OBESITY AND INSULIN RESISTANCE FROM IMPAIRED MATERNAL GLUCOSE METABOLISM

1. Meta Hypothesis

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

2. Specific Hypotheses

1. Fetal macrosomia and increased risk for obesity later in life is associated with an increased maternal supply of glucose during gestation.

2. The association between fetal macrosomia and an increased maternal supply of glucose during gestation varies by gestational period during which hyperglycemia occurs.

3. The combined interaction of maternal hyperlipidemia and hyperglycemia during gestation is more strongly associated with fetal macrosomia and increased risk for obesity later in life than either predictor alone. Recent evidence suggests the addition of hyperlipidemia to the presence of hyperglycemia improves the ability to predict the risk of large-for-gestational-age babies (Bo et al., 2004). Data analysis should consider regression models with both maternal lipid and glucose concentrations as variables.

3. Background and Justification

3.1 Public Health Importance

Prevalence/incidence

The prevalence of overweight among children is greater than 16 percent among those 6 or older, and this prevalence has increased during the past 40 years (Hedley et al., 2004; Kuczmarski, Flegal, Campbell & Johnson, 1994). Overweight children are at increased risk for being overweight in adulthood (Serdula et al., 1993), which is associated with increased risk of type 2 diabetes, hypertension, and coronary artery disease (Freedman, Khan, Dietz, Srinivasan, & Berenson, 2001). Furthermore, being overweight as a child increases the risk of developing type 2 diabetes before age 21 (Sinha et al., 2002). The total prevalence of diabetes in women ages 20-39 is 1.7 percent and 6 percent among those ages 40-49 (Harris et al, 1998, as cited in Beckles & Thompson-Reid, 2001). Among reproductive-aged women with diabetes, about one-third (35.4 percent) of women younger than 40 and about one-quarter (26.7 percent) of those 40 or older did not know that they had the disease (Harris et al, 1998, as cited in Beckles & Thompson-Reid, 2001). About 4-7 percent of pregnancies are complicated by gestational diabetes (Kjos & Buchanan, 1999).

Impaired glucose tolerance is categorized into either impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) depending upon the test used to diagnose it. In a cross-section of U.S. adults ages 40-74 tested from 1988 to 1994, 33.8 percent had IFG, 15.4 percent had IGT, and 40.1 percent had pre-diabetes (IGT, IFG, or both). Applying these percentages to the 2000 U.S. population, about 35 million adults ages 40-74 would have IFG, 16 million would have IGT, and 41 million would have pre-diabetes (National Institute of Diabetes and Digestive and Kidney Diseases, 2005). Based on data from the 1999-2000 National Health and Nutrition Examination Survey, approximately 2 percent of U.S. adults between 20-39 are affected by IFG (Centers for Disease Control and Prevention [CDC], 2003).

Economic and/or social burden

Because child obesity is a risk factor for adult obesity, child obesity contributes to the more than $40 billion annual cost of obesity in the United States (Colditz, 1992). Obese people suffering from diabetes make up about half of all such persons suffering from diabetes but are responsible for nearly 60 percent of the cost (Finkelstein, Brown, Trogdon, Segel, & Ben Joseph, 2007). Estimates from the Medical Expenditure Panel Survey, the National Health Interview Survey, and National Health Accounts estimated costs at $50 billion to $80 billion in 1998 for overweight and obesity combined (Finkelstein, Fiebelkorn, & Wang, 2003). State Behavioral Risk Factor Surveillance Surveys estimate combined expenditures at $75 billion, from $87 million in Wyoming to $7.7 billion in California. The number of hospital discharges associated with obesity-related diseases in the United States has increased during the past three decades. Childhood and adolescent obesity-associated annual hospital costs, adjusted for inflation, increased more than threefold from $35 million during 1979-1981 to $127 million during 1997-1999. The percentage of total hospital costs that these numbers represent increased from 0.43 percent to 1.70 percent, respectively (Wang & Dietz, 2002).

