representing the significance level (scaled by the negative log of corrected *p*-value). (B) The functional profile for down-regulated genes. Enriched (blue) and depleted (red) functions of inhibited genes at each time point are listed in the table, with shade representing the significance level (scaled by the negative log of corrected *p*-value).

Postnatal growth is also associated with programming of the liver tissue via gene expression, which varies across tissues [146]. Furthermore, this change in expression during early life can permanently affect lipid metabolism, fitting within programming hypotheses

of cardiovascular disease. Higher gene expression levels can also serve as a proxy for gene-environment interactions [147] lending credence to the concept of the postnatal time as a window for gene-environment interaction leading to different permanent lipid metabolism outcomes.

2.1.3.3.5 Epigenetic mechanisms

Epigenetic processes have been implicated in the relationship between abnormal postnatal growth and lipid outcomes at later ages in animal models. Methylation and histone modification count among the processes through which these epigenetic processes can occur (Fig. 2.5) [148–150]. Research uncovering evidence of methylation and histone modification as it relates to lipid metabolism is ongoing and revealing promising results. One human study of postnatal epigenetic metabolic programming measured via DNA methylation of two genes associated with growth and metabolism was associated with total cholesterol and HDL-C in early life (17 months) [42].

Methylation and subsequent transcriptions is often tissue dependent [151], and animal studies provide more accessible findings as related to liver tissue methylation as well as longitudinal changes in these events. Three different studies in mouse and sheep models randomizing maternal and postnatal diets find similar directions of association and arrive at the same biological mechanism as being linked with the exposure and responsible for the outcome.

Figure 2.5: Epigenetic programming.

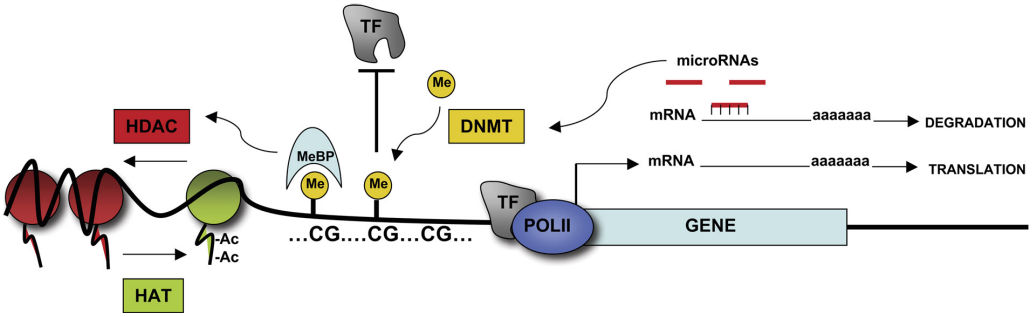


Fig. 2. General example of epigenetic regulation of gene transcription. Epigenetic regulation of gene expression is characterized by stable changes to DNA and chromatin structure that alter gene expression independent of gene sequence. The primary forms of epigenetic control involve DNA methylation by DNA methyl-transferase (DNMTs), and histone tail modifications, such as acetylation/deacetylation, by histone acetyl-transferase (HAT) and histone deacetylase (HDAC) activities, respectively. Additionally, microRNAs have recently been shown to regulate DNA methylation as well. Histone tail acetylation promotes an open-chromatin conformation, and is associated with regions of active gene expression, while histone tail deacetylation promotes a closed-chromatin conformation and is associated with gene silencing. DNA methylation of cytosine guanine (CpG) dinucleotides in the 5′ promoter region of genes generally induces transcriptional silencing, both by blocking transcription factor binding and by promoting the recruitment of transcriptional corepressors or histone-modifying complexes. MeBP, methyl-CpG binding protein; TF, transcription factor; Pol II, DNA polymerase II.

Animal models have demonstrated possible biological mechanisms triggering metabolic dysfunction and subsequent dyslipidemia. In particular, postnatal growth restriction in rats results in '... permanent elevation in circulating cholesterol and impaired glucose homeostasis' [133]. An intervention allowing a normal postnatal diet resulted in enhancing measured levels of hepatic liver X receptor (LXR), which prevented hypercholesteremia by three weeks of age [134]. This receptor, LXRα, regulates the enzyme cholesterol 7-hydroxylase (Cyp7a1) helping convert cholesterol to bile acids, and this receptor is part of cholesterol homeostasis in liver tissue. An inverse association between expression of this receptor and cholesterol levels exists in rodent models. This work is notable in that it is the first experimental work demonstrating maternal nutrition and infant growth as an exposure causing long-term cholesterol dysregulation via epigenetic processes. The results from this study sets the stage for further research to understand transcription mechanisms in cholesterol and triglyceride homeostasis [133].

Findings from a sheep study include a high fat postnatal diet in lambs following normal gestation with the resulting faster growth leads to higher TG and TC levels [152] in lambs at an age equivalent to puberty (6 months). This particular study did not test for the biological mechanism affecting the postnatal overfeeding, but mentioned that epigenetic effects may

be at play given a) the role of LDLR gene expression influenced by maternal undernutrition with a postnatal diet rich in cholesterol [153] and b) higher cholesterol levels after protein restriction during lactation in rats due to post-translational histone modification at the promoter of a gene related to the catabolism of cholesterol into bile acids [134]. Another hypothesis regarding high fat diets also implies that a high fat diet would 'initiate epigenetic changes by regulating genes that encode histone modifying enzymes.' [146]. These hypotheses at this point are conjecture and the jump from one exposure, high fat diet in this case, and the outcome, lipid metabolism, are conjecture with no evidence to support what could be a very complex biological pathway. The change in organ structure following the altered diet could also be a causal factor, to be explored in further research at the molecular level.

An earlier study in rats with a dietary intervention in the mother also proposes gene expression as the mode through which lipid programming occurs [118]. In this particular rodent study the cholesterol levels were lower after protein restriction in the mother. Further research similar to these studies will shed more light on this scenario and downstream lipid metabolic effects.

These animal models do give credibility to the notion that different levels of growth will produce different levels of transcription in liver tissue, which can permanently affect cholesterol homeostasis. Activity on a molecular level following exposure to different postnatal growth regimes ... Given this understanding that there are demonstrated biological outcomes that coincide with certain growth patterns, including overfeeding after normal birth weight, helps provide a foundation upon which to understand the role postnatal growth may play in contributing to differences in cholesterol patterns.

2.1.4 Analytic Issues

2.1.4.1 Generalizability and source populations To date very little evidence exists documenting infant growth and its association with dyslipidemia in a Hispanic sample. The two largest and most comprehensive analyses include White Europeans: ALSPAC in the

United Kingdom [14] and the Helsinki Birth Cohort in Finland [15,39]. Different ancestral groups, outside of those with European ancestry, may have a different associations between exposure and outcome. One recent study notes HDL levels in children aged 8-11 years vary across ethnic groups [154]. However, information on prevalence of risk factors is sparse in Hispanics [155] let alone the modification of risk within ethnic groups as proposed in the primary aims for this project.

