START Study Protocol

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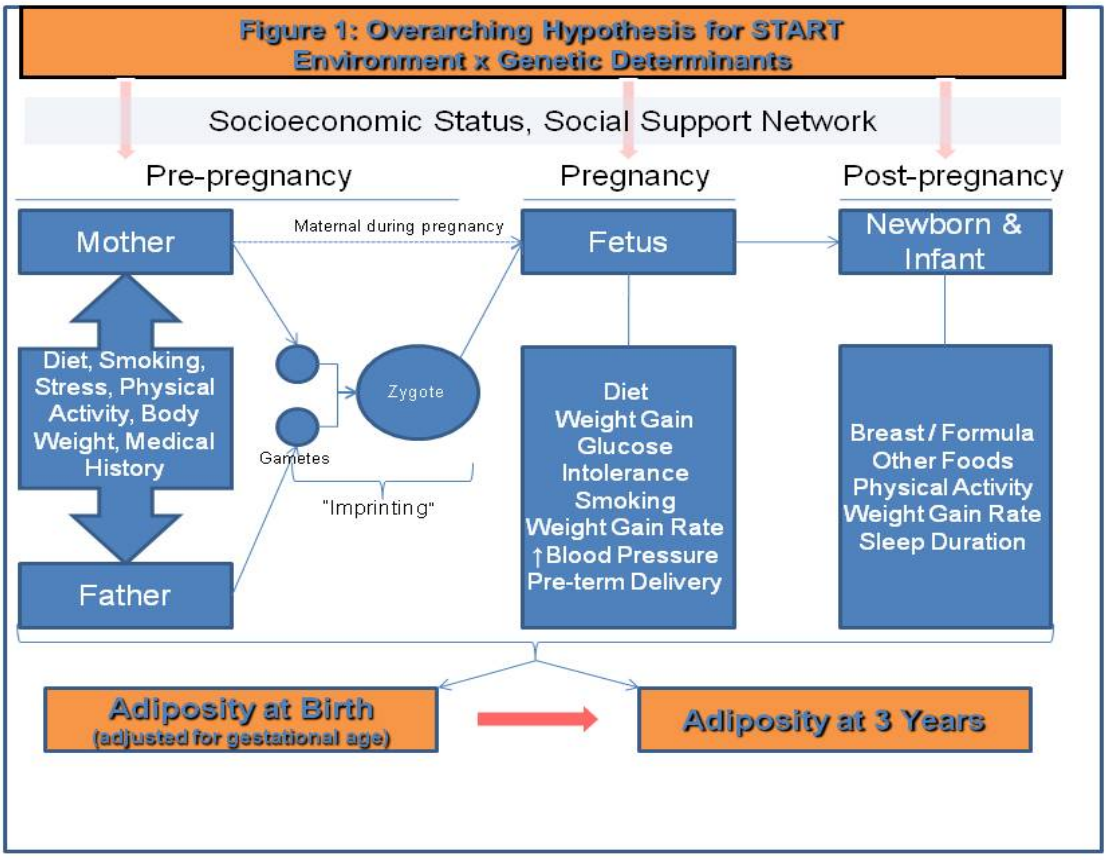
Table of Contents

1.0 Introduction:

Globally, people of South Asian origin are at high risk of developing type 2 diabetes mellitus & cardiovascular disease (CVD).[1] In India, the prevalence of type 2 diabetes is estimated to be 40 million, with an expected rise to 79 million by 2030.[2] This propensity to develop type 2 diabetes is observed among South Asians in urban environments in India as well as among South Asians living outside of India.[3] South Asians represent the fastest growing group of immigrants to Canada, & there are over 1.2 million South Asians currently living in Canada.[4] Our previous studies of South Asian adults in Canada demonstrate that South Asians have an increased prevalence of diabetes compared to white Caucasians & South Asians develop abnormal glucose, lipids, & blood pressure at a significantly lower BMI compared to white Caucasians (i.e. 21 vs 30 kg/m^2).[5] Reasons for South Asians increased metabolic sensitivity to weight gain are unknown although there is emerging evidence to suggest that these alterations in metabolic risk factors may be "programmed" early in life. [6] To better understand the development of adiposity among South Asians in Canada, we propose to study the environmental & genetic basis of adiposity among 1,000 South Asian mothers & their offspring recruited from the Peel Region in Ontario (START cohort). These mother-baby dyads will be compared to an existing white Caucasian mother-baby prospective cohort (FAMILY study) we recently recruited in Hamilton, Ontario, Canada (n=901).

2.0 Hypothesis:

Adiposity is partly programmed *in-utero*, & is influenced by maternal, paternal & fetal factors. At birth, the "programmed fetus" is exposed to a new external environment, & interactions between the programmed newborn & the new environment lead to excess adiposity & related metabolic risk factors during childhood. (Figure 1)



3.0 Objectives:

Among all four birth cohorts: rural India, urban India, South Asians from urban Canada, and white Caucasians from urban Canada (which is already recruited – FAMILY Study), the objectives of this study are to determine the primary causes of adiposity and understand the effect of diverse environments on the development of adiposity among newborns and the growing offspring during the first three years of age.

Primary objectives: The primary objectives of this study are to:

1) Determine if there are differences in birth weight and adiposity (corrected for gestational age) comparing South Asian to: i. white Caucasian newborns in Ontario, and ii. newborns in urban and rural Bangalore, India.
2) Determine the antenatal maternal factors (e.g. medical history, dietary intake, smoking exposure, & psychosocial stress), & pregnancy factors (e.g. maternal weight gain, glucose intolerance, pregnancy-induced hypertension) across diverse environments which are associated with the newborn's adiposity at birth.
3) Determine the association between early feeding practices (i.e. exclusivity of breastfeeding, formula feeding, type, frequency & duration of breast/bottle feeding, growth after weaning) and sleep patterns on post-natal growth & adiposity at 1 & 3 years after birth.
4) Determine the association between maternal micronutrient status (i.e. vitamin B12 & folate) & newborn's adiposity & insulin resistance at birth & 3 years.

Secondary Objectives:

5) Determine the relative contribution of selected candidate genes & epigenetic markers using DNA collected from mothers & newborns to adiposity at birth & adiposity accumulation in the growing offspring to 3 years.
6) To determine the impact of the home environment, including family structure, socio-economic status, health behaviours (i.e. dietary intake and physical activity) psychosocial stress factors of the mother and father, and parent-child interaction, on the development of adiposity of the growing offspring in the first three years.
7) To determine the association between birth weight and measures of adiposity at birth with morbidity events (respiratory infections, unspecified fever and diarrhoea) and its impact on anthropometric measures during the first year of life across diverse environments.
8) To validate measures of adiposity (i.e. triceps skinfold thickness) with accurate measures of body composition including deuterium dilution technique, bioelectrical impedance assessment (BIA) and DXA scanning.
9) To build research capacity in a rural setting in South India (training sessions in clinical epidemiology, nutritional and psychosocial assessments applied to cohorts), and in a community hospital in Brampton, Ontario, Canada.

4.0 Background and Significance:

4.1 South Asians Unique Risk Factor Profile:

People of South Asian origin are one of the highest risk groups for the development of type 2 diabetes and CVD.[7] The health behaviors & risk factor profile of South Asians living outside the Indian subcontinent are remarkably similar to those of South Asians living in the Indian subcontinent. South Asians develop abnormal glucose, lipids, & blood pressure at a significantly lower BMI compared to white Caucasians.[5] For example, South Asians demonstrate increased plasma glucose, apolipoprotein B & systolic blood pressure & lower apolipoprotein A as the BMI increases above 21 compared to white

Caucasians who don't develop equivalent changes until their BMI increases above 30.[5] The likely pathophysiology of South Asians' heightened sensitivity to weight gain is their increased propensity to develop visceral abdominal fat & fatty infiltration of the liver when in a state of chronic overnutrition. The accumulation of visceral & ectopic fat is associated with the "metabolic syndrome" associated risk factors (i.e. increased glucose, abnormal lipids & elevated blood pressure).[8] The reasons South Asians are prone to develop these risk factors include a predilection to develop abdominal adipocyte hypertrophy with fat overflow into ectopic sites, genetic variants predisposing to fatty liver, & early origins hypothesis that this response to chronic over nutrition is programmed *in-utero*[9].

4.2 Rationale for the creation of a South Asian Birth Cohort:

There is evidence among South Asians to support the concept that adiposity & related metabolic factors may be influenced by factors which "program" the developing fetus. Programming refers to metabolic events which occur during critical time periods of antenatal & postnatal development which have moderating effects on health in later life.[10] **There are no South Asian specific birth cohorts in Canada**, & information regarding the influence of fetal programming on adult health outcomes in this group comes from India. A birth cohort from six rural villages in India compared the anthropometric characteristics of 631 mothers/babies to 338 mothers/babies of European origin recruited from Southampton UK.[11] This comparison revealed that South Asian mothers were younger, lighter & shorter & had a lower BMI (18 vs. 23) compared to the European mothers, & South Asian babies were lighter (2.7 kg vs. 3.5 kg), shorter, yet had comparable subscapular skin fold thickness, suggesting for their weight they had relatively more adipose tissue. In summary, South Asian babies were typically "short, underweight, but fat", which is referred to as the **"thin-fat" phenotype**. These South Asian babies were also found to have increased adiposity, glucose, insulin at 6 to 8 years.[12] However these observations were <u>not</u> supported by the results of an urban-based birth cohort from Bangalore, involving 712 South Asian women (BMI 21.5) who were recruited at 12 weeks of gestation & followed up until delivery. These investigators observed that skin-fold thickness in Indian babies were similar to those reported in a Western population with comparable birth weights.[13] To date in Canada, there has been no detailed study of the anthropometric characteristics of South Asian newborns. Recently our co-investigator Ray reported that the mean birth weight for 753 South Asian babies born between 2002-2007 of 39 weeks gestational age was 3.2 kg. While this was significantly lower than the mean birth weight of European origin babies in Canada (3.4 kg), $P<0.001$, it is substantially higher than the reported birth weights from the rural & urban birth cohorts of South Asians from India. (2.7 and 2.8 kg respectively).[14] However these between center comparisons are indirect, & it's possible that differences in maternal characteristics, gestational age, & pregnancy factors (i.e. maternal nutrition, gestational diabetes, hypertension) may explain these differences. It's important to understand the determinants of birth weight & adiposity in South Asian babies to help explain the recent report from the South Asian population in Brampton, Ontario that the prevalence of low birth weight babies & still births is higher in this community compared to other health jurisdictions in Ontario.[15] **Thus the conflicting reports from urban & rural areas of India, & sparse Canadian data underscore the need for creation of a birth cohort of South Asian mothers offspring to determine if South Asian newborns are lower birth weight & relatively more adipose at birth compared to a similarly accrued white Caucasian cohort in Ontario. This information will help us to understand the role of fetal programming in the development of adiposity in South Asians.**

4.3 Early Origins of Adiposity and Related Metabolic Changes:

Childhood obesity is primarily attributed to environmental changes leading to increased energy intake & lower physical activity.[16] Despite significant population-level changes, there is increasing evidence that a complex interplay of genetics, epigenetics, & non-genetic factors also interact to "program" a newborn to be more or less prone to develop obesity depending on the environment to which it is exposed.[17] The

major lines of evidence which support the concept of fetal programming & adiposity include the observations that: 1) low birth weight babies have a higher risk of adult onset diabetes & CVD compared to normal birth weight babies, 2) maternal behaviours & metabolic characteristics (i.e. nutrition, pre-pregnancy obesity, weight gain & glucose status) are associated with newborn adiposity & adult diabetes in the offspring, 3) infant feeding & post natal growth are associated with adolescent & adult adiposity & metabolic abnormalities, & 4) genetic & possibly epigenetic imprinting of the fetus are associated with adiposity & insulin resistance in the growing offspring. We review these four lines of evidence below.