3.2 Justification for a Large Prospective Longitudinal Study

Findings from the National Children's Study (NCS) are becoming more important as the incidence of obesity increases. A 2006 review of the increased incidence of obesity revealed approximately one-third of all pregnant women in the United States are obese (King, 2006).

A longitudinal study with national representation across the United States is essential to assess the development of obesity in the offspring. A small study would likely not be sufficient to answer the question because many variables affect the development of obesity. As opposed to a small cohort investigation, the components of the NCS focus on childhood obesity, as it is related to maternal gestational glucose tolerance, will be more likely to identify factors associated with childhood obesity. In addition, the longitudinal nature of the study data will provide stronger statistical confidence in identifying specific variables on the causal pathway of childhood obesity.

3.3 Scientific Merit

Current scientific understanding

Determining the strength of the association between impaired maternal glucose metabolism during pregnancy and overweight in children is a timely issue because the prevalence of overweight among women of childbearing age has increased (Mokdad et al., 1999) and being overweight is a risk factor for impaired maternal glucose metabolism (Kjos & Buchanan, 1999). The offspring of mothers who have type 1 diabetes are at increased risk of being overweight, and this effect is often evident as young as age 4 (Whitaker & Dietz, 1998). Studies that grouped mothers with gestational diabetes with type 1 and type 2 diabetics have shown an increased risk of overweight offspring (Whitaker & Dietz, 1998). On the basis of these observations, offspring of mothers with gestational diabetes or lesser degrees of impaired glucose metabolism are suspected of being at increased risk of obesity, but there are little data available to address the issue, and those data that are available have extremely limited statistical power (Whitaker & Dietz, 1998; Dabelea & Pettit, 2001).

3.4 Potential for Innovative Research

The currently proposed study is innovative and timely because of the increased incidence of obesity in the United States. The design of the NCS provides the unique opportunity to perform a natural history study of childhood obesity, which can be more precisely followed in a prospective manner to

further identify growth trajectories that lead to obesity in adulthood. Additionally, information about maternal glucose levels during gestation can be used to identify significant risks to childhood and therefore adult obesity.

The Study will recruit a large number of women prior to pregnancy. This will allow researchers to collect observational data on both mother and the developing fetus soon after conception. By observing early fetal development, reactions of the fetus to maternal hyperlidemia and hyperglycemia during gestation at various points in childhood can be described. The design of the Study will use the contributions of body composition at birth, growth trajectory of lean and fat body mass, and fat/lean body mass ratio to adult insulin resistance can be parsed. Variation in the occurrence of insulin sensitivity due to status at birth, childhood and adolescence growth trajectory, and dietary intake may be determined.

3.5 Feasibility

The data collection is feasible. Data required to test this hypothesis and suggested specific hypotheses are included in the currently proposed study protocol. The feasibility of obtaining serial information from birth and throughout childhood adequate to characterize childhood growth, body composition, and regional body fat (in particular, omental fat) remains to be settled. Characterizing growth trajectory throughout childhood will require more frequent measurements than currently planned.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal

Physician report or preexisting or gestational diabetes

- Blood glucose and HgbA1C
- Serum lipid profiles
- Serum insulin or related samples (e.g., C-peptide)
- Genetic samples (for exploration of potentially relevant genes such as VNTR insulin, glucokinase)

4.2 Methods

Primary/maternal

Blood samples

- Medical records review
- Interviews
- Physical exams-anthropometry

4.3 Life Stage

Primary/maternal

- Prenatal (first and third trimesters) and birth

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

- Insulin resistance:
  - Serum insulin levels
  - Glucose levels
  - HgbA1C
- Obesity:
  - IGF body size
  - Habitus body composition