In terms of identifying genetic variants that underpin chronic diseases such as CVD, no one population can adequately identify all variants associated with disease risk. The composition of genetic variation across ancestral groups requires investigation with many sources of descent to quantify hypotheses relating to disease risk. Needless to say, adding information regarding the risk factor of HDL-C in a diverse sample such as SLCS, of American Indian (44-51%), European (45-51%) and African (4-6%) descent, will be integral to the mapping of determinants of CVD for the entire human population (54,55).

2.1.4.2 Current analytic approaches for growth The proposed analytic approach will employ methods determined to be among the best at estimating growth [156]. These sort of analyses may not be possible in studies with few measurements, common in prior work investigating infant growth associations with cardiovascular disease outcomes. However, the large number of observations in the first year of life for the SLCS sample allows more flexibility in modeling. Better characterizing growth will allow the ability to detect patterns in growth that may have been lost with fewer observations. In our case we propose using the SuperImposition by Translation and Rotation (SITAR) method [20] to find characteristics of individual growth including, size, tempo and velocity differences relative to the group average (Fig. 3.2).

Augmenting the SITAR summaries which directly measure aspects of the growth curve, we will also include exploratory analyses that can detect patterns in growth, latent growth mixture modeling (LGMM). These patterns will then be used to predict lipid levels in

adolescence, considered a distal outcome in the context of the LGMM models (Fig. 3.3). Latent class models produce patterns, or classes, of growth that are data driven, potentially arbitrary, and entirely dependent on the sample itself. Validation of these patterns in another sample is advantageous to confirm scientifically meaningful groups [157]. Some specific criticisms of this method include subjective decisions by the investigator to choose the number and definition of latent groups, large variation within the selected groups representing the patterns, and 'un-satisfying generalizability' [158] with different groups occurring with different samples.

2.1.4.3 Detecting gene-environment interactions Epigenetic mechanisms and liver change during the postnatal period appear to hold promise as causal factors mediating the path between postnatal growth and lipid metabolism later in life. Animal models provide a basis to detect epigenetic changes in early life, but this work is less feasible in human studies, including tissue-specific methylation measures. This limitation in human studies warrants a focus on gene-environment interactions. These interactions possess a strong association with epigenetic mediating factors [[147]; ong_measuring_2015; liu_gene-environment_2008-1], and can serve as a proxy for some phenomenon that are very difficult to directly measure. Interestingly, the argument has been made in the reverse sense with suggestions to use methylation measures to detect potential gene-environment interactions [147].

Postnatal epigenetic activity functions in the DOHaD framework to influence dyslipidemia later in life. Also, given the strong association between gene-environment interaction and epigenetic activity, there is potential for a mediating role of epigenetic activity between gene-environment interactions and dyslipidemia outcomes in adolescence. Different postnatal growth patterns, serving as an environmental exposure, may modify the association between lipid profiles and genotypes.

In light of this rationale motivating a gene-environment assessment, we will conceptualize the gene-environment interaction (GXE) as an environmental effect, growth trajectories,

modifying the relationship between genetic variants and dyslipidemia in adolescence. Choosing which variable serves as the effect modifier of the other one does not make a difference in how the effects are modeled, but does lead to an assumption of larger effect sizes compared to a genetic effect modifier of relationships between the environment and dyslipidemia [131]. Genetic variants as selected in GWAS typically have small main effect sizes.

The sample size for the proposed research is approximately 500 people. With the relatively small sample size, a careful choice of candidate genes to test a gene-environment interaction in this scenario is necessary. candidate gene-environment interaction (cGXE) interactions are not without their flaws [131] and choosing candidate genetic variants requires careful consideration. Pitfalls in this scenario include low power to detect effects, choice of variants not having undergone rigorous testing in GWAS first (i.e. low prior probabilities), and correction for multiple testing. The genetic variants in this project will be selected to have both a strong rationale for function as well as sufficient power to detect marginal differences at the GWAS level.

2.1.4.3.1 Model choice

Modeling a gene-environment interaction usually involves a cross-product term between an environmental and genetic factor. Simply including a cross-product term between the environmental variable and the genotype coded as three categories (0, 1, or 2 alleles) may not suffice when detecting an GXE and further sensitivity tests with additional model terms may be warranted to determine if better fitting models exist [131]. For example, different alleles may have differently shaped associations between the environment and phenotype and relaxing the assumption of similar distributions across alleles may be necessary to capture the observed data [159]. Some experts [159] suggest that a single cross product term should never be used when testing GXE, and what they call a 'six parameter model' should be used that accounts for a G^2 term, G representing the coded genotype of 0, 1 and 2 risk alleles. This type of model will relax the assumption that the difference between the lines representing

the environment and exposure association remain the same across genotypes. In turn, this model specification avoids bias, types I and II errors.

2.1.4.4 Genetic Risk Score

2.1.4.4.0.1 Advantages

[TO ADD]

2.1.4.4.0.2 Disadvantages

[TO ADD]

2.1.4.5 Validation in independent cohort Aim 1 provides a unique opportunity to replicate results from a Chilean birth cohort studying predictors of growth trajectories [160]. The Growth and Obesity Cohort Study (GOCS) (n=959) was one of three cohort studies used to examine the association between infant growth (including months 0, 1, 2, 4, 6 and 12) and maternal characteristics such as height, age, parity, education, pre-pregnancy BMI, smoking, gestational diabetes and hypertension. Initiation of GOCS started in 2006, approximately ten years after SLCS. Methods used in this particular research include characteristics described in the first aim: size, tempo and velocity measures. Replication of results using these two independent samples offers a chance to externally validate the prediction model in the SLCS sample. Validation can determine to what degree it is representative of Santiago, Chile and if effects are sensitive to temporal changes as the GOCS cohort occurred 10 years after SLCS. External validation using a growth model with data from a contemporary cohort such as the SLCS has not been accomplished to date, and it is an important part of scientific research [161,162].

2.1.5 Summary

CHAPTER 3

Research Plan

3.1 Research Plan

3.1.1 Overview

3.1.1.1 Outcome Assessment

3.1.1.1.1 Anthropometric Measures

Lipid measures have been assessed in the SLCS from stored blood samples using Enzymatic Colorimetric methods. When the participants were 17 years of age, they had their blood drawn (venipuncture) the morning following an overnight fasting period. Second, infant anthropometric measures are the main outcome in analyses for aim 1. During the study periods from 1991 to 1996, study nurses for the SLCS weighed the participating infant once at monthly clinic visits throughout the first year on electronic scales to the nearest 0.01 kg. Length was measured on a recumbent board to the nearest 0.1 cm. The primary measure for postnatal growth trajectories will be weight-for-length (weight (kg) divided by length (cm)) because this better represents adiposity than weight alone [16,163,164]. Also, weight and length as separate outcome measures will be included in separate models as these are frequently included in many studies of infant growth to date and will facilitate comparisons with prior studies.

