Conference on 'Getting energy balance right'
Symposium 4: Nutritional epidemiology and risk of chronic disease

The influence of maternal and infant nutrition on cardiometabolic traits: novel findings and future research directions from four Canadian birth cohort studies

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A mother's nutritional choices while pregnant may have a great influence on her baby's development in the womb and during infancy. There is evidence that what a mother eats during pregnancy interacts with her genes to affect her child's susceptibility to poor health outcomes including childhood obesity, pre-diabetes, allergy and asthma. Furthermore, after what an infant eats can change his or her intestinal bacteria, which can further influence the development of these poor outcomes. In the present paper, we review the importance of birth cohorts, the formation and early findings from a multi-ethnic birth cohort alliance in Canada and summarise our future research directions for this birth cohort alliance. We summarise a method for harmonising collection and analysis of self-reported dietary data across multiple cohorts and provide examples of how this birth cohort alliance has contributed to our understanding of gestational diabetes risk; ethnic and diet-influences differences in the healthy infant microbiome; and the interplay between diet, ethnicity and birth weight. Ongoing work in this birth cohort alliance will focus on the use of metabolomic profiling to measure dietary intake, discovery of unique diet–gene and diet–epigenome interactions, and qualitative interviews with families of children at risk of metabolic syndrome. Our findings to-date and future areas of research will advance the evidence base that informs dietary guidelines in pregnancy, infancy and childhood, and will be relevant to diverse and high-risk populations of Canada and other high-income countries.

Birth cohort: Microbiome: Birth weight: Nutrition: Diet: Pregnancy

Non-communicable diseases such as cancers, CVD, diabetes and chronic respiratory diseases are responsible for more than 35 million deaths in the world annually[1].

Common aetiologic factors such as smoking, obesiity, and (increasingly) dietary intake, link some non-communicable diseases[2]. Nutrition is a critical environmental factor that

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influences the development of the fetus, and infant and child health in early life. In low-income countries, health risks are primarily due to undernutrition, while in high-income countries such as Canada, nutrition-related health risks arise primarily from over nutrition, most strikingly for obesity and associated non-communicable diseases[1]. Furthermore, women with access to secure and plentiful food access often consume an excess of energy-dense nutrient-poor foods. Poor-quality diets can result in para-doxical 'over-nourished, malnourished' expectant mothers, and such unbalanced nutrition is associated with several adverse maternal, such as excessive weight gain during pregnancy and gestational diabetes. Evidence for developmental programming during fetal life underscores the critical influence of maternal diet on fetal growth and development. Interactions between genetic and epigenetic factors (in both mother and fetus), and sub-optimal maternal nutrition, may increase the infant susceptibility to adverse health outcomes including adiposity, metabolic syndrome-related factors, allergic disorders and asthma[3–5]. Furthermore, infant feeding patterns and alterations in the gut microbiota in the early years may accelerate the development of these adverse health outcomes[6–8].

What are birth cohort studies and why are they important?

Birth cohort studies are those which begin at or before the birth of its participants and continue to study the same individuals at later ages, on more than one occasion[9]. They are a type of observational study so there is no randomisation to exposure groups, and there is no attempt to manipulate the exposure status. They usually aim to be nationally representative but some are area-based and often such studies enrol many thousands of participants.

Birth cohorts are a powerful resource to study diet–disease associations, gene–diet interactions, epigenetic influences and the role of the microbiome because these studies involve a detailed assessment of the maternal/fetal environment before birth and include longitudinal prospective follow-up of multiple health outcomes from birth, through infancy and early childhood. This prospective measurement of maternal exposures and pregnancy characteristics is superior to the retrospective classification of exposures, as it minimises recall bias.

Many of the important birth cohort findings are consistent with advice that our mothers and grandmothers have been passing on for generations. Birth cohort studies have helped establish that 'breast is best': breast-fed children are at lower risk of becoming overweight during adolescence, experience better cognitive outcomes and are at lower risk of being diagnosed with attention-deficit/hyperactivity or autism spectrum disorders[10,11]. Through birth cohort studies, we have also come to understand the educational benefits of reading regularly to children[12]; and the benefits of supine positioning for sleep[13]. But beyond grandmothers' advice, birth cohorts can help us to uncover the role of the *in utero* environment in shaping our health trajectory through

the lifespan. Maternal energy deficit during pregnancy at key stages of development, especially when combined with overfeeding in the postnatal period, leads to a constellation of cardiometabolic risks, including altered glucose–insulin metabolism[14]. These effects on offspring phenotype seem to be partly mediated by changes in the number and function of pancreatic β-cells, possibly via epigenetic mechanisms[15].