5.2 Methods

- Blood samples, physical exams
- Anthropometry, body composition

5.3 Life stage

- All measures: Birth and periodic

6. Important Confounders, Mediators, and Effect Modifiers

- **Lipid profile:** Increased lipid levels are associated with an increased risk of insulin resistance (McGarry, 2002).
- **Glucokinase mutation:** Glucokinase mutation is associated with increased risk of maturity onset diabetes of the young (Kahn, Vicent, & Doria, 1996).
- **Hormone levels, such as cortisol, growth hormones, insulin-like growth factors:** Elevated levels of these and other hormones are associated with obesity and insulin resistance in children (Reinehr & Andler, 2004).
- **Genetic markers for obesity:** Certain genetic markers increase the risk of obesity.
- **Parent's body mass indices:** Body mass index and obesity are associated with certain genetic markers (Maes, Neale, & Eaves, 1997).

- **Family history of diabetes and obesity:** A family history of diabetes and obesity increases a child's risk (Centers for Disease Control and Prevention [CDC], 2005; Maes et al., 1997).

- **Lifestyle factors:** Less active lifestyles would increase the risk of obesity and insulin resistance (Sinha et al., 2002).

- **Nutrition:** Poor nutritional and high caloric diet would increase the risk of obesity and insulin resistance (Ogden, Flegal, Carroll, & Johnson, 2002).

- **Socioeconomic status and demographics:** Children of lower economic status and racial groups (particularly Native Americans, Hispanics, African Americans, and Asians) are at higher risk for obesity and insulin resistance (CDC, 2005).

7. Power and Sample Size

About 4-7 percent of pregnancies are complicated by gestational diabetes (Kjos & Buchanan, 1999). Assuming 100,000 children and an exposure prevalence of 5 percent:

- Smallest detectable relative risk for obesity (prevalence 10 percent, using 30 kg/m2 definition) is approximately 1.2

- Smallest detectable relative risk for metabolic syndrome (assumed prevalence 0.4 percent) is approximately 1.7

8. Other Design Issues

- Addressing this hypothesis based on obesity and insulin resistance measures at later life stages may be adversely impacted by attrition of study subjects.

- Blood studies, especially fasting, in younger children will require attention.

- Accurate characterization of growth trajectories are best when based on study measurements and not reports. This would require in-person visits more frequently during childhood than is currently planned and would introduce additional costs.

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OBESITY AND INSULIN RESISTANCE FROM INTRAUTERINE GROWTH RESTRICTION

1. Meta Hypothesis

Intrauterine growth restriction (IUGR) is associated with subsequent risk of central-body obesity and insulin resistance in offspring independent of subsequent body mass index.

2. Specific Hypotheses

1. IUGR is associated with a higher ratio of fat to lean body mass at the same body mass index beginning in childhood and persisting into adulthood and increased insulin resistance in offspring.

2. Adult insulin resistance is more likely in infants born with IUGR who:

    2.1 Demonstrate catchup growth during infancy or childhood.

    2.2 Demonstrate catchup in fat mass that exceeds catchup in linear dimensions or head size.

    2.3 Demonstrate early adiposity rebound.

    2.4 Have growth trajectories associated with insulin resistance which differ for males and females.

    2.5 Have growth trajectories leading to increased fat/lean body mass ratio which are associated with higher per kilogram or centimeter dietary intake.

3. Children born with IUGR who experience growth/body composition trajectories (see above) will also have significantly different timing of other developmental events (e.g., adrenarche, pubarche).

4. Children with IUGR, regardless of their postnatal growth trajectory, will demonstrate elevated cortisol, IGF 1, and IGF 2 levels at birth and into adulthood.

5. Measures of linear and body composition status/trajectories during fetal life will more precisely predict adult insulin resistance than size at birth.

6. Matrilineal birth size history (i.e., birth size of mother, grandmother) interacts with current intrauterine environment to affect birth size.

7. Late gestation and birth differences in body composition among IUGR infants predispose to:

    - 7.1 Differing body composition and growth trajectories throughout childhood, and
    - 7.2 Differences in the percentage demonstrating insulin resistance as adults.

