

**APPENDIX F-A
HYPOTHESES FOR THE NATIONAL CHILDREN'S STUDY**

1.0 Undesirable outcomes of pregnancy: birth defects and preterm birth

- 1.1 Among women without diabetes before pregnancy, impaired glucose metabolism during pregnancy is proportional to risk of major congenital malformations of the heart, central nervous system, musculoskeletal system, and all birth defects combined
- 1.2 Intrauterine exposure to mediators of inflammation due to infection of either vaginal, cervical, or uterine sites, or of more distal sites (e.g., periodontal disease) is associated with an increased risk of preterm birth.

2.0 Altered neurobehavioral development, developmental disabilities, and psychiatric outcomes

- 2.1 Repeated low-level exposure to nonpersistent pesticides in utero or postnatally increases risk of poor performance on neurobehavioral and cognitive examinations during infancy and later in childhood, especially, for certain agents, among those with genetically decreased paraoxonase activity.
- 2.2 Prenatal infection and mediators of inflammation are risk factors for neurodevelopmental disabilities, such as cerebral palsy and autism.
- 2.3 Infection and mediators of inflammation during pregnancy and the perinatal period are associated with increased risk of schizophrenia.

3.0 Injury

(Note: Hypotheses 3.1 and 3.2 were recently removed by the ICC but are currently being further strengthened for possible inclusion as NCS hypotheses.)

- 3.1 Exposures early in life that lead to neurotoxic effects are associated with increased risk of injury.
- 3.2 Attributes of childcare and relationship with caregivers influence risk of injury.
- 3.3 Repeated head trauma has a cumulative adverse effect on neurocognitive development.

4.0 Asthma

- 4.1 Exposure to indoor and outdoor air pollution and bioaerosols (including allergens, endotoxin, and mold) is associated with increased risk of asthma.
- 4.2 Respiratory viral infection early in life is associated with increased risk of asthma.

- 4.3 Maternal stress during pregnancy is associated with increased risk of asthma.
- 4.4 Antioxidant constituents of diet decrease risk of asthma.
- 4.5 Early exposure to bacterial and microbial products decreases risk of asthma (hygiene hypothesis).
- 4.6 Access to health care and management of asthma are strongly related to asthma hospitalization.

5.0 Obesity and altered physical development

- 5.1 Impaired maternal glucose metabolism during pregnancy is directly related to risk of obesity and insulin resistance in offspring.
- 5.2 Intrauterine growth restriction as determined by serial ultrasound examination is associated with subsequent risk of central obesity and insulin resistance in offspring, independent of subsequent body mass index.
- 5.3 Breast milk feeding, compared with infant formula feeding, and breastfeeding duration are associated with lower rates of obesity and lower risk of insulin resistance.
- 5.4 Dietary predictors of obesity and insulin resistance include reduced intake of fiber and whole grains, and high glycemic index.
- 5.5 Environmental factors such as distance to parks, availability of walking routes in the neighborhood, and neighborhood safety are associated with risk of obesity and insulin resistance.
- 5.6 Social, behavioral, and family factors that affect development of dietary preferences and physical activity patterns early in childhood determine risk of childhood obesity and insulin resistance.
- 5.7 In utero and subsequent exposure to environmental agents that affect the endocrine system (bisphenol A, atrazine, and lead) results in altered age at puberty.

APPENDIX F-B
PROPOSED MEASURES FOR HYPOTHESES BY METHOD,
LIFE STAGE, AND PARTICIPANT

TABLE 1.1.a THROUGH TABLE 5.7.g: Proposed measures for hypotheses by method, life stage, and participant

Note: Measures in **bold** are of critical importance. Those of high, but not critical importance are *italicized*. The remainder of the entries are in normal text.

Hypothesis 1.1: Among women without diabetes before pregnancy, impaired glucose metabolism during pregnancy is proportional to risk of major congenital malformations of the heart, central nervous system, musculoskeletal system, and all birth defects combined.

References for Hypothesis 1.1: Becerra et al., 1990; Casson et al., 1997; Eriksson et al., 2000; Kucera et al., 1971; Martinez-Frias et al., 1998; Schaefer et al., 1997; Tikkanen et al., 1992.

Table 1.1.a: Hypothesis 1.1 – Method: Blood

Life stage	Mother	Father	Index Child	Other Adult
Preconception (Mother) Upon Enrollment (Father)	DNA	DNA		
	Glucose tolerance	Glucose tolerance		
Necessary to verify diabetes status prior to pregnancy on Mother only. Measures on father can be collected any time prenatally and serve as covariates which account for male mediated effect.	HgbA1C	<i>HgbA1C</i>		
	Lipid profile	<i>Lipid profile</i>		
	Blood glucose levels	<i>Blood glucose levels</i>		
	Serum insulin levels	<i>Serum insulin levels</i>		
	Insulin gene VNTR	<i>Insulin gene VNTR</i>		
	Glucokinase mutation	<i>Glucokinase mutation</i>		
Could potentially use surrogate retrospective measures from medical record review or interview	Hormones -- Cortisol	<i>Hormones -- Cortisol</i>		
1st Trimester	Glycemic index			
	Insulin gene VNTR			
	Glucokinase mutation			
	Cortisol			

Life stage	Mother	Father	Index Child	Other Adult
2nd Trimester	Glucose tolerance			
	HgbA1C			
	Lipid profile			
	Blood glucose levels			
	Serum insulin levels			
3rd Trimester	Glycemic index			
	Cortisol			
	Blood glucose levels			
	Serum insulin levels			
Notes: Measures during pregnancy are required to determine a change over time, but the hypothesis would not be threatened if measured were not taken at every trimester.				

Table 1.1.b: Hypothesis 1.1 – Method: Urine

Life stage	Mother	Father	Index Child	Other Adult	Household
Notes: Urine isoprostanes could be considered for the mother during the prenatal period, but are likely redundant with other measures.					

Table 1.1.c: Hypothesis 1.1 – Method: Physical Sampling (other than blood or urine)

Life stage	Mother	Father	Index Child	Other Adult	Household
No additional physical sampling measures are necessary to support this hypothesis.					

Table 1.1.d: Hypothesis 1.1 – Method: Medical Record Review

Life Stage	Mother	Father	Index Child	Other Adult
Preconception (or Prenatal retrospectively)	Glycemic index	Glycemic index		
	Type I diabetes	Type I diabetes		
2nd Trimester	Glycemic Index			
Year 2 through 20			Birth defects	
Notes: Birth defects are assumed to be measured through direct observation from a medical professional – but could also be captured via medical record review. Observations of birth defects in the index child are assumed at Years 2, 5, 10, 15, and 20 in the tables in the main body of this report.				