The Barker hypothesis proposed in 1990 by the British epidemiologist David Barker (1938–2013) posits that in human subjects, intrauterine growth retardation, low birth weight and premature birth have a causal relationship to the origins of hypertension, type 2 diabetes and CHD in middle age[16]. Two observations provided the impetus for the development of Barker's hypothesis. Barker and Osmond reported a positive association between a county's neonatal death rate (a surrogate for low birthweight and its cardiovascular mortality rate)[17]. In 1989, Barker revisited Hertfordshire County birth records from 1911 to assess the association between birth weight and IHD, and found that low birthweight babies had three times the rate of IHD than normal-weight babies[18,19]. One long-term consequence of inadequate prenatal nutrition is impaired development of endocrine pancreas, which 'hardwires' the infant to be nutritionally 'thrifty'. If the child is nutritionally deprived, the phenotype is advantageous as it matches his/her prenatal environment; but if the same infant is exposed to a high-nutritional postnatal environment, he/she will carry an increased cardiometabolic risk[15].

To examine whether early catch-up growth following reduced intrauterine growth modifies risk of death from CHD in adulthood, Eriksson *et al.* followed 3641 boys from birth through adulthood to assess the association between birth weight and death from CHD[20]. Men who died from CHD had an above average BMI at all ages from 7 to 15 years. They found that those who were born large but were small at age 11 were at no increased risk of adult CHD, whereas those who were born small and experienced large catch-up growth were at increased risk of death from CHD later in adulthood. The highest death rates from CHD occurred in boys who were thin at birth but whose weight caught up so that they had an average or above average body mass from the age of 7 years.

The Dutch Hunger Winter occurred in West Netherlands near the end of World War 2 when food rations were limited to fewer than 3347 kJ/d (800 kcal/d)[21,22]. In a historical cohort study, 300 000 19-year-old men whose mothers were exposed to the Dutch Hunger Winter pre- and post-natally were examined at military induction to test the hypothesis that prenatal and early postnatal nutrition determines subsequent obesity[14]. The influence of maternal exposure to the famine was highly dependent on timing. Exposure to famine during the last trimester of pregnancy and the first months following delivery produced significantly lower obesity rates and better glucose tolerance. This is consistent with the inference that nutritional deprivation affected a critical period of development for adipose-tissue cellularity. Exposure to famine during the first half of pregnancy, however,
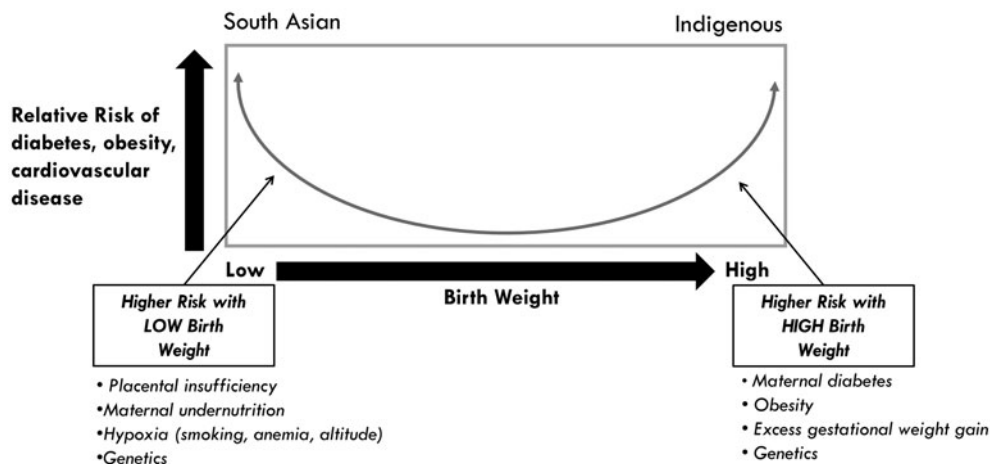
Fig. 1. The NutriGen Birth Cohort Alliance provides us with the ability to observe two distinct high-risk groups: South Asians, who incur higher risk with lower birth weight; and Indigenous Canadians, who incur higher risk with higher birth weight.

resulted in significantly higher obesity rates and poorer glucose tolerance. These data are supported by famine studies of China, Ukraine and Austria but not from the former Soviet Union or those in Africa[23–26].