3.1.1.2 Exposure Assessment Analysis of genetic information will be based on a small number of candidate genes drawn after careful consideration of replicated associations in

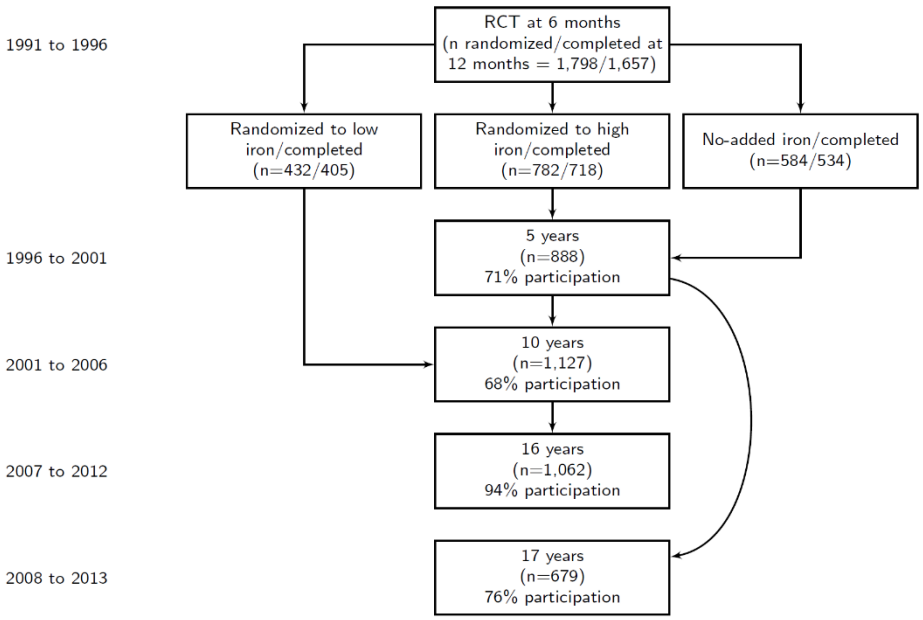
very large samples, including those from the Global Lipids Genetics Consortium with a sample size exceeding 180,000 individuals [28] (Appendices A and B). This subset of single nucleotide polymorphisms (SNPs) are directly related to lipid metabolism in adults, defined as a functional variant. Absent functional variant status, marker SNPs tested in a gene-environment relationship can yield spurious findings [**???**]. This targeted, a priori planned research study of underlying genetic variation in adverse lipid levels will conserve power in testing [165] and follows suggested protocol for gene-environment interaction analyses [131] – issues that figure prominently in analyses involving genetic variation.

Blood samples from participants currently participating in the 21-year follow-up time have been microarray genotyped for all the candidate genes associated with lipid levels using the Illumina MEGA array, an enhanced genotyping array optimized for multi-ethnic populations. With funds from UNC and AHA (15GRNT25880008; PI North), genotyping using DNA from SLCS blood samples is underway for the 679 adults. Genotyping is being conducted with the Multi-Ethnic Genotyping Array (MEGA), which was designed with a 1.25M genome-wide association (GWA) scaffold optimized for imputation accuracy in African-American, HL, Amerindian, Asian, and European populations. MEGA incorporates 400K low frequency and rare disruptive and non-synonymous variants, derived from a pan-ethnic panel of 40K whole-exome sequenced individuals. This includes >100K rare disruptive variants on American Indian backgrounds and a heavily curated catalog of over 40K variants from clinical and biomedical databases, which is by far the largest set of functional variants currently available on a commercial chip. By the time the proposed research begins, the genotyping will be complete. With this array we will analyze the genotypes for a subset of SNPs that are associated with genes having known functions influencing lipid metabolism and present in the general population at a frequency common enough to detect in our sample. Examples of such SNPs determined from Willer et al (37) include ABCA1 (SNP: rs1883025), APOA1 (SNP: rs964184), LIPG (SNP: rs7241918), LIPC (SNP: rs1532085), PLTP (SNP: rs6065906), LPL (SNP: rs12678919) and SCARB1 (SNP: rs838880) [166].

3.1.2 Data Source: Santiago Longitudinal Cohort Study (SLCS)

This study of the association between infant growth trajectories and adverse lipid levels at 17 years of age builds on an ongoing cohort that started with 1,657 infant participants from Chile [18] (Fig. 3.1). Participants were recruited during infancy and have been followed through childhood and adolescence into early adulthood (Lozoff and Gahagan, Multiple PI, R01 HD033487; Gahagan, PI, R01 HL088530). Santiago, Chile is the geographic region of origin for SLCS participants. The population of Chile exceeds 14 million residents and as a country has undergone a 'nutrition transition' in which malnutrition declined during the 1980s and levels of obesity rose [167]. Obesity levels in pregnant women rose from 12.9% to 32.7% from 1987 to 2000 [168]. Chile has had a universal public health network in place since the 1950s and the infant mortality rate is comparable to the U.S. (7 and 6 deaths per 1,000 live births in 2010-2014 [**???**], respectively). By 1998, 87 percent of infants were still breastfeed at six months of age [169].

Figure 3.1: Flow chart of sample size for Santiago Longitudinal Cohort Study.



Originally established as part of a randomized clinical trial to investigate the effects of iron supplementation in formula-fed infants [18], SLCS is still active. Sampling occurred

from 1991 to 1996 in low- to middle- income groups. Full-term infants with no iron-deficiency anemia or other major health problems were eligible to participate. There was a total of 1,657 infants who completed the study at 12 months of age by 1996. Following the infant study, 679 children (Fig. 3.1) from this cohort are participating in additional extensive cardiovascular testing at 17 and 21 years of age. This combination of factors allows for a detailed characterization of infant weight trajectories paired with high quality cardiovascular measures in adolescence.

The study team in Chile maintains frequent contact with the study participants and has an established protocol to track participants including annual postal contact.

Participants at five years of age were compared to the non-participants and no statistically significant differences existed for many socio-demographic characteristics including gestational age, birth weight, parental education, and maternal age. Non-participating children had significantly lower SES (0.20 SD, $p<0.01$). In sum, this extensive collection of information over a time spanning the childhood years in this sample is unique, and these data provide a foundation for testing hypotheses relating infant growth to adverse lipid levels at 17 years of age.

3.1.3 Statistical Analyses

The three specific aims of this research project are to 1) characterize predictors of postnatal growth curves, 2) determine the association between infant growth trajectories and adverse lipid levels, and lastly, 3) assess the influence of certain growth curve characteristics on the relationship between genetic variants and adverse lipid levels at 17 years of age.