Table 1.1.e: Hypothesis 1.1 – Method: Interview

Life stage	Mother	Father	Index Child	Other Adult
Upon Enrollment (Either Preconception or early in Pregnancy)	Age	Age		
	Residential Environment	Residential Environment		
	Residential History	Residential History		
	Diet and Nutrition	Diet and Nutrition		
	Medical Hx	Medical Hx		
	Family Hx obesity	Family Hx obesity		
	Family Hx diabetes	Family Hx diabetes		
	Family Hx dietary habits	Family Hx dietary habits		
	Medicine Usage			
	Prev child w/ birth defect	Prev child w/ birth defect		
Throughout Pregnancy (Repeated at each Trimester)	Medicine Usage			
	Residential Environment			
	Diet and Nutrition			
	Physical Measures of Stress			
	Smoking Status			
	Alcohol Consumption			
	Physical Activity			
Notes:				

Table 1.1.f: Hypothesis 1.1 – Method: Self-Administered Questionnaire

Life stage	Mother	Father	Index Child	Other Adult
Upon Enrollment	<i>Diet and nutrition -- food</i>	Diet and nutrition -- <i>food</i>		
	<i>Lifestyle factors</i>	Lifestyle factors		
Preconception (when possible) through Pregnancy	<i>Alcohol consumption</i>	Alcohol consumption		
	<i>Family Hx physical activity</i>	Family Hx physical activity		
	<i>Physical activity measure</i>	Physical activity measure		
	<i>Smoking status</i>	Smoking status		
3rd Trimester	Physical activity measure			
Notes:				

Table 1.1.g: Hypothesis 1.1 – Method: Direct Observation by a Medical Professional

Life stage	Mother	Father	Index Child	Other Adult
Preconception	<i>Abdominal girth</i>			
	<i>BMI</i>			
	<i>Blood pressure</i>			
	<i>Waist to hip ratio</i>			
	<i>Weight</i>			
	<i>Pregnancy Hx</i>			
	<i>Prev Hx of birth defect</i>			
2nd Trimester	<i>BMI</i>		Ultrasound of fetus	
	<i>Blood pressure</i>			
3rd Trimester	<i>BMI</i>			
	<i>Blood pressure</i>			
Spon Ab/Stillbirth			Birth defects (autopsy)	
Birth			Birth defects	
			Glucokinase mutation	
			Abdominal girth	
			Length	
			Neurological exam	

Life stage	Mother	Father	Index Child	Other Adult
			Weight	
4X in Yr 1			Abdominal girth	
			Length	
			Neurological exam	
			Weight	
			Birth defects	
Year 1			Abdominal girth	
			Length	
			Neurological exam	
			Weight	
Year 2 Through Childhood			Birth defects	
<p>Notes: Measures from the mother could be possible predictors of gestational diabetes. The bolded outcome measures in the child are of high priority, but the frequency could probably be altered if it better fit some design issues. It is assumed that birth defects are observed via medical record review in Tables 2 and 3 in the main body of the report.</p>				

Hypothesis 1.2: Intrauterine exposure to mediators of inflammation due to infection of either vaginal, cervical, or uterine sites, or of more distal sites (e.g., periodontal disease) is associated with an increased risk of preterm birth.

References for Hypothesis 1.2: Andrews et al., 2000; Copper et al., 1996; Eriksson et al., 1999; Gitau et al., 1998; Goldenberg et al., 2000; Hillier et al., 1988; Nelson et al., 2000; Offenbacher et al., 2001.

Table 1.2.a: Hypothesis 1.2 – Method: Blood

Life Stage	Mother	Father	Index Child	Other Adult
Preconception	WBC			
1st Trimester	Genetic markers NOS			
2nd Trimester	WBC			
Birth	Antibodies			
Notes:				

Table 1.2.b: Hypothesis 1.2 – Method: Urine

Life stage	Mother	Father	Index Child	Other Adult
Notes: No data from urine samples are required to test this hypothesis.				

Table 1.2.c: Hypothesis 1.2 – Method: Physical Sampling (other than blood or urine)

Life stage	Mother	Father	Index Child	Other Adult
Preconception	Swab -- Cytokines			
1st Trimester	Swab -- Cytokines			
2nd Trimester	Swab -- Cytokines			
	Metalloproteinase			
3rd Trimester	Swab -- Cytokines			
	Metalloproteinase			
Birth			Placenta culture/exam	
			Umbilical cord culture	
			Cytokines	

Table 1.2.d: Hypothesis 1.2 – Method: Medical Record Review

Life Stage	Mother	Father	Index Child	Other Adult
1st Trimester	<i>Medicine usage</i>			
2nd Trimester	<i>Medicine usage</i>			
3rd Trimester	<i>Medicine usage</i>			
Notes: There may be opportunities to obtain these data from other sources.				

Table 1.2.e: Hypothesis 1.2 – Method: Interview

Life stage	Mother	Father	Index Child	Other Adult
1st Trimester	Recent infection			
2nd Trimester	Recent infection			
3rd Trimester	Recent infection			
Notes: Preconception data can be collected with other hypotheses. At least one measurement to collect infection data, preferably early in pregnancy, would be required, as this is primary explanatory variable.				

Table 1.2.f: Hypothesis 1.2 – Method: Self-Administered Questionnaire

	Mother	Father	Index Child	Other Adult
Notes: No data from self-administered questionnaires are required to test this hypothesis.				

Table 1.2.g: Hypothesis 1.2 – Method: Direct Observation by Medical Professional

Life stage	Mother	Father	Index Child	Other Adult
Prenatal (early in pregnancy if possible)	Dental exams			
Birth			Gestational age	
			Birth weight	
Notes: Dental exams can reveal recent infections or mediators of inflammatory disease. Gestational age is the defining variable for pre-term birth, but birth weight may be considered as a surrogate, if necessary.				

Hypothesis 2.1: Repeated low-level exposure to nonpersistent pesticides *in utero* or postnatally increases risk of poor performance on neurobehavioral and cognitive examinations during infancy and later in childhood, especially, for certain agents, among those with genetically decreased paraoxonase activity.

References for Hypothesis 2.1: Eskenazi et al., 1999; Mendola et al, 2002; Myers et al, 2000; Ribas-Fito et al., 2001.

Table 2.1.a: Hypothesis 2.1 – Method: Blood

Life stage	Mother	Father	Index Child	Other Adult
Upon Enrollment (Preconception through Prenatal)	Pesticides			
	Genetic markers	Genetic markers		
Birth	Pesticides		Genetic markers	
Notes: Genetic markers include the paraoxonase gene and characterization of paraoxonase activity				

Table 2.1.b: Hypothesis 2.1 – Method: Urine

Life stage	Mother	Father	Index Child	Other Adult
Upon Enrollment (Preconception through Prenatal)	Pesticides - 1st AM void			
Year 1			Pesticides - Diaper sample	
Yearly (Ages 2-7)			Pesticides - Diaper sample or 1st AM void	
Notes:				

Table 2.1.c: Hypothesis 2.1 – Method: Physical Sampling (Other than blood or urine)

Life stage	Mother	Father	Index Child	Other Adult
Preconception through Prenatal Period	Detailed exposure * assessment for pesticides on limited subsample of study population			
	Time activity patterns (GPS)			
Birth through Year 1	Pesticides in breast milk		Pesticides - fetus	
Throughout Childhood and Adolescence			Detailed exposure * assessment for pesticides on limited subsample of study population (including dietary intake)	
Yearly (Ages 2-7)			Time activity patterns (GPS)	
Notes: * We will probably need to be able to relate biomarkers and questionnaire information to these measures and their changes over time. This is especially of concern for pesticides, given the temporal variability in these exposures (due to both residential usage and dietart changes over time).				

Table 2.1.d: Hypothesis 2.1 – Method: Medical Record Review

Life stage	Mother	Father	Index Child	Other Adult
Throughout Childhood and Adolescence			Other records - school grades/performance	
Notes:				

Table 2.1.e: Hypothesis 2.1 – Method: Interview

Life stage	Mother	Father	Index Child	Other Adult (Proxy for Child)
Upon Enrollment (Preconception through Prenatal)	Residential environment	Occupational data		
	Occupational data			
	Food frequency questionnaire during pregnancy	Pesticide usage log		
	Pesticide usage log			
Birth and during WBV through Year 4			Pesticide usage log	Daycare (homecare, or preschool) environment
				Residential env.
				Food frequency diary
Yearly (Ages 5-7)			Pesticide usage log	Daycare environment
				School environment and "play areas"
				Residential env
				Food frequency diary
				Occupational data
Years 10, 15, 20			Residential env. + school and "play areas" survey	
			Food frequency diary	
			Pesticide usage log	
<p>Notes: Residential environment and occupation data are important, but probably can be collected less frequently than specified. The same is true for most of the data collected from the primary caregiver, especially as the child gets older. The ages for follow-up late in childhood and adolescence were chosen to correspond with other hypotheses for efficiency – this will need careful consideration in final protocol development. Pesticide usage log is an inventory of products used in or around the home (by resident or a technician), where they were applied, when, and how much was applied.</p>				