A potential explanation is that famine that occurs early in pregnancy is relatively more potent than famine that occurs later in pregnancy[27]. This interpretation is consistent with the existence of a critical time window, one of the tenets of the developmental origins of health and disease, which posits that events during early, plastic phases of human development can have lifelong, sometimes irreversible effects[28].

The NutriGen Birth Cohort Alliance

A birth cohort study represents a considerable investment of time, personnel, and funds in order to recruit and retain participants, collect data, and store and analyse samples[29]. However, the development of common objectives across already established cohorts in the area of nutrition and health is an efficient and powerful way to address important questions that cannot be answered within any single cohort. Furthermore, pooling data across ethnically diverse birth cohorts provides diversity in exposures and may enable discovery of new insights with respect to exposure–disease associations. To do this, we have brought together four Canadian birth cohorts (described later) representing mother–child dyads from across socioeconomic gradients, and from diverse ethnic groups (including South Asians and Indigenous peoples; Fig. 1). Each cohort collected a detailed semi-quantitative FFQ from pregnant women in the late second or early third trimester, assessed infant feeding patterns in the first year of life, and has assessed or will assess the child's diet after age 1 year. All cohorts have collected or will collect maternal and newborn DNA, anthropometric measures of infant adiposity and growth, blood pressure, biologic samples for the measurement of lipids and glucose, and outcome information

on allergic disorders and asthma. We plan to follow all participants for a minimum of 3 years. Later, we briefly describe the setup of these cohorts and present physical characteristics of women and infants in Table 1.

The Family Atherosclerosis Monitoring In Early Life (*FAMILY*) *cohort* is a prospective birth cohort which includes predominantly white Caucasian mothers and their offspring and was designed to understand the early life determinants of risk factors for CVD. FAMILY includes 857 mothers and 901 infants recruited from Southwestern Ontario between 2004 and 2009[30,31]. Of the mother–infant dyads enrolled during pregnancy, >97 % pairs have provided infant measures at age 1 year and >95 % have provided child measures at age 3 years.

The Canadian Healthy Infant Longitudinal Development (CHILD) study is a four centre (Vancouver, Edmonton, Winnipeg and Toronto, Canada) longitudinal, population-based birth-cohort study which enrolled 3455 mother–child pairs between 2008 and 2012 with planned 5-year follow-up[32]. The focus of CHILD is to identify environmental and genetic determinants of allergic disorders and asthma. The follow-up to age 5 years is complete with >95 % retention to age 1 year, >93 % to age 3 year and >93 % to age 5 years.

The South Asian Birth Cohort (START) study aimed to enrol 1000 South Asian mother–child pairs from the greater Toronto area, in the province of Ontario[33]. Two sister cohorts recruiting 500 mother–child dyads are underway in rural and urban Bangalore, India. START will study the influence of diverse environments, genetics and epigenetic marks on early life adiposity, growth trajectory and cardio-metabolic factors. Recruitment in Canada began in July 2011, and presently 1012 mothers with 1002 newborns delivered have been enrolled. Follow-up is >95 % complete to age 1 and >92 % to age 3 with follow-up visits ongoing.

The Indigenous (Aboriginal) Birth Cohort study aimed to enrol 300 pregnant women and their offspring from the Six Nations Reserve in Ontario, to characterise

Table 1. Maternal and infant characteristics (through July 2018)