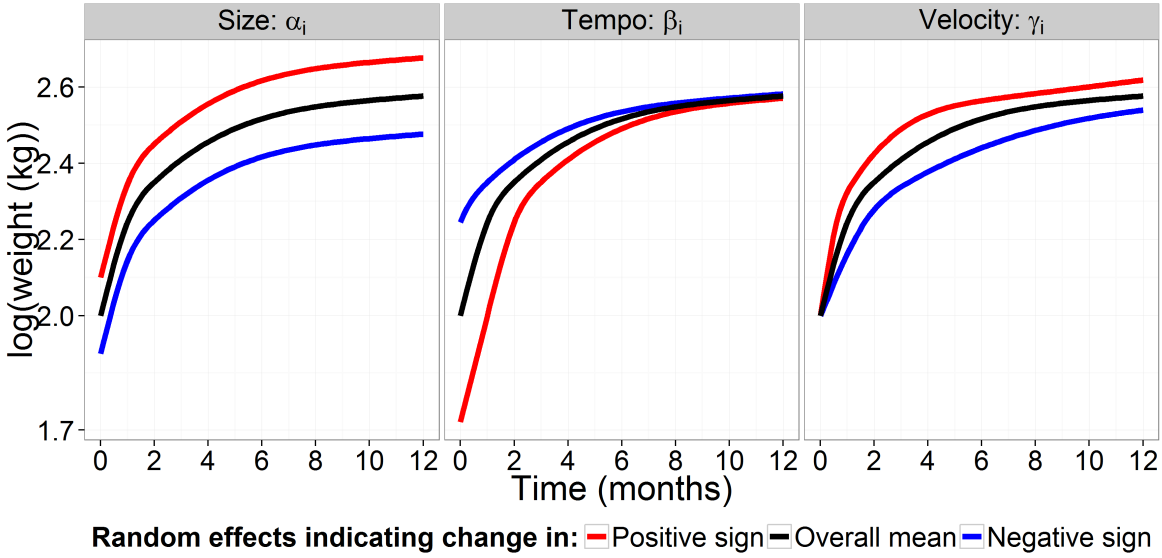
3.1.3.1 Aim 1.1 – Characterize growth trajectories with SITAR method To determine characteristics of size, tempo, and velocity of infant growth trajectories (Fig. 3.2) we will use the approach as outlined by Cole and colleagues [20,156]. This method uses a shape invariant model (SIM) for growth originally proposed by Beath [170] and extracts three

random effects from this model to characterize the growth curve for each individual. The model is:

$$y_{it} = \alpha_i + h\left(\frac{t - \beta_i}{exp(-\gamma_i)}\right) \tag{3.1}$$

where y_{it} is the observed outcome (weight-for-length) for the i^{th} person at the t^{th} time point. $h(t)$ is a natural cubic spline function of the outcome versus age, t, in this case. The three random effects in this model, $\alpha_i, \beta_i, \gamma_i$, are the random intercept for the outcome, the random intercept for age, and a random scaling factor for age, respectively. As random effects, these three variables by definition will have a mean of zero and represent deviations about a population mean (Fig. 3.2). For example, a positive value for α_i represents a shift of the growth curve upwards. A positive value for β_i represents a shift of the growth curve to the right and a growth curve starting later than the average. Lastly, a positive value for γ_i leads to shortening of the age scale and a steeper growth curve and change in outcome over a one-unit change in time.

Figure 3.2: Type of change in random effects (α, β, γ) relative to the sample mean trajectory in weight growth curve trajectories following a shape invariant model (SIM).



The other part of aim 1 is the identification of predictors of growth curves. Each of the three components of the growth curve will be regressed separately onto the selected predictors,

which include maternal pre-pregnancy BMI, height, age and education. For example, to obtain the relationship between maternal pre-pregnancy BMI, x_i, and infant velocity, γ_i , a simple linear regression model [160] would be $\gamma_i = \alpha + \beta_1 x_i + \epsilon_i$. We used simulations to calculate 90% power to detect a coefficient of 0.1 (sd=0.03) for a one unit change in maternal age assuming a mean (sd) maternal age of 27 (7) years – estimates derived from a similar study [160] .

3.1.3.2 Aim 1.2 – Characterize growth trajectories with latent class growth mixture method Another part of the first aim includes identification of latent growth trajectory patterns. At its simplest level, data in a latent class growth mixture model (LGMM) approach include a longitudinal series of measurements for an individual. An example of a series is monthly weight-for-length values in the first year of life. With these weight-for-length values, a latent class growth trajectory model [157,171,172] would estimate a discrete number of groups of infant growth trajectories in which the group members would have as similar growth characteristics as other members in their group but as different as possible from members in other groups.

This approach has been applied to child growth curves, but relating the latent classes from growth curves to lipid levels as a distal outcome (response value) remains unexplored [173].

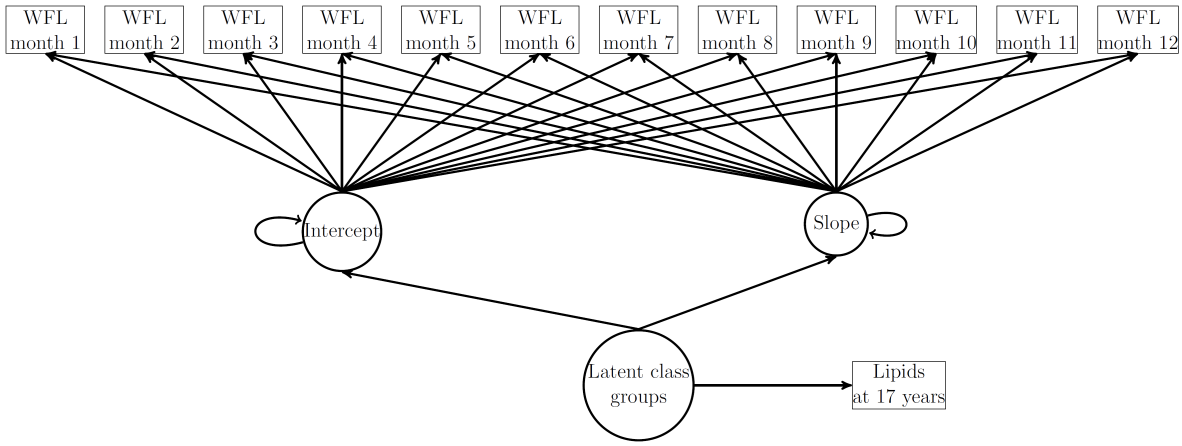
The LGMM for infant growth is adapted from an approach previously done in an asthma research study [174] and will be as follows:

$$y_i = \eta_{0i} + \eta_{1i} age_t^2 + \eta_{2i} age_t^2 + \eta_{3i} age_t^3 \tag{3.2}$$

where y_{it} is the observed weight-for-length for person at time = 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 months. Class membership is estimated from the characteristics of the growth model as specified above using an expectation-maximization (EM) algorithm [171]. The last

component in this analysis involves characterizing the association between latent groups of infant growth and lipid levels at ages 17 years as a distal outcome (Fig. 3.3).

Figure 3.3: Diagram of latent growth mixture model (LGMM) of infant growth. Circles represent latent variables, rectangles represent observed variables. Note: WFL is weight-for-length.



To estimate power with this approach we conducted simulations using Mplus software [175] [scripts available upon request]. In the simulations three different cases were considered such that the odds ratios of an adverse lipid outcome in one latent class versus the other would be 1.50, 1.65, and 1.82 with power in these three scenarios of 0.57, 0.77, and 0.90, respectively.