8. The relationship of IUGR mediated by postnatal growth trajectory differs by ethnicity.

9. Childhood adiponectin levels are increased in IUGR children showing evidence of insulin resistance (measured by HOMA-IR).

3. Background and Justification

3.1 Public Health Importance

Prevalence/incidence

Intrauterine growth retardation: IUGR occurred in 7.6 percent of births in 2000 based on birth records of more than four million births (Boulet, Alexander, Salihu, Kirby, & Carlo, 2006). Prevalence of low birth weight (less than 2,500 grams) has increased in most ethnic groups between 1995 and present. The Pregnancy Nutrition Surveillance System reported a 2004 low birth weight rate of 7.5 percent for whites, 11.7 percent for African Americans, and 6.3 percent for Hispanics (Centers for Disease Control and Prevention [CDC], 2006). Some infants are not growth retarded at this weight and some high gestational age infants are growth retarded at higher weights (Boulet et al, 2006).

Insulin resistance: About 12.6 percent of the National Health and Nutrition Examination Survey (NHANES) sample had high fasting glucose or medication use indicating treatment for abnormal glucose metabolism. Figures were highest in Mexican Americans and higher in African Americans than whites from the NHANES 1988-94 (Ford, Giles, & Dietz, 2002). About half the variation in insulin resistance is explained by body mass indices (McLaughlin, Allison, Abbasi, Lamendola, & Reaven, 2004). Prevalence of the metabolic syndrome was about 23 percent, increasing from 7 percent in early adulthood to 42 percent in 60- to 69-year-olds. The rate was about the same in men and women in whites, but higher in Mexican American and African American women. Insulin resistance by various definitions occurred in 2-20 percent of normal weight, 12-60 percent of overweight, and 48 to 80 percent of obese children in the NHANES sample 1999-2002 (Lee, Okumura, Davis, Herman, & Gurney, 2006). The prevalence of diagnosed diabetes in 2001 on BRFSS was 7.9 percent, up from 7.3 percent in 2000, and was highest in African Americans (11.2 percent).

Obesity and abdominal obesity: In the NHANES sample (Ogden et al., 2006), 13 percent of adults were overweight and 16 percent were obese. Compared with the 1999-2000 NHANES wave, in the 2003-2004 NHANES wave 17 percent of all children 2-19 and 32 percent of adults 20 and older were overweight, with significant increases for children, adolescents, and men; rates were higher in ethnic minorities (58 percent of African American women ages 40-59 are obese compared to 38 percent of white women).

Abdominal obesity (defined as waist circumference greater than 102 centimeters in men and greater than 88 centimeters in women), was 39 percent (31, 23, and 31 percent for white, African American, and Mexican American men, respectively, and 44, 62, and 63 percent in white, African American, and Mexican American women, respectively) (Gillum, 1999).

Economic and/or social burden

Obese persons suffering from diabetes comprise about half of all such persons but are responsible for nearly 60 percent of health care costs (Finkelstein, Brown, Trogdon, Segel, & Ben Joseph, 2007). Estimates from the Medical Expenditure Panel Survey, the National Health Interview Survey, and National Health Accounts estimated costs at $50 billion to $80 billion in 1998 for overweight and obesity combined (Finkelstein, Fiebelkorn, & Wang, 2003). State Behavioral Risk Factor Surveillance Surveys estimate combined expenditures at $75 billion, with states ranging from $87 million for Wyoming to $7.7 billion for California. Among hospital discharges in the United States, the number of discharges associated with obesity-related diseases has increased during the past three decades. Childhood and adolescent obesity-associated annual hospital costs, adjusted for inflation, increased more than three fold from $35 million during 1979-1981 to $127 million during 1997-1999. The percentage of total hospital

costs these numbers represent increased from 0.43 percent to 1.70 percent, respectively (Wang & Dietz, 2002).