Parameter	iABC	START-Canada	FAMILY	CHILD
Mothers				
N^*	151	1012	816	3296
Maternal age (y)[†]	26·7 (5·9)	30·2 (3·9)	32·0 (5·1)	32·2 (4·7)
Pre-pregnancy weight (kg)[†]	76·0 (19·4)	62·6 (12·1)	71·8 (18·4)	66·3 (15·0)
Pre-pregnancy BMI (kg/m^2)[†]	30·6 (16·7)	23·8 (4·5)	26·5 (6·5)	24·5 (6·2)
Gestational diabetes mellitus (self-reported)[‡]	9 (6·0 %)	177 (17·8 %)	38 (4·8 %)	168 (5·1 %)
Gestational diabetes mellitus (IADPSG)[‡,§]	15 (16·5 %)	212 (22·5 %)	110 (14·0 %)	n/a
Newborn total skinfold thickness (mm)[†]	12·7 (3·8)	11·5 (2·6)	10·4 (2·7)	n/a
Infants				
N^*	151	1002	816	3220
Male (%)[‡]	61 (51·8 %)	497 (49·7 %)	416 (51·0 %)	1694 (52·6 %)
Gestational age at birth (weeks)[†]	39·3 (1·6)	39·1 (1·5)	39·2 (1·8)	39·5 (1·4)
Birthweight (g)[†]	3605 (656)	3212 (486)	3459 (567)	3445 (482)
Newborn total skinfold thickness (mm)[†]	12·7 (3·8)	11·5 (2·6)	10·4 (2·7)	n/a
Ponderal index (kg/m^3)[†]	25·6 (4·1)	24·2 (3·2)	27·7 (2·6)	25·3 (3·6)

iABC, The Indigenous (Aboriginal) Birth Cohort; START, The South Asian Birth Cohort; FAMILY, The Family Atherosclerosis Monitoring in Early Life Cohort; CHILD, The Canadian Healthy Infant Longitudinal Development study.
* Counts reflect data collected through July 11, 2018.
† Values are mean (SD).
‡ Values are counts (%).
§ IADPSG (International Association of the Diabetes and Pregnancy Study Groups) criteria for gestational diabetes mellitus[113].

the contextual, social and biological determinants of excess adiposity, type 2 diabetes and related cardio-metabolic risk factors in Indigenous infants and children[34]. Recruitment began in December 2012, and finished in December 2017, with 157 women and infants enrolled. Follow-up is >68 % complete to age 1 year, and >59 % to age 3 years with follow-up visits ongoing.

NutriGen Birth Cohort Alliance hypotheses

The main objectives of the NutriGen Birth Cohort Alliance are: (1) to harmonise measurements of exposures and outcomes across cohorts to improve comparability and facilitate comparisons; (2) to identify the dietary patterns and specific macronutrient intake levels of pregnant mothers which predict maternal health outcomes during pregnancy (i.e. excess weight gain, gestational diabetes, hypertension, preterm labour, post-partum weight retention) and newborn/infant/child health outcomes (i.e. adiposity, growth, metabolic traits (glucose, insulin, lipids, blood pressure), allergic disorders and asthma); (3) to investigate novel gene–diet interactions (maternal diet × maternal genotype, maternal diet × newborn genotype; newborn genotype × infant diet) and diet × epigenome interactions of newborns, and relate these to infant/child outcomes including birth weight, adiposity, metabolic traits, allergic disorders and asthma; (4) to characterise the infant microbiome at age 1 year comparing diverse maternal and infant diets, and study the association between the infant microbiome with infant/child health outcomes including adiposity, growth, metabolic traits, allergic disorders and asthma. Later, we review some important early methodological advances and novel findings from the NutriGen Birth Cohort Alliance to-date that have furthered these objectives.

Example 1: Creation of harmonised dietary patterns

Methodological advances in dietary measurement in large epidemiologic studies, such as the development of valid and reproducible semi-quantitative FFQ[35,36] have facilitated the study of associations between dietary intake and health and disease outcomes, such as cancer and CVD. This is often approached with a 'reductionist' lens by examining associations between specific food items[37–40], single nutrients[40,41], or sources of nutrients[42,43] and health outcomes. This approach is reflective of public health approaches to food and nutrient recommendations, and has been quite valuable, particularly at identifying and correcting single-nutrient deficiencies such as xerophthalmia (vitamin A), beriberi (vitamin B_1), pellagra (vitamin B_3), anemia (vitamin B_{12}), scurvy (vitamin C), pernicious rickets (vitamin D) and goitre (iodine)[44], but has several conceptual and methodological limitations.

First, people do not eat isolated nutrients; they eat meals consisting of a variety of foods with complex combinations of nutrients that likely interact[45]. Secondly, the high degree of intercorrelation among some nutrients in the same foods (such as potassium and magnesium) makes it challenging and unhelpful to examine their individual effects[46]. Thirdly, the effect of a single nutrient may be too small to detect, but the joint effects of multiple nutrients as part of a dietary pattern may be measurable[47]. Fourthly, analyses based on a large number of nutrients or food items may produce chance or spurious associations[48]. Lastly, because nutrient intakes are often associated with certain dietary patterns[49,50], single-nutrient analyses may be confounded by the effect of

other nutrients in foods that are often consumed together.