Table 2.1.f: Hypothesis 2.1 – Method: Self-Administered Questionnaire

Life stage	Mother	Father	Index Child	Other Adult
<p>Notes: No self-administered questionnaire data are required to test this hypothesis.</p>				

Table 2.1.g: Hypothesis 2.1 – Method: Direct Observation by a Medical Professional

Life stage	Mother	Father	Index Child	Other Adult
Prenatal			Neuro psych testing	
Birth			Time activity patterns- GPS	
WBV2			<i>Neuro psych testing</i>	
(18-24 months)			<i>Neuro psych testing</i>	
Yearly (Ages 3-7)			<i>Neuro psych testing</i>	
Year 10			<i>Neuro psych testing</i>	
Year 15			<i>Neuro psych testing</i>	
Year 20			<i>Neuro psych testing</i>	
Notes: Frequent neuro and psych testing is justified due to the chance of measurable changes over time. The ages for follow-up late in childhood and adolescence were chosen to correspond with other hypotheses for efficiency – this will need careful consideration in final protocol development.				

Hypothesis 2.2: Prenatal infection and mediators of inflammation are risk factors for neurodevelopmental disabilities, such as cerebral palsy and autism.

References for Hypothesis 2.2: Bejar et al., 1988; Grether et al., 1997; Grether et al., 1999; Lou et al., 1994; Mattson et al., 1996; Nelson et al., 1998; Nelson et al., 2002; Roebuck et al., 1999; Yoon et al., 1997. Wu et al., 2000.

Table 2.2.a: Hypothesis 2.2 – Method: Blood

Life stage	Mother	Father	Index Child	Other Adult
1st Trimester	Cytokines			
	Infection serology			
	Interleukins			
2nd Trimester	Cytokines			
	Infection serology			
	Interleukins			
3rd Trimester	Cytokines			
	Infection serology			
	Interleukins			
Birth	Genetic markers			
	Cytokines			
	Infection serology			
	Inflammatory markers			
	Antibodies			
Notes:				

Table 2.2.b: Hypothesis 2.2 – Method: Urine

Life stage	Mother	Father	Index Child	Other Adult
Notes: There are no urine samples required to test the hypothesis.				

Table 2.2.c: Hypothesis 2.2 – Method: Physical Sampling (other than blood or urine)

Life stage	Mother	Father	Index Child	Other Adult
1st Trimester	Vaginal/cervical culture			
2nd Trimester	<i>Vaginal/cervical culture</i>			
3rd Trimester	<i>Vaginal/cervical culture</i>			
Birth	Placenta culture/pathology		Amniotic fluid analysis	
	Vaginal/cervical culture		Umbilical cord culture/pathology	
Notes:				

Table 2.2.d: Hypothesis 2.2 – Method: Medical Record Review

Life stage	Mother	Father	Index Child	Other Adult
1st Trimester	<i>Obstetric medical Hx</i>			
2nd Trimester	<i>Obstetric medical Hx</i>			
3rd Trimester	<i>Obstetric medical Hx</i>			
Birth	<i>Obstetric medical Hx</i>			
Follow-up - Year 7			Other records – school grades/performance	
Notes: It will be important to note any unusual obstetric history or current pregnancy items.				

Table 2.2.e: Hypothesis 2.2 – Method: Interview

Life stage	Mother	Father	Index Child	Other Adult
Notes: There are no interview data required to test this hypothesis.				

Table 2.2.f: Hypothesis 2.2 – Method: Self-Administered Questionnaire

Life stage	Mother	Father	Index Child	Other Adult
Notes: There are no self-administered questionnaire data required to test the hypothesis.				

Table 2.2.g: Hypothesis 2.2 – Method: Direct Observation by a Medical Professional

Life stage	Mother	Father	Index Child	Other Adult
2 nd and 3 rd Trimesters			Fetal ultrasound	
Year 2			Neurological exam	
			Screening test for autism	
			Screening test for cerebral palsy	
Follow-up - Year 7			Neurological exam	
Notes: The age at which the screening tests for autism and cerebral palsy are performed may vary with regard to symptoms or physician preference. Neurological exams will continue to be important measures of the primary outcome variable.				

Hypothesis 2.3: Infection and mediators of inflammation during pregnancy and the perinatal period are associated with increased risk of schizophrenia.

References for Hypothesis 2.3: Bresnahan et al, 2000; Buka et al., 2001a; Buka et al., 2001b; Gunnar et al., 1995; Heim et al. 1999.

Table 2.3.a: Hypothesis 2.3 – Method: Blood

Life stage	Mother	Father	Index Child	Other Adult
1st, 2nd, and 3rd Trimesters	Cytokines			
	Infection serology			
	Interleukins			
	Cortisol			
	Interleukins			
Birth	<i>Genetic markers</i>			
	Cytokines			
	Infection serology			
	Inflammatory markers			
	Antibodies			
Notes:				

Table 2.3.b: Hypothesis 2.3 – Method: Urine

Life stage	Mother	Father	Index Child	Other Adult
Notes: No urine data are required to test this hypothesis.				

Table 2.3.c: Hypothesis 2.3 – Method: Physical Exam Physical Sampling (other than blood or urine)

Life stage	Mother	Father	Index Child	Other Adult
Upon Enrollment	Genetic polymorphisms - scraping	Genetic polymorphisms - scraping		

1st Trimester	Vaginal/cervical culture			
2nd Trimester	Vaginal/cervical culture			
3rd Trimester	Vaginal/cervical culture			
Birth	Placenta /Umbilical cord culture/pathology		Amniotic fluid analysis	
	Vaginal/cervical culture		Antibodies, IVH	
Childhood Years 5, 10, 15, 20			Imaging	
Notes: The ages for follow-up late in childhood and adolescence were chosen to correspond with other hypotheses for efficiency – this will need careful consideration in final protocol development.				

Table 2.3.d: Hypothesis 2.3 – Method: Medical Record Review

Life stage	Mother	Father	Index Child	Other Adult
Birth	Obstetric complaints			
Childhood (twice) Years 5 and 10			Other records - school grades/performance	
Adolescence Years 15 and 20			Other records - school grades/performance	
Notes: Obstetric complaints might be indicators of infection and are therefore of some significance. School performance and related measures might be early indicators in identifying children with schizophrenia, but are more likely covariates. Since the disease is generally not diagnosed until the late teens or early 20's, these are not direct measures of risk. The ages for follow-up late in childhood and adolescence were chosen to correspond with other hypotheses for efficiency – this will need careful consideration in final protocol development.				

Table 2.3.e: Hypothesis 2.3 – Method: Interview

Life stage	Mother	Father	Index Child	Other Adult
Upon Enrollment	Age	Age		
	Economic status	Economic status		
	Race/ethnicity	Race/ethnicity		
	Family mental illness Hx	Family mental illness Hx		
	Mental health Hx	Mental health Hx		
1st Trimester	Residential env. survey			
	Food frequency diary			
	Smoking status			
	Medicines usage			
	Recent systemic infection Hx			
	Vaccinations			
2nd Trimester	Food frequency diary			
	Medicines usage			
	Recent systemic infection Hx			
3rd Trimester	Medicines usage			
	Recent systemic infection Hx			
Childhood Years 5 and 10			Residential environment	
Adolescence Years 15 and 20			Residential environment	
<p>Notes: Many of these variables, e.g., age, economic status, overlap with several other hypotheses. Personal and family history of mental illness are important predictors due to the likelihood of familial aggregation of some mental disorders. The ages for follow-up late in childhood and adolescence were chosen to correspond with other hypotheses for efficiency – this will need careful consideration in final protocol development.</p>				

Table 2.3.f: Hypothesis 2.3 – Method: Self-Administered Questionnaire

Life stage	Mother	Father	Index Child	Other Adult
Notes: There are no self-administered questionnaire data required to test this hypothesis.				