4.3.1 Low Birth Weight & Adult Diabetes:

In 1993 Barker described an association between low birth weight & type 2 diabetes among babies born in England.[18] He suggested that intrauterine *undernutrition* programmed the fetus for adult onset diabetes. This hypothesis has gained currency following studies documenting the relationship between early markers of growth and later risks of hypertension, diabetes and CVD in adulthood.[19,20] Subsequent population, clinical & animal studies report associations between low birth weight & the risk of dysglycemia, insulin resistance, & central obesity in adulthood. Impaired fetal growth induced by either inadequate maternal nutrition or placental dysfunction are associated with an increased risk of developing the metabolic syndrome. These observations formed the basis for the "thrifty phenotype hypothesis".[21] This hypothesis proposed that in response to a suboptimal early environment (i.e. caloric or placental insufficiency), fetal physiology adapted to maximize chances of the offspring surviving postnatally in conditions of ongoing deprivation.[21] However, if post natal environments are not devoid of or possibly have excess nutritional energy, the newborn continues to use the programmed energy-saving mechanisms, appears to be more prone to adiposity and its associated metabolic abnormalities.

4.3.2 Maternal nutrition, weight gain & glucose status:

The dietary intake of mothers before & during pregnancy influences fetal growth, & has been linked to newborn adiposity. Fetal undernutrition leads to metabolic & structural changes, which may be initially beneficial for early survival but may increase the risk of type 2 diabetes in adulthood. The Dutch Famine Birth Cohort is composed of 801 offspring of mothers exposed to famine who were born as term singletons in Amsterdam around the time of the Dutch famine during World War II. Famine during any stage of gestation was associated with glucose intolerance. Furthermore, the offspring of the famine exposed mothers had increased adiposity & poor health in later life compared to non-exposed babies.[22] Similarly in 7,874 rural offspring born to pregnant women exposed to the Chinese Famine (1959-1961) had an increased risk of hyperglycemia and type 2 diabetes in adulthood, & those who were later exposed to a nutritionally 'rich' environment had the highest risk of subsequent dysglycemia.[23] These observations are supported by animal data which demonstrate that protein restricted diet (8% protein content) given to pregnant rats during late pregnancy produces offspring with reduced beta-cell mass at birth and a reduced insulin secretion at weaning.[24,25]***Micronutrients****:* Micronutrient deficiencies during pregnancy have also been associated increased adiposity & insulin resistance.[26] Children born to South Asian mothers with low vitamin B12 & high folate concentrations were the most adipose & insulin resistant at 6 years of age compared to the offspring of mothers without this micronutrient pattern during pregnancy.[27] In addition, in a cohort from Mysore India, vitamin B12 deficiency was associated with maternal gestational glucose intolerance, & babies born to these mothers who had both vitamin B12 deficiency & gestational diabetes had an increased risk of adiposity & glucose tolerance at 5 years of age.[28] Data from Canada regarding the B12, folate & homocysteine concentrations in South Asian women are limited. In SHARE, 51% of South Asian women were vegetarian, compared to only 10% of European origin women.[29] Therefore given the high proportion of South Asian women who are vegetarian together with the fortification/supplementation of folic acid in Ontario since 1998, we anticipate that the prevalence of vitamin B12 deficiency & adequate to high folate intake among

pregnant South Asian mothers in Canada will be substantial, thereby potentially masking vitamin B12 deficiency. *Given the evidence linking vitamin B12, folate, & homocysteine metabolism to adverse birth & fetal outcomes including excess adiposity & insulin resistance in the offspring, we will carefully assess the maternal diet, collect information on vitamin supplementation during pregnancy, & assess micronutrient status during second trimester of pregnancy in all South Asian mothers.*

Maternal weight pre-pregnancy & weight gain: Pre-pregnancy maternal obesity, maternal weight gain during pregnancy, & maternal glucose control during pregnancy are all associated with increased adiposity among their offspring. Prior studies have shown that the low BMI of mothers is associated with poor pregnancy outcomes including preterm birth, & having a small-for gestational age (SGA) infant.[30,31,32] Further pre-pregnancy low BMI together with low pregnancy weight gain rate is an important risk factor for low birth weight.[33,34] On the other hand a high maternal BMI increases the risk for hypertension, preeclampsia, gestational diabetes & giving birth to a macrosomic infant.[30] Greater gestational weight gain is associated with higher child BMI, adiposity and systolic blood pressure.[35] A recent systematic review (> 1 million women; 84 studies) showed that overweight & obese women have an increased risk of preterm birth.[36] Furthermore infants of women who gained more than 24 kg during pregnancy were 148.9 g (141.7-156.0) heavier at birth than were infants of women who gained 8-10 kg.[37] Therefore pre-pregnancy weight, & maternal weight gain will be studied in relation to newborn birth weight and adiposity.

Maternal Glycemic Status: The effect of maternal gestational dysglycemia (defined as elevated glucose which includes impaired fasting glucose, impaired glucose tolerance, & diabetes) on both intrauterine growth & postnatal growth patterns is considerable. Prenatal exposure to maternal diabetes is also associated with a higher prevalence of overweight or obese children. [38] Children born to a mother with gestational diabetes or even those with mildly impaired glucose tolerance[39] are at increased risk for overweight[40] and obesity.[41] Gestational diabetes increases the concentration of glucose, free fatty acids, & amino acids in maternal blood and results in fetal hyperinsulinemia, which in turn results in a larger fat mass in the infant.[42] The effect of dysglycemia during pregnancy is particularly relevant among South Asian women in whom the prevalence of gestational diabetes or milder impaired glucose tolerance is higher than the general population. Our co-investigator Retnakaran has recently shown that maternal insulin resistance during pregnancy is higher in South Asians and is a predictor of infant weight gain & adiposity in the first year postpartum.[43] It is important to also consider the degree to which mothers with gestational diabetes receive glucose lowering treatments during pregnancy, as failure to consider this may confound associations between maternal glucose status & newborn body composition. This increased propensity to develop dysglycemia may be due to increased insulin resistance or beta cell impairment among South Asian women during pregnancy which may in part explain the observation of increased fat mass among South Asian newborns[44].

4.3.3 Postnatal factors & adiposity:

At birth, the immediate environment faced by the newborn changes dramatically from that of the intrauterine environment. Prior studies suggest that infant **nutrition exposure, breast-feeding, formula-feeding, the energy density of the feeds, activity levels** & **sleep patterns** influence the development of adiposity & related metabolic changes in the growing offspring [45,46,47,48,49] *Postnatal catch up growth & adiposity:* Prior studies have shown that infants who are growth restricted during fetal life but subsequently grow rapidly are at a higher risk to develop adiposity at childhood and in adult life.[50,51,52] Among children with low birth weight those who are most vulnerable to developing obesity are light and thin at birth & then experience a period of rapid growth in the first 7 years of life.[53,54,55] However, in a study from Brazil, rapid early weight gain was related to height & lean mass but not fatness.[56] Conversely in the ALSPAC cohort from the UK, children who showed catch-up growth between zero & two years were heavier, taller, & fatter at five years than other children.[57] These inconsistent results may partly be due to differences in food availability & physical activity in different

populations. ***Breastfeeding & adiposity:*** In infants the post natal feeding regimen greatly influences growth trajectories. The protective effects of breast feeding on later development of obesity has received much attention.[45,46] Meta-analyses do support a small but significant positive effect of breast feeding on later health outcomes[47,48]. Infant feeding practices have been implicated in modifying the risk of developing insulin resistance, obesity & increased blood pressure.[58] Some studies have shown a dose dependent relationship between duration of breast feeding & the risk of later development of obesity.[52] Feeding of enriched formula to infants has been shown to increase linear growth in SGA infants[53] although it might increase the risk for metabolic diseases. In a UK cohort study, dietary energy intake in formula fed infants at 4 months of age was positively correlated to early childhood weight gain, body weight & BMI up to 5 years of age[59,54] The potential pathways that link breast feeding & protection against obesity include reduced early postnatal weight gain, a better learned self-regulation of food intake, & the presence of leptin in breast milk.[60] Children's daytime activity levels, sleep patterns, & sedentary behaviours including hours of television viewing are also associated with adiposity among school aged children.[61] ***Infant sleep:*** Shorter sleep durations are thought to decrease the production of satiety-promoting hormone, leptin. Among infants, sleeping short durations at night (less than 12 hours) is associated with overweight and obesity later in life. Both sleep and activity levels during the first year of life may be important determinants in the adiposity trajectory among South Asian infants in Canada. ***Adipokines and adiposity:*** Adipokines (i.e. leptin and adiponectin) may play a role in energy homeostasis by being influenced by diet and sleep during infancy. Leptin plays a role in the regulation of satiety. Infants who are breastfed have demonstrated higher levels of serum leptin then formula fed infants, likely due to the content of leptin in breastmilk. It has been shown that cord blood leptin levels in South Asian newborns are higher then in white Caucasian newborns when adjusted for weight. A prospective, longitudinal study of white Caucasian infants in the United States showed serum leptin levels to be positively correlated with central adiposity at 3 years of age. Adiponectin is strongly associated with insulin sensitivity and affects lipid metabolism. In cord blood samples adiponectin is positively correlated to cord blood leptin levels and birth weight. Similar to leptin, adiponectin is found in breastmilk. The relationship between the change in adipokine profiles from birth through infancy and early determinants of adiposity in this high-risk group needs to be studied. **In START we will carefully characterize early infant feeding practices, the child's dietary intake, infant activity** & **sleep patterns** & **relate them to adiposity** & **growth trajectories.**

4.3.4 Genetics and Epigenetics:

Studies of twins have revealed that the heritability of BMI is approximately 0.70 in adults & children,[62] & similar heritability has been observed for other measures of adiposity such as waist circumference, & total & regional fat distribution.[63] Recent genome wide association studies (GWAS) of obesity have identified almost 50 SNPs for obesity & related traits (i.e. BMI, waist circumference). Most of these genes appear to alter hypothalamic pathways and are believed to affect appetite control or response to satiety. In addition to adiposity, recent GWAS have identified more than 20 loci associated with the development of type 2 diabetes. Interestingly, there appears to be little overlap between loci associated with adiposity and loci associated with type 2 diabetes which are mostly implicated in pancreatic beta cell function & metabolism.[64] Thus, while adiposity is strongly associated with type 2 diabetes, the GWAS identified pathways of adiposity & diabetes appear to be different and it is not yet clear how these genes interact with early environmental exposures to define life-long health trajectories. It's likely the interaction of genetics & environment influences the development of adiposity, & discovering these interactions may help us understand more complex molecular mechanisms that underlie the regulation of energy balance.[65] The ALSPAC birth cohort recently tested the association between 10 GWAS identified SNPs associated with BMI in adults. They used a gene score of 8 SNPs to classify children by their "genetic load" for obesity. Although the obesity-risk-allele score showed little association with birth weight (regression coefficient: 0.01 SD per allele), it had a larger positive effect on early infancy

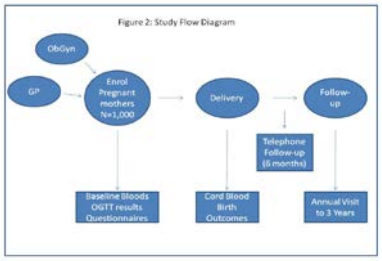
weight gain (0.12 SD/allele/year) & early infancy failure to thrive (odds ratio = 0.92 per allele; p = 0.009).[66] This cohort did not collect maternal genotypes which limits the study of maternal and offspring genotype interactions. Prior studies strongly suggest that consideration of both maternal and fetal genotypes are important in understanding the genetic determinants of birth weight, & fetal programming.[67,68] **To our knowledge the contribution of common genetic polymorphisms to the development of adiposity** & **insulin resistance has not been comprehensively investigated among South Asian newborns**. Furthermore, the modulation of the genetic effects by *in-utero* characteristics, postnatal diet, activity, & post natal rate of weight gain in relation to the growing offspring's adiposity has not been investigated. There is also emerging evidence from model systems that early exposure to environmental factors – including *in-utero* environment – produce epigenetic modifications leading to lifelong changes in gene expression, metabolic profile, & complex behaviors. Maternal malnutrition & over nutrition can induce epigenetic modifications of the fetal genome. The impact of early environmental exposures on epigenetic markers has not been systematically studied in humans partly due to technological barriers. However chip-based epigenetic measures can now be made with a high degree of precision and reproducibility.[69] Creation of the START cohort with its longitudinal assessment of nutritional & metabolic risk factors during pregnancy (i.e. fetal environment), at birth & up to 3 years of age for the index child, provides a unique opportunity to characterize how early environmental exposures interact with genetic variants that in turn program lifelong adverse health trajectories. By studying mothers & their offspring, we will be able to study gene–environment interactions for the development of risk factors and adiposity in early childhood.