To overcome these limitations of single-nutrient or single-food studies, the empirical derivation of dietary patterns, defined as the quantities, proportions, variety or combinations of different foods and beverages in diets, and the frequency with which they are habitually consumed,[51] has been proposed to more closely reflect how we consume foods and nutrients. These patterns can be assessed for their associations with health and disease[45,52–54]. In preparation for investigations into the role of maternal nutrition on maternal and newborn outcomes, we developed an approach to derive harmonised dietary patterns in pregnant women[55].

In the CHILD study, maternal diet was assessed by using a semi-quantitative FFQ, adapted from the Fred Hutchinson Cancer Center tool[56]. In the FAMILY, START and Indigenous (Aboriginal) Birth cohort studies, semi-quantitative FFQ developed for the Study of Health and Risk in Ethnic Groups Study[57] were used to assess maternal dietary intake during pregnancy, modified to capture ethnic-specific foods. Individual FFQ items from each study were combined into thirty-six smaller groups by nutrient profile and type (e.g. poultry, leafy greens, legumes, etc.) to create common food groups across the cohorts. We identified three primary dietary patterns: plant-based, Western, and health-conscious, which collectively explained 29 % of the diet variability in a principal components analysis[54]. (Table 2). This study addressed a novel challenge: the merging and harmonisation of dietary data across cohorts which used different FFQ. We described a valid approach to merging both similar and distinct FFQ datasets which could be used in other studies that combine cohorts with unique diet assessment methods.

Example 2: Understanding the causes and consequences of gestational diabetes mellitus

The reasons for the increased risk of gestational diabetes among South Asian women are not well-understood. Using data from the START study, one of the participating cohorts in the NutriGen Birth Cohort Alliance, we sought to identify the determinants of gestational diabetes and its impact on newborn health[58]. We collected health information and physical measurements from 1012 women and administered an oral glucose tolerance test. We obtained birth weight and skinfold thickness measurements from newborns, as well as cord blood glucose and insulin levels. We reported an incidence of gestational diabetes of 36·3 %; the age-standardised rate was 40·7 %. Newborns of dysglycemic mothers had increased birth weight and body fat, and reduced insulin sensitivity, which influences future risk of excess adiposity and type 2 diabetes.

Factors associated with gestational diabetes included maternal age, family history of diabetes, pre-pregnancy weight and low diet quality, which had a combined population attributable risk of 65 %. Maternal height was protective against gestational diabetes. The population attributable risk due to the modifiable risk factors, pre-

Table 2. Principal component analysis food group loading scores

Food group	Plant-based	Western	Health conscious
Fats		0·55	
Full fat dairy			
Low fat dairy	0·39	0·41	
Fermented dairy	0·61		
Red meats	(−0·35)	0·43	0·33
Eggs			0·36
Organ meats			
Fish and seafood			0·50
Processed meats		0·55	
Meat dishes			0·49
Poultry and waterfowl			0·36
Fried foods			
Leafy greens			0·38
Cruciferous vegetables			0·55
Legumes	0·62		
Fresh seasonings	0·72		
Starchy vegetables		0·43	
Vegetable medley	0·43		0·47
Other vegetables	0·70		0·32
Tofu			
Fruit			0·52
Whole grains	0·71		
Refined grains			0·35
Pasta		0·53	
Pizza		0·32	
French fries		0·47	
Non-meat dishes	0·63		
Stir-fried dishes			0·47
Snacks		0·42	
Nuts and seeds			0·35
Sweets		0·46	
Condiments		0·48	0·41
Tea	0·53		
Coffee		0·34	
Sweet drinks		0·56	
Artificial sweets			
Eigenvalue	4·02	3·30	3·05
Cumulative variation*	0·11	0·20	0·29
Maximum diet score	50	65	70

Food items with a loading score ≥ |0·30| are presented and characterise each of the three dietary patterns within the NutriGen Birth Cohort Alliance cohort (n 4880). Originally published in reference[47]
* Proportion of the total dietary variation in the dataset that is explained by considering 1, 2, or 3 underlying dietary patterns.

pregnancy BMI and low diet quality was 37·3 %. This suggests that, if South Asian women could achieve an optimal pre-pregnancy weight (i.e. BMI <23) and improve their diet quality, about one-third of cases of gestational diabetes in this population could be prevented.