3.1.3.3 Aim 2 – Determine association between infant growth trajectories and adverse lipid levels.

In a second stage of regression models, we would use the random effects separately from the SITAR method mentioned above. In our case, this second stage would be one in which a lipid level, as a continuous variable, is regressed onto each of the three random effects from the growth curve in separate models and in one model together. Some examples of this approach [176,177] include using growth parameters as predictors of obesity later in life [176] and predicting aspects of growth using infant feeding practices as a predictor [177]. The simple linear regression model we would use for this aim would be

considered **model 1**:

$$Y_i = g_0 + g_1\alpha_i + g_2\beta_i + g_3\gamma_i + \epsilon_i \tag{3.3}$$

in which the i^{th} person has an observed lipid outcome of y_i. In this model, g_0 is the intercept, g_1, g_2, g_3 are coefficients for the growth parameters indicating a change in a numeric lipid outcome for a one-unit change in the respective growth parameter, and ϵ_i is the term for random error for the i^{th} person.

One problem that may present in this analysis is collinearity between the three growth parameters when combined in one model. We will assess that model for collinearity and if present will revise the model so collinearity is absent (i.e. retain one of the two collinear variables conditional on model fit).

To estimate power we used simulation studies with R software [178] [scripts available in online repository that is available upon request]. Power to detect a change in a lipid level, in mg/dL, was determined using a sample size of 500, an $\alpha = 0.05$, mean lipid level of 40, and sd=0.25 for the parameter estimates. Power exceeds 94%, 86% and 27% to detect a change in lipid levels for a one-unit change in the size, tempo, and velocity random effects, respectively.

3.1.3.4 Aim 3 – Determine change in association between genetic variants and adverse lipid levels across characteristics of infant growth

To assess the presence of a gene-environment interaction effect we will first evaluate the association between functional genetic variants and lipid levels at 17 years of age [179] in **model 2**:

$$Y = Z_0\beta_0 + x_1a_1 + g_2\beta + g_3\eta + \epsilon \tag{3.4}$$

For model 2, Y is the vector of observed lipid levels; Z_0 is the vector of the intercept and relevant covariates outside of genetic factors that could be confounders, such as ancestry, age and sex of participant; a1 is the coefficient for the additive effects of the i^{th} single nucleotide polymorphism (SNP) [180]; G_i noted below represents the number of reference alleles and

ranges from 0 to a total of 2 at the bi-allelic SNP. The additive effect is one in which each additional allele present in the genotype results in one additional unit in the covariate. The indicator variables for the SNPs will be coded as follows so that they conform to additive effects with $x_i = -1$ for $G_i = 0$; x_i=0 for $G_i = 1$; $x_i = 1$ for $G_i = 2$.

To evaluate the interaction between the growth terms and the selected genetic variants we would expand upon the main effects model (Equation 3.4), and include interaction terms between the growth parameters as determined and analyzed from aims 1 and 2 in **model 3**:

$$Y = Z_0\beta_0 + x_1a_1 + g_1\alpha + g_2\beta + g_3\gamma + x_1g_1i_{sa} + x_1g_2i_{ta} + x_1g_3i_{va} + \epsilon \tag{3.5}$$

In model 3, i_{sa}, i_{ta}, i_{va} all represent the coefficients for the interaction terms between size (s), tempo (t) and velocity (v) with the additive (a) effects.

Lastly, we will combine all identified functional variants into a GRS by summing the number of unweighted risk alleles [181,182] and use this as a covariate in a linear regression analysis with main effects,

$$Y = \mu + GRS \times c_1 + g_1\alpha + g_2\beta + g_3\gamma + \epsilon \tag{3.6}$$

and interaction terms

$$Y = \mu + GRS \times C_1 + g_1\alpha + g_2\beta + g_3\gamma + GRS \times g_1 \times i_{GRS\times g_1} + GRS \times g_2 \times i_{GRS\times g_2} + GRS \times g_3 \times i_{GRS\times g_3} + \epsilon \tag{3.7}$$

to test an interaction between the risk score and the growth exposures via F-tests.

Aim 3 posits an interaction exists between growth trajectory characteristics and SNPs linked to lipid levels. To estimate power, we considered interaction effects as mentioned for model 3 above to measure the association between nonlinear growth trajectory random effects and a continuous lipid outcome. One important piece of information that can affect power is

the minor allele frequencies (MAF) of the SNPs of interest. To compute power, we selected a MAF of 0.19 – the lowest frequency of the suggested candidate SNPs – to determine the most conservative estimates with genotype frequencies assumed to follow Hardy-Weinberg equilibrium (0.04/0.31/0.65). Other fixed parameters included in power simulations included a sample size of 500 people (allowing for around 25% dropout or missing data), an average outcome of 40 mg/dL (standard deviation=10), an additive genetic coefficient (a1) of 1, and interaction effects (i_{as}, i_{at}, i_{av}) of 0.25. In general, estimates for power given these assumptions as applied to simulations of model 3 indicate power exceeding 0.80 for all estimates with the exception of velocity effects.

3.1.3.4.1 Individual variants – main effects

The genetic variants determined to have a strong likelihood of association with lipids (Appendices A and B) cannot all be used for single variant association tests, as specified in equations 3.4 and 3.5. A prominent factor limiting the selection of variants is power to detect an effect with such a small sample size, assuming that the association also exists in this sample with ancestry not like the source sample, which is mostly European. We determined which variants would have an *a priori* power of 0.80 given a) the minor allele frequency from the source population (Ad Mixed American), b) a sample size of 500 (approximately equal to the genotyped sample), and c) the effect size of the single variant tests as determined from the source GWAS [113]. Based on these parameters we determined that the following list of variants were appropriate for single variant testing:

Variant	Trait	Variant	Locus	Effect Size (SE) mmol/l
1	HDL	rs3764261	CETP	0.158 (0.040)[a]
2	HDL	rs1532085	LIPC	0.053 (0.038)
3	LDL	rs6511720	LDLR	-0.013 (0.158)
4	TG	rs1260326	GCKR	0.360 (0.150)[a]

Variant	Trait	Variant	Locus	Effect Size (SE) mmol/l
5	TG	rs964184	APOA1–C3-A4-A5	0.326 (0.148)[a]
6	TC	rs6511720	LDLR	0.125 (0.165)

[Add results from selected SNPs in SLCS AHA abstract. 5 snps with description of nearby gene.] "C:/Program Files/RStudio/bin/pandoc/pandoc"

3.1.4 Manuscript Preparation and Timeline

CHAPTER 4

Discussion

4.1 Discussion

The proposed work examining the modification of the association between these functional variants and the dyslipidemia outcomes across different levels of postnatal growth is research presently absent from current literature. Tests of modification of the relationship between specific variants and a range of infant growth trajectories could provide evidence supporting the importance of gene-environment interaction in the pathogenesis of CVD, and in particular relating to dyslipidemia. In turn, any associations arising from these tests would support the hypothesis that early postnatal growth permanently affects lipid metabolism via a biological mechanism including epigenetic effects [6,120,184]. ## Strengths and Limitations

4.1.0.1 Strengths The proposed research project, which includes detailed postnatal growth trajectory information and its association with HDL-C levels, can offer information on a predictor of a disease risk factor in an infrequently studied ethnic group.