Table 2.3.g: Hypothesis 2.3 – Method: Direct Observation by a Medical Professional

Life stage	Mother	Father	Index Child	Other Adult
2 nd Trimester			Fetal ultrasound	
3 rd Trimester			Fetal ultrasound	
Birth			Gestational age	
			Birth weight	
			Head circumference	
Childhood – twice Years 5 and 10			<i>Neuro psych testing</i>	
			<i>Neuro psych - function development</i>	
			<i>Social function</i>	
Adolescence Years 15 and 20			<i>Neuro psych testing</i>	
			<i>Neuro psych - function development</i>	
			<i>Social function</i>	
Notes: The ages for follow-up late in childhood and adolescence were chosen to correspond with other hypotheses for efficiency – this will need careful consideration in final protocol development. The specific neuro and/or psych tests will vary over time, depending on age-appropriateness.				

NOTE: Hypotheses 3.1 and 3.2 were recently eliminated by the ICC but are currently being further evaluated and possibly strengthened for consideration as NCS hypotheses.

Hypothesis 3.1: Exposures early in life that lead to neurotoxic effects are associated with increased risk of injury.

References for Hypothesis 3.1: CDC, 1999; Laflamme et al., 2001, Miller et al., 2000, Stiffman et al., 2002; Stoddard et al., 2001.

Table 3.1.a: Hypothesis 3.1 – Method: Blood

Life stage	Mother	Father	Index Child	Other Adult
Upon Enrollment (Preconception through Pregnancy – and while nursing)	Variety of persistent neurotoxins (e.g. lead, mercury, PCBs, persistent pesticides, etc.)			
Yearly Ages 1-5			Variety of persistent neurotoxins (e.g. lead, mercury, PCBs, persistent pesticides, etc.)	
Years 10, 15, 20				
Note: The ages for follow-up late in childhood and adolescence were chosen to correspond with other hypotheses for efficiency – this will need careful consideration in final protocol development.				

Table 3.1.b: Hypothesis 3.1 – Method: Urine

Life stage	Mother	Father	Index Child	Other Adult
Upon Enrollment (Preconception through Pregnancy – and while Nursing)	Variety of non-persistent neurotoxins or biomarkers of exposure (e.g. cotinine, OP pesticides and their metabolites)			
Yearly Ages 1-5			Variety of non-persistent neurotoxins or biomarkers of exposure (e.g. cotinine, OP	
Years 10, 15, 20				

Life stage	Mother	Father	Index Child	Other Adult
			pesticides and their metabolites)	
Note: The ages for follow-up late in childhood and adolescence were chosen to correspond with other hypotheses for efficiency – this will need careful consideration in final protocol development.				

Table 3.1.c: Hypothesis 3.1 – Method: Physical Exam Physical Sampling (other than blood or urine)

Life stage	Mother	Father	Index Child	Other Adult
Upon Enrollment (Preconception and throughout Pregnancy)	Detailed exposure assessment for variety of neurotoxins sampled from microenvironments (Pursued on a subset of study participants)			
Childhood			Detailed exposure assessment for variety of neurotoxins sampled from microenvironments (Pursued on a subset of study participants)	

Table 3.1.d: Hypothesis 3.1 – Method: Medical Record Review

Life stage	Mother	Father	Index Child	Other Adult
Pregnancy	Obstetric complaints			
Throughout Childhood (e.g. Years 2, 5, 10, 15, 20)			Injuries and ER visits (Prompted by interview)	
Notes: The type and severity of injury to be counted will have to be determined, along with the appropriate methods for retrospective evaluation of injury. The ages for follow-up late in childhood and adolescence were chosen to correspond with other hypotheses for efficiency – this will need careful consideration in final protocol development of behavioral variables that might increase or decrease the risk of injury.				

Table 3.1.e: Hypothesis 3.1 – Method: Interview

Life stage	Mother	Father	Index Child	Other Adult (Proxy for Index Child)
Upon Enrollment	Economic status	Economic status		
	Race/ethnicity	Race/ethnicity		
	<i>Occupational exposures</i>	<i>Occupational exposures</i>		
Prenatally (at 1 st , 2 nd and 3 rd Trimesters)	Residential env. survey			
	Food freq diary			
	Smoking status			
	Medicines usage			
	Activity patterns			
	Consumer product usage			
Early Childhood (Birth through Age 5)	Breastfeeding index child		Residential env. survey	Occurrence of injury
			Risk-taking behavior	Measures of social environment
			Food frequency diary	Childcare environment
			Medicines usage	Relationship with caregivers
			Activity patterns	
			Consumer product usage	
Years 10, 15, 20			Residential env. survey	Occurrence of injury
			Risk-taking behavior	Measures of social environment
			Food frequency diary	
			Medicines usage	
			Activity patterns	
			Consumer product usage	
		Injury		
<p>Notes: The type and severity of injury to be counted will have to be determined, along with the appropriate methods and schedule for measuring a range of behavioral variables that might increase or decrease the risk of injury. The ages for follow-up late in childhood and adolescence were chosen to correspond with other hypotheses for efficiency – this will need careful consideration in final protocol development.</p>				

Table 3.1.f: Hypothesis 3.1 – Method: Self-Administered Questionnaire

Life stage	Mother	Father	Index Child	Other Adult
Notes: There are no self-administered questionnaire data required to test this hypothesis.				

Table 3.1.g: Hypothesis 3.1 – Method: Direct Observation by a Medical Professional

Life stage	Mother	Father	Index Child	Other Adult (Proxy for Index Child)
During Childhood through Early Adulthood			Injury – occurrence	
			Injury - severity	
Notes: It is assumed that occurrence and severity of serious injury will be recorded by a medical professional for the index child, and that these medical records would be available for abstraction for the NCS.				

Hypothesis 3.2: Attributes of childcare and relationship with caregivers influence risk of injury.

References for Hypothesis 3.2: CDC, 1999; Cummins et al., 2001; Laflamme et al., 2001; Miller et al., 2000; Stiffman et al., 2002; Stoddard et al., 2001.

Table 3.2.a: Hypothesis 3.1 – Method: Blood

Life stage	Mother	Father	Index Child	Other Adult
Notes: There are no specific blood samples required to directly test this hypothesis – however, a variety of measures in blood from the index child (or mother) such as lead and mercury might serve as important covariates. These measures would likely be available from the assessment for a different hypothesis.				

Table 3.2.b: Hypothesis 3.2 – Method: Urine

Life stage	Mother	Father	Index Child	Other Adult
Notes: There are no specific urine samples required to directly test this hypothesis.				

Table 3.2.c: Hypothesis 3.2 – Method: Physical Sampling (other than blood or urine)

Life stage	Mother	Father	Index Child	Other Adult
Notes: There are no specific physical samples (other than blood or urine) required to directly test this hypothesis.				

Table 3.2.d: Hypothesis 3.2 – Method: Medical Record Review

Life stage	Mother	Father	Index Child	Other Adult
Throughout Childhood (e.g. Years 2, 5, 10, 15, 20)			Injuries and ER visits (Prompted by interview)	
Notes: The type and severity of injury to be counted will have to be determined, along with the appropriate methods for retrospective evaluation of injury. The ages for follow-up late in childhood and adolescence were chosen to correspond with other hypotheses for efficiency – this will need careful consideration in final protocol development. of behavioral variables that might increase or decrease the risk of injury.				