Our findings highlight the importance of public health messaging to South Asian women who are contemplating pregnancy to aim for an optimal weight before pregnancy as a potential prevention strategy against gestational diabetes[59]. To our knowledge, primary care physicians or public health specialists do not provide this message routinely, and this will require an integrated approach involving primary health care and policy initiatives[60].

Example 3: Ethnicity and diet-related differences in the healthy infant microbiome

The developing gastrointestinal microbiome in the first years of life is important for immune function, nutrient metabolism and protection from pathogens[61–63]. Microbial colonisation of the infant gut proceeds through infancy and establishment of an adult-like microbiome is estimated to occur within the first 3 years[64]. Identifying factors that shape the gut microbiome is currently an active area of research and early evidence suggests that host genetics[65] and early life exposures, including delivery method, antibiotics[66,67] and diet, influence the infant gut microbiome[68,69]. Although a stable microbiome may not be established until 1–3 years after birth, the infant gut microbiota appears to be an important predictor of health outcomes in later life, possibly influencing the progression of chronic diseases and has been associated with adverse health outcomes[70].

We analysed stool at age 1 year from a sub-sample of 173 white Caucasian and 182 South Asian infants from the CHILD and START birth cohorts to gain insight into how ethnicity, along with maternal and early infancy exposures influence the gut microbiota, using established methods of microbiome assessment[71–75]. More species richness was observed in South Asian babies than white Caucasian infants after considering breastfeeding at the time of collection[71]. The effect of ethnicity was larger than the effect of geographic location, which is typically an important source of variation[76]. Numerous studies have found the infant gut microbiome to vary between infants born by Caesarean section and those born vaginally, although the effect diminishes with age[77–79]. In our study, delivery method was not a significant predictor of the structure of the gut microbiome, but this is not surprising as the effect of delivery mode on the gut microbiome could have diminished by age 1 year. Larger analyses from the CHILD cohort have found delivery method to exert a strong influence on gut microbiome composition at 3–4 months[67,77,80].

South Asians had higher abundances of several genera within the Actinobacteria including *Bifidobacterium*, *Collinsella*, *Actinomyces* and *Atopobium* compared to white Caucasians[71]. Genera within the phylum *Firmicutes* within two distinct taxonomic groups were associated with ethnicity. Genera such as *Streptococcus*, *Enterococcus* and *Lactobacillus* (class Bacilli, order Lactobacillales) were more abundant within South Asians whereas genera such as *Blautia*, *Pseudobutyrivibrio*, *Ruminococcus* and *Oscillospira* (order Clostridiales) were more abundant in white Caucasians. The most differentially abundant bacteria were members of the Lachnospiraceae which were higher in white Caucasians. Ethnic differences in the gut microbiome may reflect maternal and/or infant dietary differences. Whether these differences are associated with future cardiometabolic outcomes will only be determined through prospective follow-up.

Example 4: The association of maternal dietary patterns with birth weight differs by ethnicity

Birth weight is an indicator of newborn health and a strong predictor of health outcomes in later life[81]. Significant variation in diet during pregnancy between ethnic groups in high-income countries provides an ideal opportunity to investigate the influence of maternal diet on birth weight. We investigated the association between maternal diet and birth weight in our multiethnic cohort using a previously developed dietary pattern analysis approach[55,82]. A total of 3997 full-term mother–infant pairs with principal component analysis-derived diet pattern scores for the plant-based, Western and health-conscious diets, along with birthweight recorded, were included. No associations were found between the Western and health-conscious diet patterns and birth weight; however, the plant-based dietary pattern was inversely associated with birth weight, and an interaction with non-white ethnicity and birth weight was observed. Among white Europeans, maternal consumption of a plant-based diet was associated with lower birth weight, increased risk of small-for-gestational and reduced risk of large-for-gestational-age. Among South Asians, maternal consumption of a plant-based diet was associated with a higher birth weight.