3.2 Justification for a Large Prospective Longitudinal Study

Most studies of the relationship of IUGR and adult insulin resistance are based on retrospective data and limited to information about size at birth and adult outcomes with no information available about different periods during prenatal development. Results of studies linking IUGR growth trajectory to adult outcomes have been contradictory because of differing definitions of key dependent and independent variables, use of different measurements, and limitations on follow-up. Many of the apparent confounders for this phenomenon (e.g., levels of such hormones as cortisol and insulin-like growth factors) are likely embedded in the same causal framework with IUGR that underlies the fetal origins of later life phenomena. Moreover, other confounders (such as genetic/familial factors, ethnicity, childhood and early adult body composition not associated with a history of IUGR) have not been sufficiently controlled.

Only a large prospective study will be able to adequately model this framework and determine the roles of the various factors embedded in it. The National Children's Study (NCS) design will provide prospective measurements of:

- Maternal nutritional deprivation or other stressors at different periods during prenatal development,
- Serial fetal growth throughout gestation (or minimally in the second and third trimesters),
- Serial fetal body composition including regional adiposity,
- Size and body composition at birth and then throughout childhood, adolescence and early adulthood,
- Dietary intake of mother during pregnancy and the offspring postnatally, and
- Key hormonal levels in
    - The mother during pregnancy, and
    - The infant at birth and then throughout childhood and adulthood.

Family factors (e.g., sibling birth size, body composition of other family members, maternal history of birth size [through report or via access to medical records]) will better control confounding.

3.3 Scientific Merit

This hypothesis addresses one of the fetal-origins hypotheses advanced by Barker (Barker & Osmond, 1987, 1988; Barker, Osmond, Golding, Kuh, & Wadsworth, 1989; Barker, 1990), which postulates an association between IUGR and features of the metabolic syndrome (including insulin resistance, dyslipidemia, hypertension, and coronary heart disease) in adulthood. Studies and commentary from many disciplines have contributed to the literature on this hypothesis (McMillen & Robinson, 2005; Armitage, Khan, Taylor, Nathanielsz, & Poston, 2004), and it has been expanded to include other adult outcomes besides metabolic syndrome, including rapid onset hypothyroidism (Kajantie et al., 2006),

immune dysfunction (McDade, 2005), chronic lung disease (Lucas et al., 2004; Gluckman, Hanson, & Pinal, 2005), maturational timing (Ibanez & d Zegher, 2006; Ibanez, Jimenez, & de Zegher, 2006), lean body mass (Li, Stein, Barnhart, Ramakrishnan, & Martorell, 2003), male reproductive dysfunction (Main, Jensen, Asklund, Hoi-Hansen, & Skakkebaek, 2006), and puberty and adrenarche (Van Weissenbruch & Delemarre-van de Waal, 2006). The preponderance of evidence supports the theory though several investigators have been unable to support it (Huxley & Neil, 2004; Desai, Gayle, Babu, & Ross, 2005; Tilling et al., 2004; Huxley, Neil, & Collins, 2002).

Initially, Barker hypothesized that the observed association was due to the "thrifty phenotype (Hales & Barker, 1992)," also called predictive adaptive response (Gluckman & Hanson, 2004) or phenotypic plasticity (Kuzawa, 2005), which is the ability of the fetus to adapt its growth to maternal constraints due to nutrient or oxygen limitation or another stressor. The observed adult outcomes were putatively associated with a mismatch between the limited prenatal environment and a postnatal environment with plentiful resources. The mechanism linking the adult outcome with fetal growth restriction was postulated to be fetal metabolic programming (the "setting" of a physiological system by an insult during a sensitive period resulting in long-term consequences). This phenomenon is observed in many animal and plant species (Kuzawa, 2005) and relates to highly conserved metabolic networks regulated by cell-level, genetically based processes (Lampl, 2005). An alternative to this hypothesis is that IUGR and the adult metabolic syndrome are secondary to genetically determined insulin resistance that results in impaired insulin mediated growth in the fetus as well as insulin resistance in adulthood (Hattersley & Tooke, 1999).