Table 3.2.e: Hypothesis 3.2 – Method: Interview

Life stage	Mother	Father	Index Child	Other Adult (Proxy for Index Child)
Early Childhood (Birth through Age 5)	Relationship with child	Relationship with child	Residential env. survey	Occurrence of injury
	Parental scales	Parental scales	Risk-taking behavior	<i>Measures of social environment</i>
			Injury	Childcare environment
				<i>Relationship with caregivers</i>
Years 10, 15, 20	Relationship with child	Relationship with child	Residential envs Survey	Occurrence of injury
	Parental scales	Parental scales	Risk-taking behavior	<i>Measures of social environment</i>
			Injury	
Notes: The type and severity of injury to be counted will have to be determined, along with the appropriate methods and schedule for measuring a range of behavioral variables that might increase or decrease the risk of injury. The ages for follow-up late in childhood and adolescence were chosen to correspond with other hypotheses for efficiency – this will need careful consideration in final protocol development. Parental scales refer to any number of social and behavioral measures of parenting skills and the relationship that the Mother and Father have with the index child.				

Table 3.2.f: Hypothesis 3.2 – Method: Self-Administered Questionnaire

Life stage	Mother	Father	Index Child	Other Adult
Notes: There are no self-administered questionnaire data required to test this hypothesis.				

Table 3.2.g: Hypothesis 3.2 – Method: Direct Observation by a Medical Professional

Life stage	Mother	Father	Index Child	Other Adult (Proxy for Index Child)
During Childhood through Early Adulthood			Injury – occurrence	
			Injury - severity	
<p>Notes: It is assumed that occurrence and severity of serious injury will be recorded by a medical professional for the index child, and that these medical records would be available for abstraction for the NCS.</p>				

Hypothesis 3.3: Repeated head trauma has a cumulative adverse effect on neurocognitive development.

References for Hypothesis 3.3: Anderson et al., 2000; CDC, 1999; Hawley et al., 2002; Laflamme et al, 2001; Salcido et al., 1992; Stiffman et al., 2002; Stoddard et al., 2001.

Table 3.3.a: Hypothesis 3.1 – Method: Blood

Life stage	Mother	Father	Index Child	Other Adult
Notes: There are no specific blood samples required to directly test this hypothesis – however, a variety of measures in blood of the index child (or mother), such as lead and mercury, might serve as important covariates. These measures would likely be available from the assessment for a different hypothesis.				

Table 3.3.b: Hypothesis 3.3 – Method: Urine

Life stage	Mother	Father	Index Child	Other Adult
Notes: There are no specific urine samples required to directly test this hypothesis.				

Table 3.3.c: Hypothesis 3.3 – Method: Physical Sampling (other than blood or urine)

Life stage	Mother	Father	Index Child	Other Adult
Notes: There are no specific physical samples (other than blood or urine) required to directly test this hypothesis. There may be some assessment of the physical environment at the residence and nearby areas for likely risks of injury.				

Table 3.3.d: Hypothesis 3.3 – Method: Medical Record Review

Life stage	Mother	Father	Index Child	Other Adult
Throughout Childhood (e.g. Years 2, 5, 10, 15, 20)			Traumatic head injuries and ER visits (Prompted by interview)	
			Other records – school grades/performance	
<p>Notes: The type and severity of injury to be counted will have to be determined, along with the appropriate methods for the retrospective evaluation of injury. The ages for follow-up late in childhood and adolescence were chosen to correspond with other hypotheses for efficiency – this will need careful consideration in final protocol development. of behavioral variables that might increase or decrease the risk of injury.</p>				

Table 3.3.e: Hypothesis 3.3 – Method: Interview

Life stage	Mother	Father	Index Child	Other Adult (Proxy for Index Child)
Early Childhood (Birth Through Age 5)	Relationship with child	Relationship with child	Residential env. survey	Occurrence of injury
	Parental scales	Parental scales	Risk-taking behavior	Measures of social environment
			Injury	Childcare environment
			Participation in sports	Relationship with caregivers
Years 10, 15, 20	Relationship with child	Relationship with child	Residential env. survey	Occurrence of injury
	Parental scales	Parental scales	Risk-taking behavior	Measures of social environment
			Injury	
			Participation in sports	
<p>Notes: The type and severity of injury to be counted will have to be determined, along with the appropriate methods and schedule for measuring a range of behavioral variables that might increase or decrease the risk of injury. The ages for follow-up late in childhood and adolescence were chosen to correspond with other hypotheses for efficiency – this will need careful consideration in final protocol development.</p>				

Table 3.3.f: Hypothesis 3.3 – Method: Self-Administered Questionnaire

Life stage	Mother	Father	Index Child	Other Adult
Notes: There are no self-administered questionnaire data required to test this hypothesis.				

Table 3.3.g: Hypothesis 3.3 – Method: Direct Observation by a Medical Professional

Life stage	Mother	Father	Index Child	Other Adult (Proxy for Index Child)
Regular Intervals Throughout Childhood (1,2,3,5,10)			Neurocognitive measures (To be Determined)	
Head Injuries During Childhood through Early Adulthood (Unplanned)			Injury – occurrence	
			Injury - severity	
At Scheduled Times Following Head Injury			Neurocognitive measures (To be Determined)	
Notes: It is assumed that occurrence and severity of serious injury will be recorded by a medical professional for the index child, and that these medical records would be available for abstraction for the NCS.				

NOTE: Due to the similarity of Hypotheses 4.1 through 4.6, the tables for these hypotheses are combined. In these tables, a measure that is unique to a specific hypothesis is followed by the number of the specific hypothesis that it supports, in square brackets.

Hypothesis 4.1: Exposure to indoor and outdoor air pollution and bioaerosols (including allergens, endotoxin, and mold) is associated with increased risk of asthma.

References for Hypothesis 4.1: Abbey et al., 1995; Boland et al., 1999; Eggleston et al., 1999; Nel et al., 1998; Schwartz, 1993; Sunyer, 2001; Weitzman et al., 1990.

Hypothesis 4.2: Respiratory viral infection early in life is associated with increased risk of asthma.

References for Hypothesis 4.2: Croner et al., 1992; Droste et al., 2000; Finn et al., 2000; Gehring et al., 2002, Lanphear et al., 2001; London et al., 2000; Martinez, 2000; Sears et al., 1991; Sherman et al., 1990; Weitzman et al., 1990.

Hypothesis 4.3: Maternal stress during pregnancy is associated with increased risk of asthma.

References for Hypothesis 4.3: Dobbin et al., 1991; Herbert et al., 1993; London et al., 2000; McEwen et al., 1998; Wright et al., 2002.

Hypothesis 4.4: Antioxidant constituents of diet decreased risk of asthma.

References for Hypothesis 4.4: Croner et al., 1992; Droste et al., 2000; Finn et al., 2000, Gehring et al., 2000; Lanphear et al., 2001; Martinex, 2002; Sears et al., 1991; Sherman et al., 1990.

Hypothesis 4.5: Early exposure to bacterial and microbial products decreases risk of asthma (hygiene hypothesis).

References for Hypothesis 4.5: Croner et al., 1992; Finn et al., 2000; Gehring et al., 2002; Gergen, 2001; Lanphear et al., 2001, Liu, 2002; London et al., 2000; Platts-Mills, 2001; Sporik, 2001; von Mutius, 2001.

Hypothesis 4.6: Access to health care and management of asthma are strongly related to asthma hospitalization.

References for Hypothesis 4.6: Carter et al., 2001; Gottlieb et al., 1995; Homer et al., 1996; Marker et al., 1992; Weitzman et al., 1990.