Having both anthropometric observations combined with genotyping in normal birth-weight groups is an asset, which can further confirm the relationship between accelerated postnatal growth and abnormal lipid metabolism – evidence currently lacking in the literature in both animal experiments and human observational studies.

4.1.0.2 Limitation [Note: should I mention how I will only be estimating the total effect. However, estimating direct and indirect effects would be of interest because estimating the

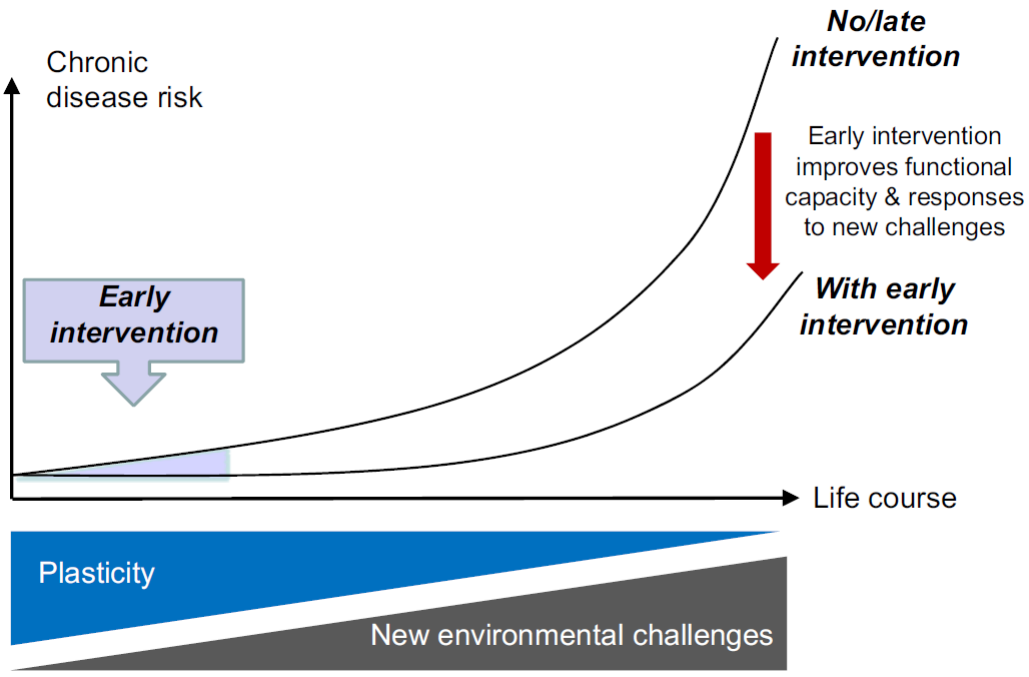
impact of growth on later weight and the subsequent consequences on TG could help explain the utility of gene expression vs adolescent environmental factors such as exercise and diet.]

[97]

[Note: add that there are arguments that early life exposures are over-stated and it is really later life exposures in the lifecourse that affect outcomes. Start by citing the Gustafsson 2010 paper. This topic can lead into the importance of early life intervention even if there are mediating factors in the lifecourse, which may be more difficult to intervene upon]. ## Contributions to Public Health

Investigation of associations between early growth and lipid outcomes can lead to modifiable interventions such as xx, xx and xx. In turn, these interventions have the potential for great public health significance because little shifts in development at an early age can lead to very large differences in health at later ages (Fig. 4.1 [136]) when efforts at modifying behavior are much harder to do.

Figure 4.1: Intervention across the life course and chronic disease.



[Add part about potential to reverse effects in animal models and potential for intervention in humans (reduce amount of feeding). See page 146 of Vo et al indicating neonatal development can stop long-term liver development damage. Also see Sohi 2015 for info on importance of postnatal diet to reverse prenatal programming effects on the liver.]

[Note: Also add piece on potential to stop generational vicious cycle of metabolic impairment (wording from the Current Opinion in Lipidology piece (Loche and Ozanne 2016)]: In an intriguing swine study, in which the exposure was food restriction or abundance, there were effects on liver growth between the control and overfed groups [185]. Findings from this study support the idea of postnatal weight change altering lipid profiles in early life. Specifically, there were also sex-specific effects on the lipid profile at sacrifice, postnatal day 28.]

[Note: points of intervention include infant feeding (formula associated with faster weight gain) as suggested in [143] and early fish oil supplementation in [186] (also mentions breastfeeding).

Note: xx paper indicates epigenetic intervention possible. In particular, suggestion to target 'lysine and arginine histone methyltrasferases' [146]. As concluding text would be good to add that evidence of intervention on these factors improving outcomes is more evidence towards a causal effect.

4.1.1 Summary

APPENDIX A

Table A.1: Results from meta-analysis of plasma lipid concentrations, Teslovich et al. 2010

Locus	Chr	Lead SNP	Lead trait	Other traits	Alleles/MAF	Effect size
Locus	Chr	Lead SNP	Lead trait	Other traits	Alleles/MAF	Effect size
LDLRAP1	1	rs12027135	TC	LDL	T/A/0.45	-1.22
PABPC4	1	rs4660293	HDL		A/G/0.23	-0.48
PCSK9	1	rs2479409	LDL	TC	A/G/0.30	2.01
ANGPTL3	1	rs2131925	TG	TC, LDL	T/G/0.32	-4.94
EVI5	1	rs7515577	TC		A/C/0.21	-1.18
SORT1	1	rs629301	LDL	TC	T/G/0.22	-5.65
ZNF648	1	rs1689800	HDL		A/G/0.35	-0.47
MOSC1	1	rs2642442	TC	LDL	T/C/0.32	-1.39
GALNT2	1	rs4846914	HDL	TG	A/G/0.40	-0.61
IRF2BP2	1	rs514230	TC	LDL	T/A/0.48	-1.36
APOB	2	rs1367117	LDL	TC	G/A/0.30	4.05
		rs1042034	TG	HDL	T/C/0.22	-5.99
GCKR	2	rs1260326	TG	TC	C/T/0.41	8.76
ABCG5/8	2	rs4299376	LDL	TC	T/G/0.30	2.75
RAB3GAP1	2	rs7570971	TC		C/A/0.34	1.25
COBLL1	2	rs10195252	TG		T/C/0.40	-2.01
		rs12328675	HDL		T/C/0.13	0.68
IRS1	2	rs2972146	HDL	TG	T/G/0.37	0.46
RAF1	3	rs2290159	TC		G/C/0.22	-1.42
MSL2L1	3	rs645040	TG		T/G/0.22	-2.22
KLHL8	4	rs442177	TG		T/G/0.41	-2.25