4.4 Contextual factors which influence adiposity:

Socioeconomic status (SES) strongly influences the home environment provided to the newborn and is associated with child health outcomes. There is strong evidence that SES influences health behaviours including dietary intake, tobacco use, & activity patterns. There is also evidence that SES is associated with maternal health post partum including mental health conditions (i.e. depression and anxiety), as well as domestic violence. Mental & physical well-being have also been linked to both stress and social support. Having an available social support network & being able to elicit & receive effective social support has consistently been found to predict improved physical & mental health & decreased mortality, & can reduce the negative impact of stress.[70] The South Asian population in Ontario is unique & we anticipate at least 10 to 15% of new mothers in the Peel region will be new immigrants to Canada (i.e. < 5 years residence in Canada). Recent migration is associated with reduced support networks & increased stress. This includes stress due to acculturation. which is the process of adjusting to a new cultural environment.[71] High levels of stress & low social support have also been associated with poorer parenting behaviours, which may in turn create a stressful environment for the infant. Thus key contextual factors (i.e. SES, maternal mental health, social support, & acculturation stress) which influence key health behaviours (i.e. infant feeding practices, sleeping patterns & activity) associated with adiposity development in the offspring will be measured.

*Summary: START is a prospective cohort study designed to **first** determine if South Asian have the "thin-fat" phenotype compared to a similar cohort of white Caucasian newborns, & **second** to identify the major determinants of adiposity among South Asian babies across diverse environments. As highlighted above, fetal programming may be influenced by maternal undernutrition & placental insufficiency and/or by maternal overnutrition, weight gain, & gestational glucose intolerance. These factors together with the fetal genotype & postnatal environments may all contribute to the development of early adiposity & associated metabolic risk factors. To unravel the various pathways by which South Asian children & adults develop central adiposity & insulin resistance, detailed phenotyping of mothers during pregnancy & their offspring from birth to early childhood is required.*

5.0 Design and Methods:



We propose to assemble three birth cohorts: 1 in urban centres in Ontario, Canada, 1 in urban Bangalore, and one in a rural environment outside of Bangalore. In Canada, we will recruit 1,000 South Asian pregnant mothers & their babies from 3 hospitals in the Peel Region, & will follow them prospectively. Similarly, our Indian collaborators will recruit 500 mothers and their babies at each of their urban and rural centre. Mothers & offspring will be followed for 10 years from birth. We currently have funds to recruit and follow 250 newborns in Canada and 500 in India for a period of 3 years and have applied for funding to recruit and additional 750 mother-baby pairs in Canada. We plan on following all subjects beyond 3 years, & additional funding will be sought at that time. (Figure 2)

The primary comparison groups for the START cohort will be i. the mothers and children recruited in the FAMILY Study - a birth cohort primarily composed of white Caucasians designed to understand the early life determinants of risk factors for CV disease.[72] The FAMILY study was initiated in 2004 in Hamilton & 859 families (901 babies & 859 mothers) were enrolled, and ii. the mothers-baby pairs recruited from urban and rural India. Our experience in FAMILY has informed the design of START as there are unique challenges in recruiting pregnant mothers & following them & their offspring prospectively. **Where possible, identical measurement tools for mother** & **the growing offspring have been chosen in START in order to overcome the limitations of previous comparisons of South Asian** & **white Caucasian newborns, who were recruited from different countries, in different decades, and in whom different measurement tools were used.** The baseline characteristics of the FAMILY cohort are shown in Table 1 a, b & in the Progress Report.

START Inclusion Criteria: Women between 18-40 years of age who are pregnant who are of South Asian origin (defined as maternal and paternal grandparents originating from India, Pakistan, Bangladesh or Sri Lanka) are eligible. **Exclusion Criteria**: Women who have had more than 4 live births previously, surrogate mothers, women who conceived the fetus using artificial methods including in-vitro fertilization or intrauterine insemination, women who suffer from severe chronic medical conditions including active cancer, severe infectious diseases including HIV, hepatitis B or C, or who are VDRL positive, will be excluded. Women who have lived in Canada for less than 9 months or for whom the father of the baby is not of South Asian origin will also be excluded. (Table 2)

Recruitment: In both countries pregnant mothers will be recruited during their antenatal visits to their primary care practitioner or obstetrician. The study will be described by the physicians to the patients and consent for participation will be obtained. The father of the fetus will also be invited to participate in the baseline examination. **In Canada:** South Asian women will be recruited from 3 hospitals in the Peel region, Brampton Civic Hospital, Trillium, & Credit Valley. Brampton Civic hospital is a high volume community hospital with 4,460 newborn deliveries/year, & an estimated 40% of which are to South Asian mothers. Credit Valley Hospital & Trillium Health Centre each reports 4,695 and 4,130 births respectively per year and approximately 20% are South Asian. In **India:** The urban cohort will be recruited from the St John's Medical-College Hospital, Bangalore. This hospital is a 1200 bed tertiary care hospital located in a busy neighborhood in the far southeastern part of the city. The rural cohort will be recruited from the Snehalaya Hospital located in the village of Solur in the South Indian state of Karnataka. Snehalaya is a rural mission hospital managed and run by the religious congregation of the Sisters of Charity of Capitanio and Gerosa which provides nearly free health care to the rural population and the hospital conducts about 1,200 deliveries per year. Pregnant women attending the routine antenatal care at these centers will be invited to participate in the study.

5.1 Data Collection Stage I:

5.1.1 Antenatal data

Pregnant mothers will be recruited during their antenatal visits to family doctors or obstetricians. Consecutive patients will be approached, a log of all potentially eligible subjects will be kept, & the main reasons for exclusion or refusal to participate will be recorded. Pregnant mothers will be provided an information package describing the study & consent to have the recruitment team contact them will be obtained. An initial visit between 24-28 weeks will be scheduled at the recruiting centre nearest to the hospital planned for delivery. Information on age, parity, medical and pregnancy history, tobacco exposure, family structure (i.e. marital status, & number of children in the house), city & country of birth, mother tongue, religious practices, & time spent living in Canada prior to pregnancy, as well as socioeconomic factors (i.e. household income, education, & employment) will be collected.[73] Health card numbers will be recorded for future linkage with administrative data sources from the Ontario Ministry of Health & Long-Term Care. It is routine practice in the 3 recruitment hospitals for women to have an ultrasound at 18-20 weeks. *All* ultrasound reports will be obtained & used to establish gestational age & to assess fetal growth characteristics. Detailed anthropometric measurements will be performed in the pregnant mother during her 2nd trimester visit. A digital scale will be used to record body weight to the nearest 100 g. Height will be measured using a stadiometer to the nearest 1 cm & mid upper arm circumference to the nearest 0.1 cm using a plastic measuring tape. Maternal BMI will be calculated using weight height at baseline (kg/m^2). The mother's pre-pregnancy weight will be recorded. Weekly maternal weight gain during the 2nd trimester will be calculated as the average weekly weight gain between weight at baseline & weight at the 24-28 week visit. Similarly weekly maternal weight gain during the 3rd trimester will be calculated as the average weekly weight gain between weights measured at the 2nd and 3rd trimester visits. Skinfold thickness (triceps & subscapular) will be measured to the nearest 0.2 mm, using skinfold calipers (Holtain, UK), for the prediction of body fat using prediction equations.[74] Systolic and diastolic blood pressure will be measured using an automated BP monitor (OMRON Intelli Sense, Model HEM-757). (Table 3)

5.1.2 Maternal Glucose Status:

The diagnosis of maternal glycemic status is critical to determine glucose-metabolic status during the second trimester of pregnancy. All non-diabetic mothers will undergo the 75 oral glucose tolerance test (OGTT) between 24-28 weeks of gestational age. This test is chosen to avoid the high false negative rate using the 50 gram Glucose Challenge Test among South Asians.[44,75] Glycosylated hemoglobin, HbA1c will be measured at time of delivery. HbA1c is correlated with glycemic status during pregnancy as seen from the FAMILY study where those women with glucose intolerance or GDM had higher HbA1c measured at time of delivery. However HbA1c cannot be substituted for a glucose challenge test during the late second trimester.

5.1.3 Dietary and Physical Activity assessment:

We have previously developed & validated an FFQ for South Asians in Canada as part of SHARE.[76] However FFQ's are subject to recall bias, & are not sensitive to recent changes in diet that may occur in pregnancy or early post partum. **For these reasons we will assess mother's diet in pregnancy & in follow-up with a combination of the SHARE FFQ <u>and</u> 24 hour diet recalls.** The 24-hour diet recall will be used to assess the immediate & recent diet intake i.e. over the last 24 hours & will be administered during the 2nd trimester visit, as well as at 6 months & 1 year postpartum. (Table 4) Information on maternal activity during pregnancy will be collected for activities in 5 domains – occupational, discretionary exercise, household chores, sedentary activities, hobbies and sleep, as will sedentary behaviours, such as screen time per day (computer, television, video games). Both will be collected at baseline, & each annual visit.

5.1.4 Assessment of Maternal Depression, Social Support and Acculturation Stress:
Depression in the mother will be assessed by the Kessler-10 scale (K-10) which is a 10-item scale with five response categories ranked on a 5-point scale. Adequacy of **social support** to the mother will be measured using a questionnaire to evaluate the emotional, instrumental, informational, & appraisal components of social support. [77] The K-10 & social support questionnaires will be administered in the 2nd trimester & then at 12 months post-partum. **Acculturation stress** will be measured using the Vancouver Inventory Assessment which is a validated questionnaire that has been used among people of South Asian origin living in the United States. This questionnaire will be self-administered during the initial enrollment visit. [78,79] (Table 5)

5.1.5 Laboratory Assessments:
A 75 gram OGTT will be carried out in the 2nd trimester to screen for the gestational diabetes. Three blood samples will be collected: Fasting, 60 minutes & 120 minutes. A blood sample will also be collected from the pregnant mothers during the 2nd trimester. Some local analysis will be performed immediately (i.e. glucose, complete blood count) using standardized assays, & the remainder will be processed, shipped, & stored at the Hamilton Liquid nitrogen facility (Hamilton Health Sciences) for the future analysis, & the buffy coat for DNA extraction. (Table 6a) Insulin resistance will be measured by using the HOMA-IR a validated index which uses the fasting glucose & insulin values.[80]

5.2 Data Collection Stage 2: Delivery
At the time of delivery, details including birth outcomes for the mother & baby (type of delivery, Apgar scores, problems during delivery, length of stay) will be collected. A cord blood sample for basic biochemistry (i.e. glucose, insulin), DNA & additional serum & plasma aliquots for future analysis will be taken from each baby. (Table 6b) The method of cord blood collection is found in Appendix 1. A section of the placenta will be collected & stored in RNAlater - a solution which preserves the tissue RNA for future analysis. (Appendix 1) The newborn's physical characteristics- birth weight, triceps & sub-scapular skin fold thickness, length, abdominal, head, & arm circumference will be measured by a trained research assistant within 72 hours after delivery.