In *post-hoc* analyses conducted separately in white Europeans and South Asians, we identified fifteen food groups for which the distribution differed between the individuals in the first and fourth quartiles of the plant-based diet. When we included terms for these food groups in the multivariable models, only the addition of cooked vegetables reduced the magnitude of the plant-based dietary pattern association in South Asians by 6 %. No other foods, nor multi-vitamin use influenced the association of plant-based diet and birth weight in either ethnic group. Differences in food preparation methods can significantly alter the chemical and nutritional composition of dishes[83], notably the addition of fat for frying. Higher fat intake has been associated with newborn length and adiposity in other studies of South Asian pregnant women[84]. These results require replication to elucidate potential mechanisms that underlie these ethnic-specific associations.

Ongoing research topics within the NutriGen Birth Cohort Alliance

In addition to dietary data, all four cohort studies collected maternal blood samples during the second trimester, which were processed in <24 h, aliquoted, and stored immediately at −70°C, and before being transferred to long-term storage in liquid nitrogen. Three of four cohorts collected fasting samples. Three of four studies have serum aliquots of infants at ages 1 and 5 years, which were processed in the same fashion. The exceptions are that CHILD did not collect a fasting maternal sample; FAMILY did not collect an infant sample at age 1 year; and the Indigenous (Aboriginal) Birth Cohort does not plan to collect an infant sample at age 5 years. With these samples, we will conduct metabolite, gene–environment and epigenetic analyses.

Metabolomics

The existing literature for both maternal and infant diet reveals inconsistent associations between foods, nutrients

and various health outcomes, which arise from the methodological challenges and errors associated with estimating dietary intake and assessing the contribution of nutrition independent of other lifestyle and biological confounders[85,86]. Recognising these limitations we look to use a more direct measurement of nutrient status, function and effect. Metabolomics-based approaches offer an unprecedented opportunity to derive a more precise measurement of dietary intake, reflecting many steps including gut absorption and liver metabolism. We will use previously identified candidate metabolites found to be associated with (1) adolescent body weight[87]; (2) gestational diabetes mellitus or hyperglycemia[88,89]; (3) low birthweight[90] or (4) childhood risk of obesity[91], together with untargeted metabolites which pass a pre-specified statistical threshold. We will conduct analyses separately for each cohort (CHILD, FAMILY and START) and pool results using appropriate meta-analytic techniques[92].

Gene–environment interactions

Complex interactions between genetic variants and selected environmental factors likely exist with cardiometabolic traits, allergic disorders and asthma. Investigation of gene–environment interactions has been challenging because optimal studies require large sample sizes, careful measurement of environmental factors and robust clinical outcomes[5]. Most investigations which have identified gene–environment interactions have selected candidate SNP identified through genome-wide association studies and tested their association with the outcome together with an environmental exposure which is also related to the outcome. Gene–environment interactions have been demonstrated and replicated in myocardial infarction, type 2 diabetes, obesity and asthma[93,94]. However gene–environment interaction studies among infants/children which necessitate large sample sizes and finely phenotyped cohorts are only recently underway. The NutriGen Birth Cohort Alliance will facilitate the study of gene–diet interactions on maternal and infant health outcomes. Our analysis of gene–diet interactions will be prioritised in three steps: Step (1) SNP demonstrated to be significant in genome wide studies will be prioritised for testing for gene–diet interactions. Step (2) a genome wide analysis of mother genotype against maternal traits and baby genotype against infant/child traits will be performed. Significant SNP will be tested with dietary patterns and highly prioritised dietary factors of mother and infant to determine if a gene–diet interaction exists. Step (3) any main effect of SNP, diet, or interaction will be tested for replication in partner cohorts.

Epigenetics

There is increasing evidence that maternal diet modifies maternal/fetal DNA (DNA methylation, histone structure and small non-coding RNA) which affects gene expression in the offspring[95]. This represents the most likely biological explanation for the persistent effects of environmental exposures during pregnancy through successive generations[96]. We aim to investigate maternal nutrition in conjunction with newborn epigenetic marks to determine their interactions and potential influences on an array of health outcomes in the offspring. Rather than performing genome-wide methylation in all samples, which is expensive and unfocused, we will use an 'omics approach to target specific methylation sites guided by gene expression information[97,98].