Locus	Chr	Lead SNP	Lead trait	Other traits	Alleles/MAF	Effect size
SLC39A8	4	rs13107325	HDL		C/T/0.07	-0.84
ARL15	5	rs6450176	HDL		G/A/0.26	-0.49
MAP3K1	5	rs9686661	TG		C/T/0.20	2.57
HMGCR	5	rs12916	TC	LDL	T/C/0.39	2.84
TIMD4	5	rs6882076	TC	LDL, TG	C/T/0.35	-1.98
MYLIP	6	rs3757354	LDL	TC	C/T/0.22	-1.43
HFE	6	rs1800562	LDL	TC	G/A/0.06	-2.22
HLA	6	rs3177928	TC	LDL	G/A/0.16	2.31
		rs2247056	TG		C/T/0.25	-2.99
C6orf106	6	rs2814944	HDL		G/A/0.16	-0.49
		rs2814982	TC		C/T/0.11	-1.86
FRK	6	rs9488822	TC	LDL	A/T/0.35	-1.18
CITED2	6	rs605066	HDL		T/C/0.42	-0.39
LPA	6	rs1564348	LDL	TC	T/C/0.17	-0.56
		rs1084651	HDL		G/A/0.16	1.95
DNAH11	7	rs12670798	TC	LDL	T/C/0.23	1.43
NPC1L1	7	rs2072183	TC	LDL	G/C/0.25	2.01
TYW1B	7	rs13238203	TG		C/T/0.04	-7.91
MLXIPL	7	rs17145738	TG	HDL	C/T/0.12	-9.32
KLF14	7	rs4731702	HDL		C/T/0.48	0.59
PPP1R3B	8	rs9987289	HDL	TC, LDL	G/A/0.09	-1.21
PINX1	8	rs11776767	TG		G/C/0.37	2.01
NAT2	8	rs1495741	TG	TC	A/G/0.22	2.85
LPL	8	rs12678919	TG	HDL	A/G/0.12	-13.64
CYP7A1	8	rs2081687	TC	LDL	C/T/0.35	1.23

Locus	Chr	Lead SNP	Lead trait	Other traits	Alleles/MAF	Effect size
TRPS1	8	rs2293889	HDL		G/T/0.41	-0.44
		rs2737229	TC		A/C/0.30	-1.11
TRIB1	8	rs2954029	TG	TC, LDL, HDL	A/T/0.47	-5.64
PLEC1	8	rs11136341	LDL	TC	A/G/0.40	11.4
TTC39B	9	rs581080	HDL	TC	C/G/0.18	-0.65
ABCA1	9	rs1883025	HDL	TC	C/T/0.25	-0.94
ABO	9	rs9411489	LDL	TC	C/T/0.20	2.24
JMJD1C	10	rs10761731	TG		A/T/0.43	-2.38
CYP26A1	10	rs2068888	TG		G/A/0.46	-2.28
GPAM	10	rs2255141	TC	LDL	G/A/0.30	1.14
AMPD3	11	rs2923084	HDL		A/G/0.17	-0.41
SPTY2D1	11	rs10128711	TC		C/T/0.28	-1.04
LRP4	11	rs3136441	HDL		T/C/0.15	0.78
FADS1-2-3	11	rs174546	TG	HDL, TC, LDL	C/T/0.34	3.82
APOA1	11	rs964184	TG	TC, HDL, LDL	C/G/0.13	16.95
UBASH3B	11	rs7941030	TC	HDL	T/C/0.38	0.97
ST3GAL4	11	rs11220462	LDL	TC	G/A/0.14	1.95
PDE3A	12	rs7134375	HDL		C/A/0.42	10.4
LRP1	12	rs11613352	TG	HDL	C/T/0.23	22.7
MVK	12	rs7134594	HDL		T/C/0.47	-0.44
BRAP	12	rs11065987	TC	LDL	A/G/0.42	-0.96
HNF1A	12	rs1169288	TC	LDL	A/C/0.33	1.42
SBNO1	12	rs4759375	HDL		C/T/0.06	0.86
ZNF664	12	rs4765127	HDL	TG	G/T/0.34	0.44
SCARB1	12	rs838880	HDL		T/C/0.31	0.61

Locus	Chr	Lead SNP	Lead trait	Other traits	Alleles/MAF	Effect size
NYNRIN	14	rs8017377	LDL		G/A/0.47	1.14
CAPN3	15	rs2412710	TG		G/A/0.02	7
FRMD5	15	rs2929282	TG		A/T/0.05	5.13
LIPC	15	rs1532085	HDL	TC, TG	G/A/0.39	1.45
LACTB	15	rs2652834	HDL		G/A/0.20	-0.39
CTF1	16	rs11649653	TG		C/G/0.40	-2.13
CETP	16	rs3764261	HDL	TC, LDL, TG	C/A/0.32	3.39
LCAT	16	rs16942887	HDL		G/A/0.12	1.27
HPR	16	rs2000999	TC	LDL	G/A/0.20	2.34
CMIP	16	rs2925979	HDL		C/T/0.30	-0.45
STARD3	17	rs11869286	HDL		C/G/0.34	-0.48
OSBPL7	17	rs7206971	LDL	TC	G/A/0.49	0.78
ABCA8	17	rs4148008	HDL		C/G/0.32	-0.42
PGS1	17	rs4129767	HDL		A/G/0.49	-0.39
LIPG	18	rs7241918	HDL	TC	T/G/0.17	-1.31
MC4R	18	rs12967135	HDL		G/A/0.23	-0.42
ANGPTL4	19	rs7255436	HDL		A/C/0.47	-0.45
LDLR	19	rs6511720	LDL	TC	G/T/0.11	-6.99
LOC55908	19	rs737337	HDL		T/C/0.08	-0.64
CILP2	19	rs10401969	TC	TG, LDL	T/C/0.07	-4.74
APOE	19	rs4420638	LDL	TC, HDL	A/G/0.17	7.14
		rs439401	TG		C/T/0.36	25.5
FLJ36070	19	rs492602	TC		A/G/0.49	1.27
LILRA3	19	rs386000	HDL		G/C/0.20	0.83
ERGIC3	20	rs2277862	TC		C/T/0.15	-1.19

Locus	Chr	Lead SNP	Lead trait	Other traits	Alleles/MAF	Effect size
MAFB	20	rs2902940	TC	LDL	A/G/0.29	-1.38
TOP1	20	rs6029526	LDL	TC	T/A/0.47	1.39
HNF4A	20	rs1800961	HDL	TC	C/T/0.03	-1.88
PLTP	20	rs6065906	HDL	TG	T/C/0.18	-0.93
UBE2L3	22	rs181362	HDL		C/T/0.20	-0.46
PLA2G6	22	rs5756931	TG		T/C/0.40	-1.54

Table B.1: Results from meta-analysis of plasma lipid concentrations, Willer et al. 2013