5.2.1 Assessment of Body Composition in Newborn & Infants:
Traditionally body fat mass in children has been measured by underwater weighing but this is not practical to perform in small children, as it requires exhaling & staying under water for several seconds. Recently, dual energy x-ray absorptiometry (DXA) has been employed to estimate whole body fat, lean & bone mass in infants & children. However DXA is associated with some radiation exposure & is difficult to perform in large epidemiologic studies. In infants % body fat can also be estimated by a prediction equation derived from four skinfold measures.[81] This method has been validated against DXA in newborns[82] & among children aged 4-10 years.(80) The correlation coefficient of equation-estimated % body fat in newborns compared to DXA is 0.92 & among children aged 4-10 years, 0.88.[82] The reliability of these estimates ranges from 99.5 to 99.8% .[83] More recently, Shaikh et al derived a similar formula in South Asian infants & preschoolers living in Kolkata, India. In cross-validation against D2O dilution (deuterium oxide), the equation-based estimates of % body fat showed a correlation of 0.98.[84] Additionally, comparing the South Asian equation[84] to the Slaughter equation produces virtually the same estimate of mean % body fat (15% and 14%, respectively). In START all newborns & infants will have skinfold thickness measured (triceps and subscapular) and mid arm circumference measured at birth & each annual visit.

5.3 Data Collection Stage 3: Follow-up after Delivery:
After delivery, mother & child dyad will be further followed by e-mail or telephone at 3 & 6 months to collect information on the infants weight & feeding practices, & in a face to face clinic visit at 1, 2, & 3 years after birth. We will offer use of a secured study website for participants to enter the baby's weight, length, & head circumference at 3 & 6 months. A detailed schedule outlining study visits & the information collected at each visit for mothers & babies is found in Table 3.

5.3.1 Assessment of growth & **body composition of the infant:**
Anthropometric measurements of the child will be made annually. Infants will be weighed to the nearest 10 g on a standard beam scale balance; length will be measured on an infantometer. Head, chest & mid upper arm circumference of the baby will be measured to the nearest 0.1 cm using a plastic measuring tape. Skinfold measurements will be measured to the nearest 0.2 mm, using skinfold calipers (Holtain, UK) for prediction of body composition. The detailed measures of growth & body composition are found in Table 7. All measures will be done by trained personnel, & inter-observer reliability testing will be conducted. Crown-heel length which will be measured using a regularly maintained & calibrated length board until 18 months of age, & height will be measured using a Harpenden stadiometer after 18 months of age. Weight will be measured with an electronic scale.

5.3.2 Infant Feeding Practices and Sleep and Activity Patterns:
Information on infant feeding practices and sleep will be collected at 3 & 6 months, & then annually by interviewing the mother of the infant/child. Diet information including initiation of breastfeeding, exclusivity of breastfeeding, duration of breast feeding, prelacteals & introduction of complementary foods will be collected. A validated Infant Feeding Form will be used and is a closed ended questionnaire with information about breastfeeding, other feeds and complementary feeds taken during last 7 days. At the final visit at 3 years, the mother will complete a 24 hour recall for the child. Sleep patterns are a measure of activity in the young infant and information will be collected by a validated infant sleep questionnaire, the Brief Infant Sleep questionnaire. An activity assessment in the growing child at each annual visit will be performed using a 24 hour activity recall developed & validated for use in young children. (Table 5)

5.4 Genetic Analysis: Mothers & newborn DNA will be extracted from the buffy coats. We will genotype a panel of 384 SNPs using Illumina's GoldenGate technology on a BeadXpress platform in Dr. Pare's laboratory at McMaster University. The SNPs will be chosen based on GWAS identified SNP associated with adiposity including fat mass, BMI, insulin resistance & type 2 diabetes. GoldenGate assays are based on hybridization of allele-specific oligonucleotides with subsequent amplification & detection using fluorescent signals. Standard quality control procedures will be applied to genotypes. Briefly, genotype completeness rate >90% will be required from every DNA sample while SNPs with call rate <95% will be excluded from analysis. SNPs with minor allele frequency < 1% will be excluded from analysis. Deviation from Hardy-Weinberg equilibrium in controls will be derived from an exact method[85]. Any sample with discordance between self-reported & genetic sex will also be excluded. RNA from leukocytes & placenta will be stored for future gene expression analysis.

6.0 Statistical Considerations:

Statistical Power: We estimate that 1,000 babies & mothers will provide substantial power to address the main objectives of this study with high precision. The primary outcome of the study is newborn adiposity measured by skin fold thickness from 4 locations (triceps, biceps, subscapular, and suprailiac). **For Objective 1** comparing body fat percentage in South Asian newborns to white Caucasian newborns from the FAMILY study we will calculate body fat from the skin fold thickness measures, & after

adjusting for difference in gestational age, we will compare the % body fat per kg of birth weight. Normalizing body composition measurements (eg, skinfold thickness & body fat) by birth weight has been used as a standard approach to make comparisons across groups of infants of varying body size, including across ethnic categories and sex.[86,87] The estimate of % body fat in the FAMILY study among white Caucasians (n=868) is 17% (SD:4.3%) or 5.1% (1.5%)/kg of birth weight. Therefore with 1,000 South Asian newborns, we will have > 80% power to detect an absolute difference in % body fat/kg birth weight of at least 0.20%, & we have similar high power to detect absolute differences in birth weight as low as 85 grams between South Asians & white Caucasians. (Table 8ab) For **Objectives 2 and 3:** For continuous predictors, with 1000 newborns we have >80% power to detect an absolute change in % body fat of 0.4 per 1 SD increase in a given predictor (i.e. maternal blood pressure) (two-tailed alpha=0.05), & we have >90% power to detect a change of 0.5 in % body fat. For categorical predictors (i.e. maternal gestational diabetes), with 1000 newborns we have >80% power to detect a difference in % body fat of 1.0% when at least 20% have the exposure of interest. There is also sufficient power to detect a difference of 1.1% body fat between top & bottom quartile group extremes (e.g., maternal glucose values or dietary factors). Similar high power is present to test postnatal factors against change in adiposity from birth to age 3 years. **Objective 4***:* For micronutrient associations with adiposity we used data for vitamin B12 & serum folate from SHARE. We have >80% power to detect an increase in % body fat of 0.2/ 250 pmol/L (2 SD) increase in vitamin B12 (two-tailed alpha=0.05), & 90% power to detect a difference in % body fat of 0.3. For folate (18.9 (9.5) nmol/L), we will have >80% power to detect an increase in % body fat of 0.2/19 nmol/L (2 SD) increase in folate, and 90% power to detect a difference in % body fat of 0.3**. Objective 5:** *Genetics:* We anticipate that the minor allele frequency (MAF) of SNPs of selected candidate genes will be similar to the MAFs we have observed in other South Asian cohorts. [88] Therefore with 1,000 babies of South Asian origin we will have high power to study associations of selected candidate SNPs with their respective quantitative traits. For example, with 1,000 babies we have more than 80% power to detect an additive genetic effect as low as 1.2% per allele change in body fat for MAFs as low as 20%, using a type I error threshold of 0.0005. For less common variants (MAF of 5-10%), we have sufficient power to detect an additive genetic effect as low as 2.2% per allele change in body fat. (Table 9) *While costs for epigenetics are precluded by the budget of this application, leukocytes & placental tissue will be collected & stored for future analysis.*

6.1 Statistical Analysis:

Descriptive statistics characterizing maternal & newborn characteristics will be generated. Continuous variables will be reported as means & SD for the normally distributed variables otherwise median & inter-quartile ranges will be reported. Categorical variables will be reported using percentages. Normality of the variables will be examined & appropriate transformations applied if required. The comparison of adiposity (% body fat) between South Asian & white Caucasian newborns will be adjusted for key covariates including gestational age, body weight, maternal glucose tolerance, weight gain in pregnancy, and SES. Linear regression will be used to assess the relationship between the micronutrient status with adiposity at birth. Multivariate regression analysis will be used to identify the determinants of adiposity at birth after adjusting for maternal factors & pregnancy factors. Changes in adiposity measurements over time will be compared by groups adjusting for confounders using linear mixed effects regression models. All analysis will be considered statistically significant at 5% level. As **an example** of the multiple sources of data we will accrue from this study a detailed statistical analysis plan is provided. We propose to study, the contributions of maternal, newborn, & post-natal factors to the offspring's adiposity at birth, & the change over time of body fat in the offspring. To do so we will study the effect of multiple types of data (i.e. maternal characteristics such as diet, hypertension, micronutrient status, maternal weight gain, presence of gestational diabetes), infant characteristics (gestational age, feeding type, amount, duration), certain contextual factors (i.e. socioeconomic status), & selected genetic variants (i.e. using a gene score of all known common genetic variants associated

with adiposity) on the outcome of adiposity, & change in body fat from birth until 3 years. Our goal will be to identify those determinants & their interactions, which predict change in adiposity as the child grows. A two-staged analytic plan will be used to accomplish this goal. **Stage 1: Examining the strongest influences (maternal & newborn characteristics) on adiposity:** First, the relationship within each group of determinants (maternal & newborn) & adiposity will be assessed using multi-level growth curve models.[89] **Measurement of adiposity over time for the same children will be modeled to show trajectories or slopes (linear or non-linear patterns) as a function of strongly associated & significant influences identified within each factor group (i.e. maternal, infant, genetic).** Potential covariates (i.e. contextual factors) will be examined to identify those that are highly correlated with other potential covariates; & only those which have an independent influence will be included. Covariates known to be associated with adiposity within each factor group will be included *a priori* & then other covariates will be identified using a backward elimination technique. Once a reasonable predictive model has been chosen, all excluded covariates will be added into the model one at a time to identify any missing confounders. We will also apply a stepwise regression approach to assess robustness of our modeling strategy. **Stage 2: Interactions of maternal, infant, & contextual determinants on adiposity:** Given that the best predictive model is identified within each group of factors (i.e. maternal, infant, childhood), the final model will include each of the significant determinants from each determinant grouping, & we will examine interactions between these determinants using the same analytic techniques as detailed above. For example, any identified biochemical marker (i.e. maternal fasting glucose) & the incremental contribution of maternal weight gain, & early childhood feeding practices to adiposity over time to age 3 years will be determined using growth curve models to determine main effects and interactions. All models will be validated internally using bootstrap re-sampling. Previous studies in which models are validated in independent data sets have shown that a fitted regression model is likely to be reliable when the number of independent predictors is less than the total sample size divided by 20.[89] This means that in the START cohort with 1000 offspring we have high power to test the effects of approximately 30 to 40 independent variables and interactions.