Table 4.0.a: Hypotheses 4.1-4.6 – Method: Blood

Life stage	Mother	Father	Index Child	Other Adult
Prenatal to Birth **Note: Some measures from mother would be useful if collected preconception, but it is not critical if not collected preconception.	DNA sample	DNA sample	DNA sample Genetic markers measured once after birth	
	Cortisol**	Lymphocytes		
	Lymphocytes			
	Infection/inflammation	Infection/inflammation	Cord blood at birth	
	Cytokines	Cytokines		
	IgE	IgE		
	Inflammatory markers	Interleukins		
	Interleukins			
	Genetic markers			
	Selenium			
	Vitamin C			
	N-3 N-6 Fatty acids			
WBV 1, 2, 3, 4, and Years 1, 2, 3, 6, 10 Once positive diagnosis of asthma is made, blood sampling or analyses may be reduced			IgE	
			Inflammatory markers	
			Allergic sensitization	
			Lymphocytes	
			Cytokines	
			Interleukins	
			Viruses	
			Caliciviruses	
			Paramixo	
			Pneumo	
			Rotavirus	
			Measures of hazardous air pollutants such as PAHs to correlate with environmental measures	
			Selenium	
		Vitamin C		
		Bifidobacteris		

Life stage	Mother	Father	Index Child	Other Adult
			BN-3 and N-6 fatty acids	
			Clostridia	
			Enterococci	
			Staphylococcus aureus	
			Bacteroides	
Years 15 and 20			Lymphocytes	
			Cytokines	
			IgE	
			Viruses	
			Caliciviruses	
			Paramixo	
			Pneumo	
			Rotavirus	
<p>Notes: Biological markers obtained from the mother and father and from the child at birth are very important as predictor variables. Some may be useful in the preconception phase, such as cortisol, which may provide a good baseline measure for maternal stress, if available. Infection and inflammatory markers of the mother are important predictor variables for Hypothesis 4.2. Because of the high prevalence of asthma, it may be possible to reduce cost and introduce efficiency by sampling children for testing after the age of 2 years (or beyond). The ages for follow-up late in childhood and adolescence were chosen to correspond with other hypotheses for efficiency – this will need careful consideration in final protocol development.</p>				

Table 4.0.b: Hypotheses 4.1-4.6 – Method: Urine

Life stage	Mother	Father	Index Child	Other Adult
Upon Enrollment and Through Pregnancy	Urine cotinine			
	Urine isoprostanes			
Years 1, 2, 3, 6, 10			Urine cotinine	
			Urine isoprostanes	
<p>Notes: Urine cotinine is an important marker of smoking, which can be an asthma risk factor. It may be desirable to measure this in the mother during pregnancy as a direct measure of tobacco consumption or exposure to secondhand smoke. Similarly, it may be desirable to measure urine cotinine in the index child for measures of secondhand smoke during Years 1, 2, 3, 6, and 10. These measures could also be considered as measures of reliability for self-reported exposure to tobacco smoke.</p>				

Table 4.0.c: Hypotheses 4.1-4.6 – Method: Physical (other than blood and urine) and Environmental Sampling

Life stage	Mother	Father	Index Child	Microenvironments of Child
2 nd Trimester			Amniocentesis -- cortisol	
Birth	Allergic skin test**	Allergic skin test [4.6]		Environmental sampling for indoor and outdoor air pollution measures, e.g., PM, diesel exhaust, NO₂, various allergens including fungal allergen [4.1] and for indicators of bacterial and microbial products [4.5] (Environmental sampling could be done on a subset of study participants with questionnaire data collected on all participants. Matrix sampling may also be considered to reduce burden so that not all samples are collected from each participant)
(** Preconception, if available)	Vitamin E+carotenoids			
	Oxidative stress response			
WBV 3,4			Infection swab	
Years 1, 2, 3, 6, 7, 10, 15, 20 Sampling may be less frequent or discontinued after positive diagnosis of asthma			Allergic skin test	
			Viruses	
			Caliciviruses	
			Paramixo	
			Pneumo	
			Rotavirus	
			Vitamin E+carotenoids swab	
			Oxidative stress response	
			Staphylococcus aureus	
			Bacteroides	
			Enterococci	
Periodically			Cytokines swab	
Notes: Allergic skin tests at birth are highly important. For Hypothesis 4.3, cortisol will be an important marker of maternal stress; periodic cytokine swabs for the index child are of high importance as an explanatory variable; and skin tests are important co-variables. All of the measures related to dietary intake are of high importance as predictor variables for Hypothesis 4.4. Infections during the index child's early years are very important, but the need to collect all the detailed information decreases over time. The ages for follow-up late in childhood and adolescence were chosen to correspond with other hypotheses for efficiency – this will need careful consideration in final protocol development.				

Table 4.0.d: Hypotheses 4.1-4.6 – Method: Medical Record Review

Life stage	Mother	Father	Index Child	Other Adult
Years 1, 2, 3, 6, 7, 10, 15, 20			Infections/inflammation	
			Bacterial enteritis	
			Bronchiolitis	
			Gastrointestinal illnesses	
			Pneumonia	
			Respiratory illnesses	
			Vaccinations	
			Type 1 diabetes diagnosis	
			Medicine usage & antibiotics	
			Atopic dermatitis	
Hospitalizations and other medical treatments (e.g., ER or urgent care)				
All data collection about history of infection is important, but the frequency and schedule may vary without introducing problems. Medicine usage, antibiotics, and vaccinations help indicate significant illnesses. The ages for follow-up late in childhood and adolescence were chosen to correspond with other hypotheses for efficiency – this will need careful consideration in final protocol development.				

Table 4.0.e: Hypotheses 4.1-4.6 – Method: Interview

Life stage	Mother	Father	Index Child	Other Adult
Prenatal through Birth	Family size/composition	Family size/composition		
	Residential history	Residential history		
	Allergy Hx	Allergy Hx		
	Asthma Hx	Asthma Hx		
	Atopic dermatitis Hx	Respiratory illnesses Hx		
	Respiratory illnesses Hx			
	Breastfeeding			
	Medicines usage Hx			

Life stage	Mother	Father	Index Child	Other Adult
	Diet and nutrition measures			
	Respiratory illness Hx			
	Atopic dermatitis Hx			
	Occupational data			
Years 1, 2, 3, 6, 7, 10, 15, 20			Residential history	
			Child diet & nutrition measures	Diet & nutrition measures
			Asthma symptom survey	
			Child psychological Hx	
			Health care usage - quantity/quality	
			Health insurance	
			Occupational history	
			Daycare/school attendance	
			Smoking exposure/status	
			Psycho-social stress	
			Hospitalizations and other medical treatments (e.g., ER or urgent care) [4.6]	
			Child infection history	
			Hygiene factors	
		Housing survey – moisture/mold/allergens/pets/farm animals		
<p>Notes: Allergy and asthma history of parents are important explanatory variables. Residential history and outdoor environmental variables will also be useful as explanatory variables or covariates. Data related to economic status and health care access will be useful primarily as covariates. Data from the primary caregiver may be a surrogate of what the child eats, but only through young childhood. All variables related to health insurance, health care access, are highly important predictors. Since these variables can change over time, it is important to measure them frequently. The ages for follow-up late in childhood and adolescence were chosen to correspond with other hypotheses for efficiency – this will need careful consideration in final protocol development.</p>				

Table 4.0.f: Hypotheses 4.1-4.6 – Method: Self-Administered Questionnaire

Life stage	Mother	Father	Index Child	Other Adult
Upon Enrollment (Preconception through Pregnancy)	Smoking status	Smoking status		
	Stress indicators			
	Occupational data	Occupational data		
Birth	Smoking status	Smoking status		
	Breastfeeding			
Yearly throughout Childhood Years 1, 2, 3, 6, 7, 10, 15, 20	Smoking status	Smoking status	Time activity patterns - diary	Vacuum with filter
	Occupational data	Occupational data	Smoking status during adolescent years	Smoking status
Notes: There is substantial overlap with the interview data. These could probably be combined without loss of validity. Alternatively, some persons could be interviewed while others use a self-administered questionnaire. The ages for follow-up late in childhood and adolescence were chosen to correspond with other hypotheses for efficiency – this will need careful consideration in final protocol development.				