Genome wide chip-based methylation analysis has a high degree of reproducibility. However, the challenge of multiple testing and generation of false-positive results with poor replication remains[99,100]. Target regions will be identified by (1) performing whole genome expression analysis using RNA in 500 cord blood samples from the START and CHILD birth cohorts, (2) investigating gene expression differences in newborn samples based on maternal dietary extremes i.e. Western diet v. Prudent diet, (3) performing genome wide methylation analysis using the Illumina 450 K Infinium methylation assay to interrogate >450 000 methylation sites per sample at single-nucleotide resolution in the same cord blood samples, and (4) investigating if genes differentially expressed in newborns have corresponding variation in methylation patterns specifically in the promoter region. These targeted methylation sites will then be tested in the remaining cord blood samples (n 4000) from infants across four cohorts and analysed in relation to maternal dietary patterns and selected infant outcomes including adiposity, cardiometabolic traits, allergy and asthma.

Bridging 'omics data

Underlying associations between the genome (an organism's complete set of DNA, including its genes), epigenome (compounds that attach to and 'mark' the genome, altering its function but not sequence) and metabolome (the totality of metabolites present within an organism) can provide additional insights regarding the transmission of the metabolome from mother to infant[101,102]. Recently, independent studies identified lipids and amino acids as being highly heritable[103]. Understanding the role of epigenetic modification as a method of controlling heritability of certain traits will help advance our understanding of childhood adiposity and other metabolic syndrome traits.

Family health behaviours

Among young children, poor diet quality along with physical inactivity contribute to the risk of excess body weight and metabolic syndrome[104–106]. Metabolic syndrome is a cluster of conditions: increased blood pressure, high blood sugar, abdominal fat and abnormal cholesterol or TAG levels that occur together, increasing the risk of heart disease, stroke and diabetes[107–109]. There is increasing evidence that a child's energy balance is mediated by their family environment because the home environment is where a child's food habits are established[110–112]. With the long-term goal of developing an intervention to prevent the metabolic syndrome in children aged 10 years, we will use quantitative information collected from questionnaires from children in the NutriGen Birth Cohort Alliance (i.e. self-reported

dietary intake and physical activity) along with semi-structured interviews to guide inquiries among families to understand their health behaviour experiences.

Strengths

Our study has several strengths. First, with up to 5000 mother–infant pairs with precise phenotypes, we have high statistical power to analyse diet–disease associations that have important implications for maternal and childhood health. Secondly, the diverse methodologies we will use provides an opportunity to investigate areas of emerging research by conducting a series of conceptually linked projects using cutting-edge technology and novel methodologies: epigenetics, metabolomics and microbiome profiling. Thirdly, we have collected data using similar assessment tools of maternal dietary patterns and health outcomes. Fourthly, the ethnic diversity of our participants ensures a wide variation in dietary exposure and environmental characteristics due to our multiple sub-populations.

Limitations

As with many observational studies, our work has limitations. First, between-study differences in the choice of data collection instruments, including questionnaires, means that data must be harmonised across cohorts to ensure between-cohort differences reflect true ethnic or cultural diversity, rather than diversity of data collection methods. Secondly, we have measured maternal diet with a single administration of self-administered FFQ, which are subject to recall and misclassification biases. Thirdly, ethnicity is a multidimensional construct which includes some within-group heterogeneity, and differences attributable to ethnicity may reflect a broad range of factors which are not purely biological. Fourthly, as with all observational studies, there is always the potential for residual confounding.

Conclusions

Several studies have shown that early life environmental exposures and genetics/epigenetics influence cardiometabolic risk factor trajectories. Birth cohorts designed to determine the relative contribution of genetic, epigenetic and environmental exposures on key health outcomes will provide major advances in this area. Furthermore, engaging high risk and diverse populations will provide information on aetiology and assist in the future design of interventions to improve health outcomes in vulnerable groups. However, studies of this nature require an interdisciplinary approach involving researchers with unique and complementary expertise in constant dialogue and an attitude of learning from each other. Harmonisation of data across multiethnic birth cohorts is unique in Canada and provides the opportunity to develop evidence-based dietary recommendations that jointly consider multiple key health outcomes. Including culturally diverse populations with disparate dietary intakes will greatly enhance our ability to detect important dietary patterns and unique diet–gene interactions and enable the assessment of whether dietary recommendations can be generalised across multiple Canadian populations.

Conflict of Interest

None.

Authorship

R. J. de S. wrote the manuscript. S. S. A., M. G., M. R. S., P. S., K. T., and G. W. led or co-led the NutriGen Alliance cohort studies that provided the data. All authors read the manuscript, provided critical feedback, and approved the final manuscript.

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