7.0 Possible Problems:

Given that there are over 4,000 South Asians birth per year in Peel Region, we are confident that the enrollment for the study can be met over the planned two year recruitment period. Our research group has considerable experience conducting large prospective epidemiologic studies & birth cohorts in Canada, and globally. We've recently completed the first phase of the FAMILY study, follow-up visits are ongoing & the attrition rate is low i.e. 10%. (Progress report) *Sample Selection:* Ideally START would include random selection of pregnant South Asian women from the Peel Region. However this would be extremely challenging as there is no comprehensive list of pregnant South Asian women in their first trimester. We will attempt to accrue a representative sample & have chosen 3 community-based hospitals (as opposed to tertiary care) which include South Asian women across a range of household incomes (i.e. lower SES in Brampton & higher SES in Credit Valley). Consecutive subjects will be approached & the main reasons for refusal to participate will be recorded. While we will try our best to achieve a representative sample, nevertheless the results of our associations between exposures & outcomes will be ***internally valid. The key factor to maintain high validity of a prospective cohort study is to minimize the lost to follow-ups***. To ensure this we will maintain frequent contact with study participants via telephone & our study website. Although much effort will be expended to maintain the cohort, we expect that a maximum of 5 – 10% of the families will not participate in all stages of the study, & by recruiting 1,000 mother-baby dyads into the study have ensured that we maintain our power.

8.0 Investigators:

The Co-Principal investigators of this proposal are Dr. Sonia Anand, Professor of Medicine & Epidemiology, McMaster University, & Dr. Milan Gupta, McMaster University who is a cardiologist, & Director of The Brampton Cardiology Research Group. **Co-Investigators from McMaster:** Dr. Koon Teo, Professor of Medicine & the PI of the FAMILY study. Dr. Sarah McDonald (Obstetrics), Dr. Katherine Morrison (Pediatrics), Dr. Guillaume Pare (Genetics), Dr. Andrew Mente (nutritional epidemiology), Dr. Joseph Beyene (Statistical Genetics and Biostatistics), Dr. James Dunn (Social determinants). Dr. Gita Wahi is a pediatrician and Clinical Scholar at McMaster who is working with Dr. Anand. **Additional co-Investigators include**: Dr. K. Srinivasan and Dr. Anura Kurpad from St John's Research Institute & St. John's Medical College, Bangalore, India are the leading the urban and rural India birth cohorts, Dr Dr. Joel Ray (Maternal-Fetal Medicine), Dr. Vidiya Persad (Maternal-Fetal Medicine), Dr. Jane Irvine (psychosocial determinants), Dr. Michaela Hynie (acculturation & social networks), Dr. Baiju Shah (health services research) & Dr. Ravi Retanakaran (endocrinology). The team assembled is multidisciplinary in nature & has been convened to address the full spectrum of the potential determinants of adiposity from genetics to social determinants. **Collaborators include**: **Dr. David Mowat**, Medical Officer of Health for the Peel Region who will be instrumental in promoting the study in Brampton, & assisting in knowledge translation of the study results within the Brampton region. **Dr. Padmaja Subbarao** from Sick Kids Hospital who is co-PI of the CIHR funded CHILD birth cohort, **Dr. Carol Wade** Chief of Obstetrics & **Dr. Ann Bayliss** Chief of Pediatrics, Credit Valley Hospital, **Dr. Gopal Bhatnagar** Chief of Staff, Trillium Hospital.

9.0 Anticipated Results & Conclusions:

There is an urgent need to understand maternal & child factors that underlie the early development of adiposity in South Asian children given the high risk metabolic profile experienced by South Asian adults in Canada. *This study is important because people of South Asian origin represent the fastest growing immigrant group in Canada, & they suffer high rates of early onset type 2 diabetes & coronary heart disease.* The information generated in START will enable the development of early screening and testing of intervention to prevent early adiposity in South Asian offspring and ultimately will be used to minimize the emerging epidemic of childhood obesity & adult onset type 2 diabetes among people of South Asian origin who live in Canada.

References

1. Sanderson, J.; Mayosi, B.; Yusuf, S.; Reddy, S.; Hu, S.; Chen, Z.; et al. Global burden of cardiovascular disease. *Heart* **2007,** *93*, 1175.

2. Diabetes Atlas Second Edition Executive Summary. http://www.eatlas.idf.org/ (accessed February 27).

3. Chadha, S.; Gopinath, N.; Sekhawat, S. Urban-rural differences in prevalence of coronary heart disease and its risk factors in Delhi. *WHO Bulletin* **1997,** *75*, 31-38.

4. Ethnocultural Portrait of Canada Highlight Tables, 2006 Census. http://www12.statcan.ca/english/census06/data/highlights/ethnic/index.cfm?Lang=E (accessed February 27).

5. Razak, F.; Anand, S.; Shannon, H.; Vuksan, V.; Davis, B.; Jacobs, R.; et al. Defining obesity cut-points in a multiethnic population. *Circulation* **2007,** *115* (2111-2118).

6. Yajnik, C. Nutrition, growth, and body size in relation to insulin resistance and type 2 diabetes. *Curr Diab Rep.* **2003,** *3* (2), 108-114.

7. Misra, A.; Vikram, N. Insulin resistance syndrome (metabolic syndrome) and obesity in Asian Indians: evidence and implications. *Nutrition* **2004,** *20*, 482-491.

8. Prabhakaran, D.; Anand, S. The metabolic syndrome: an emerging risk state for cardiovascular disease. . *Vasc Med* **2004,** *9*, 55-68.

9. Sniderman, A.; Bhopal, R.; Prabhakaran, D.; Sarrafzadegan, N.; Tchernof, A. Why might South Asians be so susceptible to central obesity and its atherogenic consequences? The adipose tissue overflow hypothesis. *Int J Epidemiol* **2007,** *36* (1), 226-227.

10. Barker, D.; Osmond, C.; Golding; Kuh, J.; Wadsworth, M. Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. *BMJ* **1989,** *289* (6673), 564-567.

11. van Steijn, L.; Karamali, N.; Kanhai, H.; Ariens, G.; Fall, C.; Yajnik, C.; et al. Neonatal anthropometry: thin-fat phenotype in fourth to fifth generation South Asian neonates in Suriram. *Int J Obes* **2009,** *33*, 1326-1329.

12. Yajnik, C.; Fall, C.; Coyaji, K.; Hirve, S.; Rao, S.; Barker, D.; et al. Neonatal anthropometry: the thin-fat Indian baby. The Pune Maternal Nutrition Study. *Ind J Obes Relat Metabol Disord* **2003,** *27* (2), 173-180.

13. Muthayya, S.; Dwarkanath, P.; Thomas, T.; Vaz, M.; Mhaskar, A.; Mhaskar, R.; et al. Anthropometry and body composition of south Indian babies at birth. *Public Health Nutr* **2006,** *9* (7), 896-903.

14. Ray, J.; Jiang, D.; Sgro, M.; Shah, R.; Singh, G.; Mamdani, M. Thresholds for small for gestational age among newborns of East Asian and South Asian ancestry. . *J Obstet Gynaecol Can.* **2009,** *31* (4), 322-330.

15. Mowat, D. Comprehensive Report on Health in Peel 2008. http://www.peelregion.ca/health/health-status-report/chsr/index.htm.

16. Anderson, P.; Butcher, K. Childhood obesity: trends and potential causes. *Future Child* **2006,** *16* (1), 19-45.

17. Ludwig, D. Childhood obesity--the shape of things to come. . *N Engl J Med* **2007,** *357* (23), 2325-2327.

18. Barker, D.; Gluckman, P.; Godfrey, K.; Harding, J.; Owens, J.; Robinson, J. Fetal nutrition and cardiovascular disease in adult life. *Lancet* **1993,** *341* (8850), 938-41.

19. Mcmillen, I.; Robinson, J. Developmental Origins of the Metabolic Syndrome: Prediction, Plasticity, and Programming *Physiol Rev* **2005,** *85*, 571-633.

20. Singhal, A.; Lucas, A. Early origins of cardiovascular disease: is there a unifying hypothesis? *Lancet* **2004,** *363* (9421), 1642-1645.

21. Hales, C.; Barker, D. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. . *Diaetologia* **1992,** *35*, 595-601.

22. de Rooij, S.; Painter, R.; Holleman, F.; Bossuyt, P.; Roseboom, T. The metabolic syndrome in adults prenatally exposed to the Dutch famine. *Am J Clin Nutr*. **2007,** *86* (4), 1219-24.

23. Li, Y.; He, Y.; Qi, L.; Jaddoe, V.; Feskens, E.; Yang, X.; et al. Exposure to the Chinese famine in early life and the risk of hyperglycemia and type 2 diabetes in adulthood. *Diabetes* **2010**.

24. Fetoui, H.; Garoui, M.; Zeghal, N. Protein restriction in pregnant- and lactating rats-induced oxidative stress and hypohomocysteinaemia in their offspring. *J Anim Physiol Anim Nutr (Berl).* **2009,** *93*, 263-270.

25. Chen, J.; Martin-Gronert, M.; Tarry-Adkins, J.; Ozanne, S. Maternal protein restriction affects postnatal growth and the expression of key proteins involved in lifespan regulation in mice. *Plos One* **2009,** *4* (3).

26. Yajnik, C. Fetal programming of diabetes: still so much to learn! *Diabetes Care* **2010,** *33* (5), 1115-21.

27. Yajnik, C.; Deshpande, S.; Jackson, A.; Refsum, H.; Rao, S.; Fisher, D.; et al. Vitamin B12 and folate concentrations during pregnancy and insulin resistance in the offspring: the Pune Maternal Nutrition Study. *Diaetologia* **2008,** *51* (1), 29-38.

28. Krishnaveni, G.; Hill, J.; Veena, S.; Bhat, D.; Wills, A.; Karat, C.; et al. Low plasma vitamin B(12) in pregnancy is associated with gestational 'diabesity' and later diabetes. *Diabetologia* **2009,** *52* (11), 2350-2358.

29. Anand, S.; Yusuf, S.; Vuksan, V.; Devanesen, S.; Teo, K.; Montague, P.; et al. Differences in risk factors, atherosclerosis, and cardiovascular disease between ethnic groups in Canada: the Study of Health Assessment and Risk in Ethnic groups (SHARE). . *Lancet* **2000,** *356*, 279-284.

30. Jensen, D.; Daum, P.; Sorenson, B.; Molsted-Pedersen, L.; Westergaard, J.; Oversen, P.; et al. Pregnancy outcome and prepregnanc body mass index in 2459 glucose-tolerant Danish women. *Am J Obstet Gynecol* **2003,** *189* (1), 239-244.

31. Heinrich, C.; McCulloch, A.; Archer, L.; Hansen, M. The association of prepregnancy Body Mass Index and Adverse Maternal and Perinatal outcomes. *Health Watch, Colorado Department of Public Health and Environment* **2006,** *69*.

32. Kanadys, W. Maternal underweight and pregnancy outcome:prospective cohort study. *Archives of Perinatal Medicine* **2007,** *13* (3), 23-26.

33. Borkowski, W.; Mielniczuk, H. The influence of social and health factors including pregnancy weight gain rate and pre-pregnancy body mass on low birth weight of the infant. *Ginekol Pol* **2008,** *79* (6), 415-421.

34. Nohr, E.; Vaeth, M.; Baker, J.; Sorenson, T.; Olsen, J.; Rasmussen, K. Combined associations of prepregnancy body mass index and gestational weight gain with the outcome of pregnancy. *Am J Clin Nutr* **2008,** *87* (6), 1750-1759.