Table 4.0.g: Hypotheses 4.1-4.6 – Method: Direct Observation by Medical Professional

Life stage	Mother	Father	Index Child	Other Adult
WBV 1, 2, 3			Chest auscultation	
WBV 4			Pulmonary function	
Upon indication of asthma symptoms from interview or medical record data, additional physical testing to confirm diagnosis and follow-up testing would be required if not already conducted			Chest auscultation	
			Pulmonary function	
			Airway reactivity to bronchoprovocation	
			Exhaled gases and condensate analysis	
(Upon death)			Autopsy data	
Notes: Airway reactivity to bronchoprovocation may be limited to older children.				

NOTE: Due to the similarity of Hypotheses 5.1 through 5.6, the tables for these hypotheses are combined. In these tables, a measure that is unique to a specific hypothesis is followed by the number of the specific hypothesis that it supports, in square brackets.

Hypothesis 5.1: Impaired maternal glucose metabolism during pregnancy is directly related to risk of obesity and insulin resistance in offspring.

References for Hypothesis 5.1: Brand-Miller et al., 2002; Breier et al., 2001.

Hypothesis 5.2: Intrauterine growth restriction as determined by serial ultrasound examination is associated with subsequent risk of central obesity and insulin resistance in offspring, independent of subsequent body mass.

References for Hypothesis 5.2: Breier et al., 2001; Whitaker et al., 1998.

Hypothesis 5.3: Breast milk feeding, compared with infant formula feeding, and breastfeeding duration are associated with lower rates of obesity and lower risk of insulin resistance.

References for Hypothesis 5.3: Dietz, 2000; Gillman et al., 2001, Hediger et al., 2003, von Kries et al., 1999.

Hypothesis 5.4: Dietary predictors of obesity and insulin resistance include reduced intake of fibre and whole grains, and high glycemic index.

References for Hypothesis 5.4: Kuzmarski et al., 1994; Mokad et al., 1999, Ogden et al., 2002, Serdula et al., 1993.

Hypothesis 5.5: Environmental factors such as distance to parks, availability of walking routes in the neighborhood, and neighborhood safety are associated with risk of obesity and insulin resistance.

References for Hypothesis 5.5: Birch et al., 1998; Colditz, 1992; Handy et al., 2002; Kohl et al., 1998; Mokad et al., 1999.

Hypothesis 5.6: Social, behavioral, and family factors that affect development of dietary preferences and physical activity patterns early in childhood determine risk of childhood obesity and insulin resistance.

References for Hypothesis 5.6: Birch et al., 1998; Colditz, 1992; Freeman et al., 2001; Handy et al., 2002; Kuzmarski et al., 1994; Mokad et al., 1999; Ogden et al., 2002; Serdula et al., 1993.

Table 5.0.a: Hypotheses 5.1-5.6 – Method: Blood

Life stage	Mother	Father	Index Child	Other Adult
Upon Enrollment	Genetic markers	Genetic markers		
	Insulin resistance	Insulin resistance		
1st Trimester	Glucose tolerance			
	Lipid profile			
	Insulin resistance			
2nd Trimester	Glucose tolerance	Insulin resistance		
	Insulin resistance			
	Glucose levels			
3rd Trimester	Glucose tolerance			
	Insulin resistance			
	Glucose levels			
Birth	Glucose tolerance		Insulin resistance	
	Insulin resistance			
WBV 1 & 4 and Years 2, 5, 10			Insulin resistance	
			Serum insulin levels	
			Glucose levels	
			Growth hormone/IgF	
			HgbA1C	
Years 15, 20			Insulin resistance	
			Serum insulin levels	
			Glucose levels	
			Growth hormone/IgF	
			HgbA1C	
			Cytokines	
			IgE	
<p>Notes: Glucose tolerance is a primary explanatory variable and therefore of high importance. Genetic markers are important, but the hypothesis could still be tested in their absence. Measures of insulin resistance include variables such as serum insulin levels and growth hormone. Serum insulin levels are valuable predictors of the obesity outcome, or may also be important covariates. At least once before age 21, a fasting blood sample and blood pressure measurement would be needed to make the diagnosis of metabolic syndrome. Periodic physical exams will be required to assess the occurrence of obesity. The frequency of exams might differ without threatening the hypothesis. The ages for follow-up late in childhood and adolescence were chosen to correspond with other hypotheses for efficiency – this will need careful consideration in final protocol development.</p>				

Table 5.0.b: Hypotheses 5.1-5.6 – Method: Urine

Life stage	Mother	Father	Index Child	Other Adult
Notes: No urine samples are expected to be collected to test these hypotheses.				

Table 5.0.c: Hypotheses 5.1-5.6 – Method: Physical Sampling (other than blood or urine)

Life stage	Mother	Father	Index Child	Other Physical Sampling
Birth	Breast milk		<i>Physical exam– body size and habitus</i>	
WBV1	Breast milk		Fecal sample	
WBV 2, 3, 4	Breast milk		<i>Physical exam– body size and habitus</i>	
Year 2	Breast milk (<i>twice</i>)			Visual inspection – neighborhood assessment
Years 5, 10, 15, 20			<i>Physical exam– body size and habitus</i>	
Notes: One or more breast milk samples during infancy are needed. The parameters for physical exams need to be specified and may change over time. The ages for follow-up late in childhood and adolescence were chosen to correspond with other hypotheses for efficiency – this will need careful consideration in final protocol development.				

Table 5.0.d Hypotheses 5.1-5.6 – Method: Medical Record Review

Life stage	Mother	Father	Index Child	Other Adult
2nd Trimester	Bio sample records [5.2]			
3rd Trimester	Bio sample records [5.2]			
Birth	Bio sample records [5.2]			
WBV 4			Medical Hx [5.2]	
Year 5, 10, 15, 20			Medical Hx [5.2]	
Notes: Medical history of the index child will be useful data, but there may be opportunities to collect these data in the context of other hypotheses to reduce redundancy and burden. The ages for follow-up late in childhood and adolescence were chosen to correspond with other hypotheses for efficiency – this will need careful consideration in final protocol development.				

Table 5.0.e: Hypotheses 5.1-5.6 – Method: Interview

Life stage	Mother	Father	Index Child	Other Adult
Upon Enrollment Prenatal	Age	Age		
	Economic status	Economic status		
	Education	Education		
	Family size/composition	Family size/composition		
	Race/ethnicity	Race/ethnicity		
	Appearance cultural norms	Appearance cultural norms		
	Values on diet	Values on diet		
	Appearance	Appearance		
	Health-related knowledge	Health-related knowledge		
	Health-related quality of life	Health-related quality of life		
	Lifestyle factors (general)	Lifestyle factors (general)		
	Alcohol consumption	Alcohol consumption		
	Smoking status	Smoking status		
	Social function	Social function		
	Social/health policies	Social/health policies		
	Neighborhood characteristics	Neighborhood characteristics		
	Residential Hx	Residential Hx		
	Family Hx physical activity	Family Hx physical activity		
1st Trimester	Lifestyle factors (general)	Lifestyle factors (general)		
	Medical Hx	Medical Hx		
	Family Hx diabetes	Family Hx diabetes		
	Occupational data	Occupational data		
	Pregnancy Hx			
2nd Trimester	Diet & nutrition measures			
	Lifestyle factors (general)			
3rd Trimester	Diet & nutrition measures			
	Lifestyle factors (general)			
Birth	Breastfeeding		Economic status	
	Lifestyle factors (general)		Neighborhood	

			characteristics	
	Appearance cultural norms	Appearance cultural norms	Parents' work schedules	
	<i>Values on diet</i>	<i>Values on diet</i>	Race/ethnicity	
	Appearance	Appearance	Residential environment	
	Occupational data	Occupational data	Type of child care	
			Transportation methods	
			Health-related quality of life	
			Lifestyle factors (general)	
			Obesity - consequences	
WBV 1, 2, 3, 4	Breastfeeding		Diet & nutrition measures	
	Diet & nutrition measures		Time activity patterns	
	Appearance cultural norms	Appearance cultural norms	Diet & nutrition measures	Appearance cultural norms
	Values on diet	Values on diet	Time activity patterns	Values on diet
	Appearance	Appearance	Medical Hx	
	Occupational data	Occupational data	Economic status	
			Neighborhood characteristics	
			Neighborhood physical activity locations	
			Parents' work schedules	
			Race/ethnicity	
			Residential environment	
			Type of child care	
			Transportation methods	
			Housing survey	
			Social function	
			Health-related quality of life	
			Lifestyle factors (general)	
			Physical activity measure	
			Obesity - consequences	
			School grades (when appropriate)	
Years 2, 5, 10, 15, 20				