Studies during the last decade have led to a more detailed and broadened understanding of this hypothesis. The important observations documented:

- Adult outcomes vary depending upon body composition at birth among IUGR infants and differ by gender and in a different way by ethnicity (Levy-Marchal & Czernichow, 2006; Eriksson, Forsen, et al., 1999; Jaquet et al., 2005; Landmann et al., 2006; Modi et al., 2006; Forsen, Eriksson, Tuomilehto, Osmond & Barker, 1999; Hack et al., 2003).

- The relationship between lean body and fat mass in adulthood better predicts insulin resistance (Landmann et al., 2006; Hediger et al., 1998; Ong, 2006; Gale, Martyn, Kellingray, Estell, & Cooper, 2001; Levitt, Lambert, Woods, Seckl, & Hales, 2005; Loos et al., 2001; Phillips, 1995; Kensara et al., 2005) and is likely related to the limitation on postnatal muscle cell hyperplasia (Eriksson, Forsen, Osmond, & Barker, 2003).

- Growth trajectories (catchup in linear growth and fatness) mediate observed adult outcomes (Ong, 2006; Barker, Osmond, Forsen, Kajantie, & Eriksson, 2005; Dulloo, 2006).

- Maternal causes of IUGR are one subset of fetal "stressors" and metabolic programming is likely driven by early effects on the hypothalamic-pituitary axis that result in excess glucocorticoid secretion (by fetus, mother and placenta) that upregulates production of cortisol during postnatal life (Dulloo, 2006; Seckl & Meaney, 2006; Vaag et al., 2006; Ward, Syddall, Wood, Chrousos, & Phillips, 2004; Lesage, Blondeau, Grino, Breant, & Dupouy, 2001; Worthman & Kuzara, 2005).

- The hypothalamic-pituitary-adrenal axis reprogramming is due to altered pancreatic development, which is the metabolic shift that allows the brain to be preferentially

- This may specifically affect development of organ systems in the fetus that in turn affect subsequent metabolism during postnatal life (Armitage, Khan, Taylor, Nathanielsz, & Poston, 2004).

- The combined effects of fetal programming and postnatal growth (linear and body composition trajectory) lead to metabolic alteration in adipose tissue hormonal secretions (e.g., adiponectin and leptin), which also affects the development of insulin resistance in adulthood or earlier.

- The presence or magnitude of the fetal programming effect differs depending upon polymorphic genes (Eriksson, Lindi, et al., 2003; Heude, Petry, Pembrey, Dunger & Ong, 2006; Ong et al., 1999) and in different population groups (Freedman, Khan, Serdula, Ogden, & Dietz, 2006) and may be regulated at least in part by gene imprinting (Dunger, Petry, & Ong, 2006; Fowden, Sibley, Reik, & Constancia, 2006; Young, 2001; Reik et al, 2003).

- Transgenerational effects may affect this adaptive process, with the fetus assessing the quality of the maternal environment relative to information about past intrauterine environments (Heude et al., 2006).

3.4 Potential for Innovative Research

The design of NCS provides the unique opportunity to perform a natural history study of IUGR, which can be more precisely characterized (using ultrasound measurements of size and body composition) prospectively related to the occurrence of IUGR and, in turn, to adult outcomes. Information about family disposition to birth size (based on maternal birth size history and birth size of siblings) can be incorporated into the definition of IUGR to develop a more precise definition of the disorder. Similarly, detailed information about growth during childhood in relation to expected growth (based on anthropometric and body composition information about family members) will allow more precise determination of expected status and trajectory.