35. Oken, E.; Taveras, E.; Klienman, K.; Rich-Edwards, J.; Gillman, M. Gestational weight gain and child adiposity at age 3 years. *Am J Obstet Gynecol* **2007,** *196* (4(322)), e 1-8.

36. McDonald, S.; Han, Z.; Mulla, S.; Beyene, J. Overweight and obesity in mothers and risk of preterm birth and low birth weight infants: systematic review and meta-analyses. *BMJ* **2010,** *341*.

37. Ludwig, D.; Currie, J. The association between pregnancy weight gain and birthweight: a within-family comparison. *Lancet* **2010**.

38. Huang, J.; Lee, T.; Lu, M. Prenatal Programming of Childhood Weight and Obesity. *Maternal and Child Health Journal* **2007,** *11* (5), 461-473.

39. Oken, E.; Gillman, M. Fetal Origins of Obesity. *Obes Res* **2003,** *11* (496-506).

40. Gillman, M.; Rifas-Shinman, S.; Berkey, C.; Field, A.; Colditz, G. Maternal Gestational Diabetes, Birthweight and Adolescent Obesity. *Pediatrics* **2003,** *111*, e221-e226.

41. Wroblewska-Seniuk, K.; Wender-Ozegowska, E.; Szczapa, J. Long-term effects of diabetes during pregnancy of the offspring. *J Pediatr Diabetes* **2009,** *10* (7), 432-440.

42. Catalano, P.; Kirwan, J.; Haugel-de Mouzon, S.; King, J. Gestational diabetes and insulin resistance: role in short- and long-term implications for mother and fetus. *J Nutr* **2003,** *133*, 1674S-83S.

43. Hamilton, J.; Odrobina, E.; Hanley, A.; Zinman, B.; Retnakaran, R. Effect of maternal insulin sensitivity during pregnancy on infant weight gain and adiposity. *Obesity* **2010,** *18*, 340-346.

44. Retnakaran, R.; Hanle, A.; Connelly, P.; Sermer, M.; Zinman, B. Ethnicity modifies the impact of obesity on insulin resistance in pregnancy: a comparison of Asian, South Asian and Caucasian women. *Journal of Clinical Endocrinology and Metabolism* **2006,** *91*, 93-97.

45. Toschke, A.; Vignerova, J.; Lhotska, L.; Osancova, K.; Koletzko, B.; von Kries, R. Overweight and obesity in 6-14 year old Czech children in 1991: protective effect of breast-feeding. *J Pediatr* **2002,** *141*, 764-769.

46. Bergmann, K.; Bergmann, R.; Von Kries, R.; Bohm, O.; Richter, R.; Dudenhausen, J.; et al. Early determinants of childhood overweight and adiposity in a birth cohort study: role of breast feeding. *Int J Obes Relat Metabol Disord* **2003**, 162-172.

47. Arenz, S.; Von Kries, R. Protective effect of breast-feeding against obesity in childhood. Can a meta-analysis of observational studies help to validate the hypothesis? *Adv Exp Med Biol* **2005,** *569*, 40-48.

48. Dewey, K. Is breast feeding protective against child obesity? *J Hum Lact* **2003,** *19*, 9-18.

49. Campbell, K.; Hesketh, K.; Crawford, D.; Salmon, J.; Ball, B.; Mccallum, Z. The Infant Feeding Activity and Nutrition Trial (INFANT) an early intervention to prevent childhood obesity: Cluster-randomised controlled trial. *BMC Public Health* **2008**.

50. Ong, K.; Dunger, D. Birth weight, infant growth and insulin resistance. *Eur J Endocrinol* **2001,** *151* (Suppl 3), U131-139.

51. Ong, K.; Loos, R. Rapid infancy weight gain and subsequent obesity: systematic review and hopeful suggestions. *Acta Pediatr* **2006,** *95*, 904-908.

52. Harder, T.; Bergmann, R.; Kallischnigg, G.; Plagemann, A. Duration of breastfeeding and risk of overweight: a meta-analysis. *Am J Epidemeol* **2005,** *162* (5), 397-403.

53. Fewtrell, M.; Morley, R.; Abbott, R. Catch up growth in small for gestational age term infants: a randomized trial. *AJCN* **2001,** *74*, 516-523.

54. Owen, C.; Martin, R.; Whincup, P.; Smith, G. Effect of Infant feeding on the risk of obesity across the life course: A Quantitative Review of Published Evidence. *Pediatrics* **2005,** *115*, 1367-1377.

55. Ekelund, U.; Ong, K.; Linne, Y.; Neovius, M.; Brage, S.; Dunger, D.; et al. Upward weight percentile crossing in infancy and early childhood independently predicts fat mass in young adults: the Stockholm Weight Development Study (SWEDES). *Int J Obes* **2006,** *29* (1192-1198).

56. Wells, J.; Hallal, P.; Wright, A.; Singhal, A.; Victoria, C. Fetal, infant and Childhood Growth: relationships with body composition in Brazilian boys aged 9 years. *Int J Obes* **2005,** *29* (1192-1198).

57. Ong, K.; Ahmed, M.; Emmett, P.; Preece, M.; Dunger, D. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. *BMJ.* **2000,** *320* (7240).

58. Knip, M.; Åkerblom, H. Early nutrition and later diabetes risk. *Adv Exp Med Biol* **2005,** *569*, 142-150.

59. Ong, K.; Emmett, P.; Noble, S. Dietary energy intake at the age of 4 months predicts postnatal weight gain and childhood body mass index. *Pediatrics* **2006,** *115* (1367-1377).

60. Miralles, O.; Sanchez, J.; Palon, A.; Pico, C. A physiological role of breast milk leptin in body weight control in developing infants. *Obesity* **2006,** *14*, 1371-1377.

61. Taveras, E.; Rifas-Shinman, S.; Oken, E. Short sleep duration in infancy and risk of childhood overweight. *Arch Pediatr Adolesc Med* **2008,** *162*, 305-311.

62. Hasselbalch, A.; Benyamin, B.; Visscher, P.; Heitmann, B.; Kyvik, K.; Sørensen, T. Common Genetic Components of Obesity Traits and Serum Leptin. *Obesity* **2008,** *16*, 2723-2729.

63. Lee, J.; Chen, L.; Snieder, H.; Chen, D. F. Heritability of obesity-related phenotypes and association with adiponectin gene polymorphisms in the Chinese national twin registry. *Ann Hum Genet.* **2010,** *74*, 146-154.

64. O'Rahilly, S. Human genetics illuminates the paths to metabolic disease. *Nature* **2009**, 307-314.

65. Pomeroy, J.; Soderberg, A.; Franks, P. Gene-lifestyle interactions and their consequences on human health. *Med Sport Sci.* **2009,** *54*, 110-35.

66. Elks, C.; Loos, R.; Sharp, S.; Langenberg, C.; Ring, S.; Timpson, N.; et al. Genetic markers of adult obesity risk are associated with greater early infancy weight gain and growth. *PLoS Med.* **2010,** *7* (5).

67. Freathy, R.; Weedon, M.; Bennett, A.; Hypponen, E.; Relton, C.; Knight, B.; et al. Type 2 diabetes TCF7L2 risk genotypes alter birth weight: a study of 24,053 individuals. *Am J Hum Genet.* **2007,** *80* (6).

68. Hattersley, A.; Tooke, J. The fetal insulin hypothesis: an alternative explanation of the association of low birthweight with diabetes and vascular disease. *Lancet* **1999,** *353* (9166).

69. Tappy, L. Adiposity in children born small for gestational age. *International Journal of Obesity* **2006,** *30*, S36-40.

70. House, J.; Landis, K.; Umberson, D. Social relationships and health. *Science* **1988,** *241*, 540-545.

71. Organista, P.; Organista, K.; Kurasaki, K. The relationship between acculturation and ethnic minority mental health. In *Acculturation: Advances in Theory, Management and Applied Research*, Chun, K.; Organista, P.; Marin, G., Eds. American Psychological Association: Washington, DC, 2003; pp 139-161.

72. Morrison, K.; Atkinson, S.; Yusuf, S.; Bourgeois, J.; McDonald, S.; McQueen, M; et al. The Family Atherosclerosis Monitoring In EarLY Life (FAMILY) Study: Rationale, design and baseline data of a study examining the early determinants of atherosclerosis. . *Am Heart Jour* **2009,** *158*, 533-539.

73. Anand, S.; Razak, F.; Davis, A.; Jacobs, R.; Vuksan, V.; Teo, K.; et al. Social disadvantage and cardiovascular disease: development of an index and analysis of age, sex, and ethnicity effects. *Int J Epidemiol* **2006,** *35*, 1239-45.

74. Durnin, J.; Womersley, J. Estimates of total body fat from skinfold thickness: measurements on 481 men and women aged from 16-72 years. *Br J Nutr* **1974,** *32*, 77-79.

75. Retnakaran, R.; Hanley, A.; Raif, N.; Connelly, P.; Sermer, M.; Zinman, B. Hypoadiponectinaemia in South Asian women during pregnancy: evidence of ethnic variation in adiponectin concentration. *Diabet Med* **2004,** *21* (4), 388-92.

76. Kelemen, L.; Anand, S.; Vuksan, V.; Yi, Q.; Teo, K.; Devanesen, S.; et al. Development and evaluation of cultural food frequency questionnaires for South Asians, Chinese, and Europeans in North America. *J Am Diet Assoc* **2003,** *103* (9), 1178-84.

77. Malda, M.; van de Vijver, F.; Srinivasan, K.; Transler, C.; Sukumar, P.; Rao, K. Adapting a cognitive test for a different cultures: an illustration of qualitative procedures. *Psychol Sci Quarterly* **2008,** *50*, 451-68.

78. Asvat, Y.; Malcarne, V. Acculturation and depressive symptoms in Muslim University students: Personal family acculturation match. *International Journal of Psychology* **2008,** *43* (2), 114-124.

79. Ryder, A.; Alden, L.; Paulhus, D. Is acculturation unidimensional or bidemnsional? A head to head comparison in the prediction of personality, self-identity and adjusment. *Journal of Personality and Social Psychology* **2000,** *79*, 49-65.

80. Vogeser, M.; König, D.; Frey, I.; Predel, H.; Parhofer, K.; Berg, A. Fasting serum insulin and the homeostasis model of insulin resistance (HOMA-IR) in the monitoring of lifestyle interventions in obese persons. *Clin Biochem* **2007,** *40* (13).

81. Slaughter, M.; Lohman, T.; Boileau, R.; Horswill, C.; Stillman, R.; Van Loan, M.; et al. Skinfold equations for estimation of body fatness in children and youth. *Hum Biol* **1988,** *60* (5), 709-23.

82. Schmelzle, H.; Fusch, C. Body fat in neonates and young infants: validation of skinfold thickness versus dual-energy X-ray absorptiometry. *Am J Clin Nutr* **2002,** *76* (5), 1096-1100.

83. Moreno, L.; Rodríguez, G.; Guillén, J.; Rabanaque, M.; León, J.; Ariño, A. Anthropometric measurements in both sides of the body in the assessment of nutritional status in prepubertal children. *Eur J Clin Nutr* **2002,** *56* (12), 1208-15.

84. Shaikh, S.; Mahalanabis, D. Empirically derived new equations for calculating body fat percentage based on skinfold thickness and midarm circumference in preschool Indian children. *Am J Hum Biol.* **2004,** *16* (3), 278-88.