Primary trait	Locus	Lead SNP	Chr	hg19 Position (Mb)	Traits GWS	Nearest Gene	Nearest Gene (kb)	No. of Genes within 100kb	Literature Candidate
HDL	ANGPTL1*	rs4650994	1	178.52	HDL	C1orf220	0	3	
	CPS1	rs1047891	2	211.54	HDL	CPS1	0	2	
	ATG7	rs2606736	3	11.4	HDL	ATG7	0	2	
	SETD2	rs2290547	3	47.06	HDL	SETD2	0	4	
	RBM5	rs2013208	3	50.13	HDL	RBM5	0	4	
	STAB1	rs13326165	3	52.53	HDL	STAB1	0	10	STAB1, NISCH
	GSK3B	rs6805251	3	119.56	HDL	GSK3B	0	3	GSK3B, NR1I2
	C4orf52*	rs10019888	4	26.06	HDL	C4orf52*	131.5	0	
	FAM13A	rs3822072	4	89.74	HDL	FAM13A	0	2	

Primary trait	Locus	Lead SNP	Chr	hg19 Position (Mb)	Traits GWS	Nearest Gene	Nearest Gene (kb)	No. of Genes within 100kb	Literature Candidate
	ADH5	rs2602836	4	100.01	HDL	ADH5	4.9	4	
	RSPO3	rs1936800	6	127.44	HDL, TG	RSPO3	4	1	
	DAGLB	rs702485	7	6.42	HDL	DAGLB	0	5	DAGLB
	SNX13	rs4142995	7	17.92	HDL	SNX13	0	1	SNX13
	IKZF1	rs4917014	7	50.31	HDL	IKZF1	0	1	IKZF1
	TMEM176A	rs17173637	7	150.53	HDL	ABP1	20.1	5	
	MARCH8-ALOX5	rs970548	10	46.01	HDL, TC	MARCH8	0	3	ALOX5
	OR4C46	rs11246602	11	51.51	HDL	OR4C46	3.2	2	
	KAT5	rs12801636	11	65.39	HDL	PCNXL3	0	12	KAT5
	MOGAT2-DGAT2	rs499974	11	75.46	HDL	MOGAT2	12.7	4	MOGAT2, DGAT2

Primary trait	Locus	Lead SNP	Chr	hg19 Position (Mb)	Traits GWS	Nearest Gene	Nearest Gene (kb)	No. of Genes within 100kb	Literature Candidate
	ZBTB42-AKT1	rs4983559	14	105.28	HDL	ZBTB42	6.2	7	AKT1
	FTO	rs1121980	16	53.81	HDL, TG	FTO	0	2	
	HAS1	rs17695224	19	52.32	HDL	FPR3	0	6	HAS1
LDL	ANXA9-CERS2	rs267733	1	150.96	LDL	ANXA9	0	10	CERS2
	EHBP1	rs2710642	2	63.15	LDL	EHBP1	0	1	EHBP1
	INSIG2	rs10490626	2	118.84	LDL, TC	INSIG2	10.2	2	INSIG2
	LOC84931	rs2030746	2	121.31	LDL, TC	LOC84931	85.6	1	
	FN1	rs1250229	2	216.3	LDL	FN1	3.6	2	FN1
	CMTM6	rs7640978	3	32.53	LDL, TC	CMTM6	0	3	

Primary trait	Locus	Lead SNP	Chr	hg19 Position (Mb)	Traits GWS	Nearest Gene	Nearest Gene (kb)	No. of Genes within 100kb	Literature Candidate
	ACAD11	rs17404153	3	132.16	LDL, HDL	DNAJC13	0	2	
	CSNK1G3	rs4530754	5	122.86	LDL, TC	CSNK1G3	0	2	
	MIR148A	rs4722551	7	25.99	LDL, TC, TG	MIR148A	2.2	1	
	SOX17	rs10102164	8	55.42	LDL, TC	SOX17	48.2	1	
	BRCA2	rs4942486	13	32.95	LDL	BRCA2	0	5	
	APOH-PRXCA	rs1801689	17	64.21	LDL	APOH	0	3	APOH, PRKCA
	SPTLC3	rs364585	20	12.96	LDL	SPTLC3	26.9	1	SPTLC3
	SNX5	rs2328223	20	17.85	LDL	SNX5	76.3	2	SNX5
	MTMR3	rs5763662	22	30.38	LDL	MTMR3	0	2	
TC	ASAP3	rs1077514	1	23.77	TC	ASAP3	0	6	
	ABCB11	rs2287623	2	169.83	TC	ABCB11	0	4	ABCB11

Primary trait	Locus	Lead SNP	Chr	hg19 Position (Mb)	Traits GWS	Nearest Gene	Nearest Gene (kb)	No. of Genes within 100kb	Literature Candidate
	FAM117B	rs11694172	2	203.53	TC	FAM117B	0	2	
	UGT1A1	rs11563251	2	234.68	TC, LDL	UGT1A1	0	12	UGT1A1/3/4/5 UGT1A6/7/8/9
	PXK	rs13315871	3	58.38	TC	PXK	0	4	PXK
	KCNK17	rs2758886	6	39.25	TC	KCNK17	15.9	4	
	HBS1L	rs9376090	6	135.41	TC	HBS1L	35.2	2	
	GPR146	rs1997243	7	1.08	TC	C7orf50	0	7	
	VLDLR	rs3780181	9	2.64	TC, LDL	VLDLR	0	3	VLDLR
	VIM-CUBN	rs10904908	10	17.26	TC	VIM	10	3	VIM, CUBN
	PHLDB1	rs11603023	11	118.49	TC	PHLDB1	0	7	
	PHC1-A2ML1	rs4883201	12	9.08	TC	PHC1	0	4	A2ML1

Primary trait	Locus	Lead SNP	Chr	hg19 Position (Mb)	Traits GWS	Nearest Gene	Nearest Gene (kb)	No. of Genes within 100kb	Literature Candidate
	DLG4	rs314253	17	7.09	TC, LDL	DLG4	1.6	13	ACADVL, CTDNEP1, SLC2A4
	TOM1	rs138777	22	35.71	TC	TOM1	0	4	HMOX1
	PPARA	rs4253772	22	46.63	TC, LDL	PPARA	0	6	PPARA
TG	LRPAP1	rs6831256	4	3.47	TG, LDL, TC	DOK7	0	4	LRPAP1
	VEGFA	rs998584	6	43.76	TG, HDL	VEGFA	3.7	1	VEGFA
	MET	rs38855	7	116.36	TG	MET	0	1	
	AKR1C4	rs1832007	10	5.25	TG	AKR1C4	0	2	AKR1C4

Primary trait	Locus	Lead SNP	Chr	hg19 Position (Mb)	Traits GWS	Nearest Gene	Nearest Gene (kb)	No. of Genes within 100kb	Literature Candidate
	PDXDC1	rs3198697	16	15.13	TG	PDXDC1	0	4	
	MPP3	rs8077889	17	41.88	TG	MPP3	0	6	
	INSR	rs7248104	19	7.22	TG	INSR	0	1	
	PEPD	rs731839	19	33.9	TG, HDL	PEPD	0	2	CEBPG

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