The Study will recruit a large number of women prior to pregnancy which will allow researchers to collect observational data on both mother and the developing fetus soon after conception. By observing early fetal development, reactions of the fetus (in terms of whole body growth, segmental and organ specific growth, and body composition) to intrauterine environment at various points in gestation can be described. The design of the study will use the contributions of body composition at birth, growth trajectory of lean and fat body mass, and fat/lean body mass ratio to adult insulin resistance can be parsed. Variation in the occurrence of insulin sensitivity due to status at birth, childhood and adolescence growth trajectory, and dietary intake may be determined.

3.5 Feasibility

Data that would be required to test this meta hypothesis and suggested specific hypotheses are included in the current protocol. The feasibility of obtaining serial information throughout fetal life (by ultrasounds completed in each trimester) adequate to characterize fetal growth, body composition, and regional body fat (in particular, omental fat) remains to be settled. Characterizing growth trajectory

throughout childhood will require more frequent measurements than are currently planned. Though of lesser quality, abstraction of primary care provider records for anthropometric measurements might be substituted. Body composition using a more direct method than anthropometry (e.g., DEXA) would increase the testability of the body composition related hypotheses. Body mass index does not adequately separate lean and fat body mass (Kensara et al., 2005). Hormonal measurements at birth using cord blood can be completed. While the inclusion of this measure may be prohibitively expensive for inclusion within the entire study population, it may be possible to include more precise body composition examinations, such as DEXA scans, within a subsection of the study population. Doing so could also be used to test hypotheses related to the adequacy of using approximate body composition estimates such as body mass index. Making use of cortisol measurements in the mother and child, included as key measures within other study hypotheses, is also planned.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal/family

- Fetal size measures (e.g., long bones, head and organ sizes)
- Cortisol
- Maternal body composition measures
- Sibling birth and fetal sizes, where available
- Maternal birth size history
- Nutrient (micro and macro) intake
- Placental hormonal levels
- Documentation of maternal insulin resistance

Primary/child

- Birth
  - Cord blood hormonal levels (cortisol, insulin-like growth factors)
  - Birth anthropometry and body composition (direct or anthropometry)
  - Observations and examination documenting IUGR
- Childhood
  - Anthropometry and body composition measures
  - Dietary intake

4.2 Methods

Maternal

- Anthropometry
- Ultrasounds
- Interviews
- Placental analysis

Child

- Anthropometry
- Direct body composition measurements
- Neonatal examinations

4.3 Life Stage

Primary/maternal

- Prenatal, second and third trimester

Primary/child

- Birth
- Childhood

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

- Measures of obesity
- Regional body fat
- Insulin resistance

5.2 Methods

- Blood sample (blood glucose levels, fasting blood glucose levels, and fasting challenge blood glucose levels), physical exam anthropometry (for body mass index), and body composition

5.3 Life Stage

- Birth, 6, 12, 18, 30, and 36 months; yearly throughout childhood

6. Important Confounders, Mediators, and Effect Modifiers

- **Glucokinase mutation:** Glucokinase mutation is associated with an increased risk of maturity onset diabetes of the young (Hattersley et al., 1998); however, evidence suggests this mutation does not result in programming in later life (Velho, Hattersley, & Froguel, 2000).

- **Hormone levels such as cortisol, growth hormone, and insulin-like growth factors:** Elevated levels of these and other hormones are associated with obesity and insulin resistance in children (Reinher & Andler, 2004).

- **Genetic markers for obesity:** Certain genetic markers increase the risk of obesity, such as the insulin variable number of tandem repeats polymorphism, located in the insulin gene promoter and variants of the gene ENPP1, known to inhibit insulin receptors.

- **Parent's body mass indices:** Body mass index and obesity are associated with certain genetic markers (Maes et al., 1997).

- **Family history of diabetes and obesity:** A family history of diabetes and obesity increases a child's risk (Maes et al., 1997, CDC, 2005).

- **Lifestyle factors:** Less active lifestyles would increase the risk of obesity and insulin resistance (Kuczmarski, Flegal, Campbell, & Johnson, 1994).