85. Wigginton, J. E.; Cutler, D. J.; Abecasis, G. R. A note on exact tests of Hardy-Weinberg equilibrium. *Am J Hum Genet* **2005,** *76* (5), 887-93.

86. Rodríguez, G.; Samper, M.; Ventura, P.; Moreno, L.; Olivares, J.; Pérez-González, J. Gender differences in newborn subcutaneous fat distribution. *Eur J Pediatr.* **2004,** *163* (8), 457-61.

87. Oldroyd, J.; Heald, A.; Bansal, N.; Vyas, A.; Siddals, K.; Gibson, M.; et al. Inflammatory markers and growth in South Asian and European origin infants in Britain: the Manchester Children's Growth and Vascular Health Study. *Atherosclerosis* **2009,** *207* (1), 227-31.

88. Anand, S.; Xie, C.; Paré, G.; Montpetit, A.; Rangarajan, A.; McQueen, M.; et al. Genetic Variants Associated With Myocardial Infarction Risk Factors in Over 8000 Individuals From Five Ethnic Groups. *Circulation: Cardiovascular Genetics.* **2008,** *2*, 16-25.

89. Cohen, J.; Cohen, P., Applied Multiple Regression/Correlation Analysis for the Behavioral Sciences. 1983.

Tables and Figures:

Table 1A: Baseline characteristics of mothers in Family Study

	Baseline (during pregnancy) N=859
Demographics	
Age (yrs), mean (SD)	32.1 (5.2)
Ethnicity (%)	
European	84.4
Non-European	15.6
Education (%)	
0 -13 yrs	16.2
>13 yrs	83.8
Household income <$50,000 (%)	23.2
First child (%)	55.9
Smoking history	
Smoker, baseline visit (%)	6.3
Smoker, last year (%)	33.1
Never smoked (%)	60.6
CV Risk Factors (before pregnancy)	
Hypertension (%)	3.0
Abnormal cholesterol levels (%)	4.8
Diabetes (%)	4.3
Heart attack/angina/stroke (%)	0.4

Table 1B: Physical and Laboratory Measurements for Babies at Birth and Years 1,2, 3, 5 in the FAMILY Study

Measurements	At Birth N=865*	Year 1 N=755	Year 2 N=648	Year 3 N=512	Year 5 N=157
Physical Measurements – Mean (SD)					
Height (cm)	49.6 (3.0)	75.8 (3.3)	88.2 (3.4)	96.1 (3.8)	111.7 (3.9)
Weight (kg)	3.36 (0.65)	10.1 (1.3)	12.8 (1.5)	14.9 (1.8)	19.5 (2.2)
Waist circumference (cm)	29.5 (2.9)	44.4 (3.2)	47.2 (3.1)	47.9 (3.1)	50.9 (3.0)
Hip circumference (cm)	27.6 (3.0)	44.9 (3.3)	48.7 (3.3)	51.2 (3.0)	57.7 (3.3)
Waist to hip ratio	1.07 (0.08)	0.99 (0.07)	0.97 (0.06)	0.93 (0.04)	0.88 (0.03)
Tricep Skinfold Thickness	5.30 (1.44)	11.78 (2.86)	11.73 (2.84)	12.17 (2.98)	12.88 (3.57)
Subscapular Skinfold Thickness	5.03 (1.46)	7.38 (2.21)	6.84 (2.11)	7.00 (2.48)	7.08 (2.84)
Systolic BP (mm Hg)**	69.4 (9.6)	96.9 (11.3)	97.6 (9.3)	96.7 (9.0)	99.8 (6.1)
Diastolic BP (mm Hg)**	39.1 (7.9)	60.1 (7.2)	60.8 (6.2)	60.1 (5.8)	61.5 (4.7)
Laboratory Measurements – Mean (SD)					
Glucose (mmol/L)	4.26 (1.0)	-	-	4.50 (0.4)	

*Total n = 901; Physical measurements and cord blood samples were not available for 36 babies due to various reasons.

Table 2: Inclusion and Exclusion Criteria for START

Inclusion Criteria	Exclusion Criteria
Women Age: 18-40 years South Asian Origin Pregnant	Expected Multiple Births Artificial/Assisted Conception of Fetus > 4 Live Births* Surrogate Mothers Chronic Medical Conditions - Active Cancer - HIV - Hepatitis B or C - VDRL Positive - Rheumatic Heart Disease - Seizure Disorder on Medication - Living in Canada < 9 months - Father of the baby is not of South Asian origin

Table 3: Proposed Measures and Timing of Measure in START

Measures	Antenatal Visit	Birth Visit	6 months post Delivery*	1 year Visit	2 Year Visit	3 year Visit
Demographics -	X			X	X	X
Age	X			X	X	X
HCN	X			X	X	X
Address/Postal Code	X			X	X	X
Family Doctor - Info	X			X	X	X
Midwife/ObGyn Info	X					
Expected Delivery Date	X					
Medical and Obstetric History	X					
Diabetes	X					X
Increased blood pressure	X					X
Increased cholesterol	X					X
Other Major Medical Hx - Checklist	X					X
Family History	X					
Medications Used	X					X
Past Pregnancy Info	X					
GTPAL	X					
Live Births	X					
Still Births	X					
Abortions	X					
Past Gest. DM	X					
Pre-Eclampsia	X					
Low Birth Weight	X					
Premature Delivery	X					
Multivitamin Use	X					
Social Determinants	X					
Self reported Ethnicity - for self, parents and grandparents	X					
Mother tongue/Language read	X					
Years in Canada	X					
Place Immigrated from	X					
Place of Birth	X					
Religious Practices	X					
Annual Household Income	X					X
Occupation	X					X
Marital Status	X					X
Education	X					X
Social Support	X			X	X	X
Acculturation Stress	X					X
Depression	X			X	X	X
Diet	X		X	X	X	X
Physical Activity	X		X	X	X	X
Physical Exam	X			X	X	X
Blood Pressure	X	X		X	X	X
Height/Length	X	X	X	X	X	X
Weight	X	X	X	X	X	X
Waist and Hip Circumference	X	X		X	X	X
Skin Folds	X	X		X	X	X
Head Circumference (baby)	X	X	X	X	X	X
Fetal Ultrasound	X					
Blood Analysis	X					
Hemoglobin	X					
Fasting Glucose	X	X				
75 g OGTT	X					
Insulin	X	X				
Vit B12, RBC Folate	X					
Aliquots for Future Analysis	X*	X**				
DNA/Long-term Storage	X	X	X	X	X	X
Birth Visit						
Type of Delivery		X				
Duration of Labour		X				
Premature Labour		X				
Blood Loss		X				
Birth Weight		X				
APGAR scores (1 and 5min)		X				
Adverse outcomes		X				
Placenta & Cord Blood		X				

Table 4: Dietary Measures:

Domains	Canada	Characteristics (Validity and reliability coefficients)	Time
Maternal Diet during Pregnancy	SHARE- FFQ* + 24 hr recall**	Validity: tested against 14 day dietary record. Energy adjusted de-attenuated Pearson correlation coefficients ranged from 0.32 to 0.83 Reliability: between FFQ1 and FFQ2: Energy adjusted interclass correlation coefficients ranged from 0.38 to 0.88 Use multiple pass 24 hour recall method developed by USDA for dietary recall.	25 min
Mothers Diet during breastfeeding	24 hr recall** 6 month phone visit	Use multiple pass 24 hour recall method developed by USDA for dietary recall.	15 min
Mothers Diet 1 year after weaning	24 hr recall**	As Above	15 min
Mothers Diet at Final study visit	SHARE FFQ + 24 hr recall	As Above	15 min
Infant Diet at 1, 6, 12 months of life	Detailed information on feeding pattern ***	Detailed information on feeding pattern including: 1. breast vs. formula 2. early introduction of non-milk liquids 3. introduction of solids, snacks, confectionaries 4. age of introduction 5. frequency of consumption Not used for nutrient intake estimates	10 min
Child diet at 2,3 year visit	24 hour recall	Mother or caregiver will answer questions regarding child's food intake in prior 24 hours	10 min
Antenatal Dietary Supplements	Collected via self report		<1 min

*Kelemen L, Anand S, Vuksan V, et al. Development and evaluation of cultural food frequency questionnaires for South Asians, Chinese, and Europeans in North America. Journal of the American Dietetic Association 103(9):1178-1184; 2003.
**Guenther PM, DeMaio TJ, Ingwersen LA, Berline M. The multiple-pass approach for the 24-hour recall in the Continuing Survey of Food Intakes by Individuals (CSFII) 1994-1996. Presented at the International Conference on Dietary Assessment Methods, January 1995; Boston, Mass.
***Ness A. The Avon Longitudinal Study of Parents and Children (ALSPAC) – a resource for the study of the environmental determinants of childhood obesity. European Journal of Endocrinology 115:U141-U149; 2004.

Table 5 : Psychosocial Measures

Domains	Instrument	Characteristics (Validity and reliability coefficients)	Time
Maternal Depression	K-10^	1. Good precision in the 90th-99th percentile range of population distribution. 2. Strongly discriminates between community cases and non-cases of DSM-IV/SCID disorders 3. Validated for postnatal depression assessment^^	10 min
Socio-Economic Status	Education, Occupation, Income, Household income, Marital status	SHARE Social Disadvantage Index**	5 min
Social Support	Number of Family members in the household Ability to call people in during time of need	Instrument measures emotional, instrumental, informational, and appraisal components of social support St. John's Research Institute, India Questionnaire***	5 min
Acculturation Stress	V.I.A.****	VIA: Vancouver Index of Acculturation population in Canada	8 min

^Kessler R, Andrews G, Colpe L, et al. Short screening scales to monitor population prevlence and trends in non-specific psychological distress. Psychological Medicine 32:959-976; 2002.
**Anand S, et al. Social disadvantage and cardiovascular disease: development of an index and analysis of age, sex and ethnicity effects. International Journal of Epidemiology 35:1239-1245; 2006.