Notes: Family history of diabetes and breastfeeding of the index child are important predictive factors. **Diet & nutrition measures** are important, but the intervals of data collection could vary. A child's age has a large effect on the validity of diet and activity measures. Physical activity and diet assessments will need to be specially designed for children, depending on age. The ages for follow-up late in childhood and adolescence were chosen to correspond with other hypotheses for efficiency – this will need careful consideration in final protocol development. Pregnancy history could be moved to 3rd trimester. Diet and lifestyle factors could be measured once in the 2nd or 3rd trimester; would be acceptable if not measured twice. There is potential overlap with other hypotheses, which can influence the design of prenatal interview forms. Most of the data about the index child is less important in infancy than in later childhood. However, values on diet will be important throughout the 21 year follow-up interval. Many of these variables can be collected once during the specified time interval during childhood and adolescence. Others, e.g., type of child care, become irrelevant after a certain age.

Table 5.0.f: Hypotheses 5.1-5.6 – Method: Self-Administered Questionnaire

Life stage	Mother	Father	Index Child	Other Adult (Proxy for Child)
Early Childhood (Years 1, 2, 3, 5, 10)				Time activity patterns
Older Children (Years 15, 20)			Time activity patterns	
Notes: Time activity patterns will help evaluate physical activity and its direct or indirect relationship to obesity. Frequency and precise ages could vary without creating problems in testing the hypothesis. The ages for follow-up late in childhood and adolescence were chosen to correspond with other hypotheses for efficiency – this will need careful consideration in final protocol development.				

Table 5.0.g: Hypotheses 5.1-5.6 – Method: Direct Observation by Medical Professional

Life stage	Mother	Father	Index Child	Other Adult
Upon Enrollment	BMI	BMI		
1st Trimester	Obstetric physical exam	Physical exam		
	Fetal ultrasound			
2 nd and 3rd Trimesters	Obstetric physical exam			
	Fetal ultrasound			
Years 2, 5, 10, 15, 20			Physical exams (e.g., height, weight, BMI, blood pressure, waist-to-hip ratio)	
Notes: Fetal ultrasound will probably be part of routine prenatal care and therefore relatively easy to capture. Periodic physical exams will be required to assess the occurrence of obesity. The frequency of exams might differ without threatening the hypothesis. The ages for follow-up late in childhood and adolescence were chosen to correspond with other hypotheses for efficiency – this will need careful consideration in final protocol development.				

Hypothesis 5.7: In utero and subsequent exposure to environmental agents that affect the endocrine system (bisphenol A, atrazine, and lead) results in altered age at puberty.

References for Hypothesis 5.7: Parsons et al., 1999; Whitaker et al., 1998.

Table 5.7.a: Hypothesis 5.7 – Method: Blood

Life stage	Mother	Father	Index Child	Other Adult
Preconception	Dioxins			
	Env. PCBs			
1 st , 2 nd , 3rd Trimesters	Lead			
	Dioxins			
	PCBs			
Birth	Lead		Lead	
	Dioxins			
	PCBs			
Nursing	Dioxins			
	PCBs			
Yearly Ages 1-5			Lead	

Table 5.7.b: Hypothesis 5.7 – Method: Urine

Life stage	Mother	Father	Index Child	Other Adult
Upon Enrollment and 1 st , 2 nd , 3rd Trimesters of Pregnancy	Urine Cotinine			
	Atrazine			
	Bisphenol A			
Birth and throughout Nursing	Atrazine		Atrazine	
	Bisphenol A		Bisphenol A	
Yearly Ages 1-5			Atrazine	
Yearly Ages 8-21			Male Spermarchy	
<p>Notes: Urine cotinine is an important marker of smoking, which can be an important risk factor. It may be desirable to measure this in the mother during pregnancy for a direct measure of tobacco consumption or exposure to secondhand smoke. These measures could also be considered as measures of reliability for self-reported exposure to tobacco smoke. Tables 2 and 3 of the main body of this report list the ages for follow-up in late childhood and adolescence to correspond with other hypotheses for efficiency – this will need careful consideration in final protocol development, as the sampling of urine for sperm in male index children may need to be completed with higher frequency to address this hypothesis.</p>				

Table 5.7.c: Hypothesis 5.7 – Method: Physical Exam

Life stage	Mother	Father	Index Child	Other Adult
Notes: No physical samples other than blood or urine are required to test this hypothesis.				

Table 5.7.d: Hypothesis 5.7 – Method: Medical Record Review

Life stage	Mother	Father	Index Child	Other Adult
Notes: No medical record review data are required to test this hypothesis.				

Table 5.7.e: Hypothesis 5.7 – Method: Interview

Life stage	Mother	Father	Index Child	Other Adult
Preconception (if available)	Env. tobacco smoke	Alcohol consumption		
	Alcohol consumption	Smoking status		
	Smoking status			
	Medicines used			
	Menstrual Hx			
Prenatal	Reproductive Hx			
	Diet & nutrition measures			
1st Trimester	Env. tobacco smoke			
	Alcohol consumption			
	Smoking status			
(embryo/post em.)	Medicines usage			
	Pregnancy Hx			
2 nd and 3 rd Trimesters	Diet & nutrition measures			
	Env. tobacco smoke			
	Alcohol consumption			
	Smoking status			
	Medicines usage			

Life stage	Mother	Father	Index Child	Other Adult
Birth	Diet & nutrition measures	Env. tobacco smoke		
	Env. tobacco smoke	Alcohol consumption		
	Alcohol consumption	Smoking status		
	Smoking status			
	Medicines usage			
Nursing	Env. tobacco smoke			
	Alcohol consumption			
	Smoking status			
Infant			Diet & nutrition measures	
Yearly Ages 8-18			Menstrual Hx - female	
Adult			Menstrual Hx - female	
Notes: Mother's menstrual history is an important predictor variable.				

Table 5.7.f: Hypothesis 5.7 – Method: Self-Administered Questionnaire

Life stage	Mother	Father	Index Child	Other Adult
Notes: No self-administered data are required to test this hypothesis.				

Table 5.7.g: Hypothesis 5.7 – Method: Direct Observation by Medical Professional

Life stage	Mother	Father	Index Child	Other Adult
1 st , 2 nd , 3 rd Trimesters	Physical - anthropometric measures			
Birth	Physical - anthropometric measures		Gestational age	
Yearly Ages 6-Adulthood			Physical - anthropometric measures	
			Tanner stage reprod. exam	
Notes: Tables 2 and 3 of the main body of this report list the ages for follow-up in late childhood and adolescence to correspond with other hypotheses for efficiency – this will need careful consideration in final protocol development, as the Tanner Stage reproductive exam may need to be completed with higher frequency to address this hypothesis.				