*** Malda, M.; van de Vijver, F.; Srinivasan, K.; Transler, C.; Sukumar, P.; Rao, K., Adapting a cognitive test for a different cultures: an illustration of qualitative procedures. *Psychol Sci Quarterly* **2008,** *50*, 451-68.
****Ryder A et al. Is acculturation unidimensional or bidimensional? A head-to-head comparison in the prediction

Table 6A: Laboratory Measurements:

Blood Measurement	Method of Analysis	Method of Storage
Maternal:		
Fasting Glucose	The Becton Dickenson Unicell DxC 600 Synchron Clinical System using a timed endpoint method to determine glucose.	Local Analysis
1+ 2 hr post 75 grams Glucose	The Becton Dickenson Unicell DxC 600 Synchron Clinical System using a timed endpoint method to determine glucose.	Local Analysis
CBC	Beckman Coulter LH750	Local Analysis
Vitamin B12	Roche® E-170 Folate II method is based on the Competition Principle using chemiluminescent detection	Central Analysis
RBC Folate	Roche® E-170 Folate II method is based on the Competition Principle using chemiluminescent detection.	Central Analysis
Insulin fasting	Serum Insulin is measured on the Roche Elecsys® 2010 immunoassay analyzer using a electrochemiluminescence immunoassay. All reagents purchased from Roche Diagnostics GmbH, Indianapolis, IN,USA	Central Analysis
Homocysteine	Immulite Homocysteine is a solid-phase, competitive chemiluminescent enzyme immunoassay performed on the Siemens® Immulite System	Storage
Adiponectin	Adiponectin is measured using a manual qualitative sandwich immunoassay technique (ELISA) kit manufactured by R& D systems Inc. USA.	Storage
Leptin	Leptin is measured using a manual qualitative sandwich immunoassay technique (ELISA) kit manufactured by R& D systems Inc. USA.	Storage
Buffy Coat	Extraction of DNA using Autopure	Central Extraction and Genotyping
Baby cord blood glucose, insulin buffy coat	As above	Central Analysis

Table 6B: Proposed Biospecimen Collection Protocol:

Source	Visit	Tube	Frozen Aliquots	Analytes	Local Testing
Adult	Fasting	1 x 10 mL Red Clot	3 x 1.5 mL Serum	Glucose, B12, insulin, adiponectin	-
		1 x 6mL Lav EDTA	1 x 1 mL Whole Blood*	RBC Folate	1 x 3mL Lav EDTA for CBC
			Buffy Coat	DNA	-
		1 x 6mL Lav EDTA **ON ICE**	2 x 1.5 mL EDTA Plasma	Homocysteine	-
		PaxGene (2.5 mL blood for consent; total volume with additive is 10 mL)	Entire tube**	RNA	-
	1 hr***	1 x 4mLRed Clot	1 x 1.8 mL serum	Glucose, insulin	-
	2 hr***	1 x 4mL Red Clot	1 x 1.8 mL serum	Glucose, insulin	-
Cord Blood	Birth	1 x 10mL Red Clot	2 x 1.5 mL serum	Glucose	-
		1 x 6 mL Lav EDTA	Buffy Coat	DNA	-

* remove BEFORE centrifugation, RBC Folate may be stored at -20 Celcius prior to Central analysis
** Frozen PaxGene tubes will be shipped as per standardized protocol used by the central lab in other projects.
***after consumption of 75 g Glucose load

Table 7: Growth and Body Composition:

Subject	Measurement	Description
Mother	Blood Pressure (systolic and diastolic)	Participant must be resting for > 5 minutes, and should not have smoked for at least 30 minutes before this measurement. Using oscillometric model blood pressure machines (Omron Model HEM-757 for adults). Ensure adequate cuff size. Bladder should encircle and cover 2/3 of length of arm with the bladder over the brachial artery. Its lower border should be 1 inch (2-3 cm) above the antecubital space. Take two readings on the right arm, at least 5 minutes apart, and record exact values.
	Weight	Weight is measured in light clothing using an electronic scale, to the nearest 200 grams. The scales must be standardized to 0 before each use.
	Height	Standing height is measured with the subject in bare feet, back square against the wall and eyes looking straight ahead. A Harpenden stadiometer is used to measure height to the nearest 0.5 cm. Bring the horizontal board down slowly to rest on top of the participants' head. Repeat procedure and record both readings.
	Waist	Waist circumference is measured to the nearest 0.1 cm using a non-stretchable standard tape measure attached to a spring balance exerting a force of 750 gm.* Take measurement over the unclothed abdomen at the smallest diameter between the costal margin and the iliac crest. The tape measure must be kept horizontal. Subject should relax with arms held loosely at sides. Record 2 measurements.
	Hip	Hip circumference is measured to the nearest 0.1 cm using a non-stretchable standard tape measure attached to a spring balance exerting a force of 750 gm. Take measurement over light clothing at the level of the greater trochanters (usually the widest diameter around the buttocks). The tape measure must be kept horizontal. Record 2 measurements.
	Skin fold thickness (Subscapular, triceps)	Skinfold thickness will be measured using skinfold calipers (Holtain, Crymmych, UK) to the nearest 0.2 mm by trained study personnel using standardized protocol. **Triceps skin fold thickness** is measured at the midline of the posterior aspect of the right arm, over the triceps muscle, at the mid-point of the arm identified with elbow flexed 90 degrees. Elevate the fold of the skin. Make sure to separate the fat from the muscle. Apply the calipers with the right hand approximately 1 cm above the pinch. Ensure that the pincers of the caliper are placed so that the thickness of the skinfold is measured perpendicular to the long axis of the arm. **Subscapular skin fold thickness** is measured with subject is standing tall with arms comfortably hanging at the side. Palpate the scapula by running your fingers inferiorly and laterally along its vertebral border until the inferior angle is identified. Pick up the skinfold with the left thumb and index finger such that the fold is slightly inferior to the angle of the scapula. Apply the caliper 1 cm infero-lateral to the raised fold. (As per NHANES study procedures)
	Body Fat	Percent Body Fat is measured in light clothing, with the participant standing bare feet on the Tanita body fat scale. The scales will be used by trained study personnel.
Baby at birth	Weight	Baby's weight at birth will be taken from the hospital chart.
	Blood pressure	Using oscillometric model blood pressure machines, Dinamap for neonates/children, blood pressure should be measured when the baby is sleeping or feeding. Ensure adequate cuff size. Bladder should encircle and cover 2/3 of length of arm with the bladder over the brachial artery. Its lower border should be 1 inch (2-3 cm) above the antecubital space. Take two readings on the right arm, at least 5 minutes apart, and record exact values.
	Length	Length is measured in infants < 18 months of age using the infantometer. Length is measured twice to the nearest 0.1 cm.
	Head, chest and mid upper arm circumference	Head, chest and mid upper arm circumference will be measured using non-stretchable measuring tape to the nearest 0.1 cm by trained study personnel using standardized protocol.
	Skinfold thickness	Skinfold thickness will be measured using skinfold calipers (Holtain, Crymmych, UK) to the nearest 0.2 mm by trained study personnel using standardized protocol. Four sites tricep, bicep, subscapular, suprailiac will be measured using the standardized protocol used by Slaughter et al *
Infant/Child > Annually	Length/Height	Length (0-24 month) – O'Leary Pediatric Length Board (Ellard Instrumentation) Height (>24 month) – Stadiometer (Harpenden)
	Weight	Weight is measured in light clothing using an electronic scale, to the nearest 200 grams. The scales must be standardized to 0 before each use.
	Blood pressure	Using oscillometric model blood pressure machines, Dinamap for neonates/children, blood pressure should be measured when the baby is sleeping or feeding. Ensure adequate cuff size. Bladder should encircle and cover 2/3 of length of arm with the bladder over the brachial artery. Its lower border should be 1 inch (2-3 cm) above the antecubital space. Take two readings on the right arm, at least 5 minutes apart, and record exact values.
	Skinfold thickness	Skinfold thickness will be measured using skinfold calipers (Holtain, Crymmych, UK) to the nearest 0.2 mm by trained study personnel using standardized protocol of Slaughter et al. *

***** Slaughter MH, Lohman TG, Boileau RA. Hum Biol 1988;60:709-23

Table 8A: Power to detect Difference in Body Fat %/kg birth weight comparing South Asians to White Caucasians

Difference in % body fat per kg birth weight	Absolute difference in % body fat	Power (1 – B)
0.15%	0.51%	0.57
0.20%	0.68%	0.81
0.228%	0.77%	0.90
0.25%	0.84%	0.95
0.30%	1.01%	0.99
0.40%	1.35%	>0.99
0.50%	1.69%	>0.99
0.60%	2.03%	>0.99

* Assumptions: N1=868 Caucasian newborns; N2=1000 South Asian newborns; Mean (SD) percent body fat per kg birth weight in Caucasians = 5.09 (1.5); Mean (SD) estimates obtained after applying calculation formula to data from the FAMILY study in Caucasians using skinfold measures at four sites.
Note: In an adolescent cohort differences of % body fat of 0.60% body fat (Equivalent to 0.20%/kg birth weight) in early life are increases in insulin resistance (i.e. 0.6 units HOMA) which is equivalent to a 15% statistically significant increase in risk of incident diabetes after 10 years . We have high power to detect such differences between South Asian and White Caucasian babies

Table 8B: Power to detect Difference in Birth weight (grams) comparing South Asians to White Caucasians

Difference in birth weight (g)	Relative difference in birth weight (%)	Power (1 – B)
80	2.4%	0.77
85	2.5%	0.82
90	2.7%	0.86
95	2.8%	0.89
100	3.0%	0.92
105	3.1%	0.94
110	3.3%	0.96
120	3.6%	0.98
130	3.8%	0.99
140	4.1%	>0.99

* Assumptions: N1=868 Caucasian newborns; N2=1000 South Asian newborns;
Mean (SD) birth weight in Caucasians = 3379 (640);
Mean (SD) estimates obtained using data from the FAMILY study in Caucasians.

Table 9: Genetic Power Calculation of gene variant effects on percent body fat (continuous outcome variable) by minor allele frequency.

MAF	Per Allele Change in % body fat = 1.2	Per Allele Change in % body fat = 1.4	Per Allele Change in % body fat = 1.6	Per Allele Change in % body fat = 1.8	Per Allele Change in % body fat = 2.0	Per Allele Change in % body fat= = 2.2)
5%	0.15	0.26	0.41	0.57	0.72	0.84
10%	0.45	0.67	0.84	0.94	0.98	0.99
15%	0.70	0.89	0.97	0.995	0.99	>0.99
20%	0.84	0.96	0.99	>0.99	>0.99	>0.99
25%	0.92	0.99	0.999	>0.99	>0.99	>0.99
30%	0.95	0.99	>0.99	>0.99	>0.99	>0.99
35%	0.97	0.998	>0.99	>0.99	>0.99	>0.99
40%	0.98	0.999	>0.99	>0.99	>0.99	>0.99
45%	0.98	0.999	>0.99	>0.99	>0.99	>0.99

Assumptions: N=1000 South Asian babies; 2-sided alpha = 0.0005;Inheritance mode = additive; Mean % body fat = 17% (using estimates obtained after applying calculation formula to data from the FAMILY study in Caucasians using skinfold measures at four sites); Standard deviation of % body fat based on data from the FAMILY study in Caucasians.

Appendix 1: Labour and Delivery Room Specimen Collection Protocol for Cord Blood:

1. At admission of the patient (pregnant woman in labour), L&D nurse sees that the pre-registration chart is flagged re: the patient's participation in the START study.
2. Then, the L&D nurse selects the corresponding patient plastic sheet and a bucket, which contains one START Study Birth Package-Cord Blood Baby.
3. The obstetrician or the midwife cuts the cord and collects cord blood into the 60cc syringe found in study collection package. Record date and time of cord blood draw on requisitions, as well as baby's gender and DOB.
4. L&D nurse takes the syringe of cord blood and gives it to START team member to put into the tubes (if he or she is present); otherwise L&D nurse should aliquot blood in the following order of importance: Lavender 6 ml, red 10ml.
5. The L&D nurse affixes labels on the appropriate tubes.
6. Business clerk notifies START team to pick up the blood samples.
7. Take the tubes and place them in Labour and Delivery fridge if the START team member is not available. Otherwise the START person will send appropriate samples.
8. Either the L&D nurse or START team member affixes the placenta labels on the placenta bucket.
9. Either the L&D nurse or START team member attaches remaining placenta label on pathology requisition and completes requisition form.
10. If START team member is not available, put the placenta in Labour and Delivery Fridge.
11. Within 30 minutes of placenta being delivered, the placenta must be stabilized in RNAlater
12. The placenta must be washed thoroughly with ice-cold distilled water that is RNAase and DNAase free to make it completely blood/mucus free to avoid any contamination of samples.
13. Avoiding the peripheries and the vessels, take full depth samples at 3 different sites.
14. Take each sample and stabilize in RNAlater. The sample stabilized in RNAlater would be used for epigenetics and gene expression analysis. The sample stabilized in RNAlater should then be stored in a refrigerator until next day for storage at -80c
15. Placenta (5g) will be stored in RNA*later* solution and shipped to the core lab in Hamilton (Appendix 2)