- **Nutrition:** Poor nutritional and high caloric diet would increase the risk of obesity and insulin resistance.

- **Socioeconomic status and demographics:** Children of lower economic status, and particular ethnic and racial groups (especially Native Americans, Hispanics, African Americans, and Asians), are at a higher risk of obesity and insulin resistance (CDC, 2005).

7. Power and Sample Size

The prevalence of high fasting glucose tolerance or the history of diabetes or glycemic medication use for adults age 20 years and older (Ford et al., 2002) are indicators for the outcome variable, insulin resistance (Table 1), and prevalence of infants born weighing fewer than 2,500 grams, according to data from the Pediatric and Pregnancy Nutrition Surveillance System (CDC, 2006) (Table 2), is used as an indicator for the risk factor, intrauterine growth retardation in calculation of sample size.

Table 1. Prevalence of NHANES adults with high fasting glucose or diabetes or glycemic medication use by gender and ethnicity

Ethnicity	Males	Females
Whites	15.6	8.5
African Americans	14.5	15.5
Mexican American	21.4	18.5

Table 2. Percent of infants born < 2,500 gm from the Pediatric and Pregnancy Nutrition Surveillance System by ethnicity (genders combined)

Ethnicity	Percent
Whites	7.5%
African Americans	11.7%
Mexican American	6.3%

Of births in the United States in 2005, 55 percent were non-Hispanic white, 14 percent were non-Hispanic black. Data for Mexican Americans comes from 2004: 16.5 percent were Mexican American. Using these proportions and correcting for sample attrition over 20 years (yearly retention rate of 98 percent assumed), in an analysis restricted to specific races, the smallest odds-ratios that can be reliably detected (with a power of 80 percent and a two-sided 95 percent confidence interval) by race and gender are presented in Table 3.

Table 3. Smallest odds ratios for insulin resistance (as indicated by high fasting glucose levels or medication use) given a birth weight < 2,500 gm by gender for three ethnicities

Ethnicity	Males	Females
Non-Hispanic Whites	1.30	1.58
Non-Hispanic Blacks	1.49	1.47
Mexican American	1.52	1.55

8. Other Design Issues

- Blood studies, especially fasting, in younger children will require careful attention. Obtaining consent for the use of DNA may be an issue.
- Addressing this hypothesis based on central obesity and insulin resistance measures at later life stages may be adversely impacted by attrition of study subjects.
- While one ultrasound may be included as part of routine prenatal care, additional ultrasounds may introduce added cost.
- Accurate characterization of growth trajectories should be based on study measurements and not reports. This would require in-person visits more frequently during childhood than is currently planned and would introduce additional costs.
- Because of problems using body mass index, direct body composition measurements are necessary for accurate characterization of fat and lean body mass. Direct body composition measures (with the exception of bioelectrical impedance) are costly.

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BREAST-FEEDING ASSOCIATED WITH LOWER RATES OF OBESITY AND LOWER RISK OF INSULIN RESISTANCE

1. Meta Hypothesis

Breast milk feeding compared with infant formula feeding is associated with lower rates of obesity and lower risk of insulin resistance.

2. Specific Hypotheses

1. Breast-feeding is associated with lower rates of childhood obesity in a dose response relationship in the United States.

2. The protective effect of breast-feeding on the risk of overweight is evident from infancy through adolescence and persists after controlling for confounding effects of other environmental factors.

3. Lower amounts of protein early in life ("the early protein hypothesis") (Koletzko, 2006) is positively associated with childhood obesity.

4. Protein and energy intake are different for infants who receive exclusive breast-feeding, breast-feeding plus complementation (formula feeding), and exclusive formula feeding. These differences are associated with obesity risk with higher cumulative protein and energy intake in infancy being associated with childhood obesity.

5. Serum leptin and adiponectin concentrations are higher in breast-fed than formula-fed infants and are associated with less rapid weight gain and lower risk of childhood obesity and insulin resistance.

6. Termination of breast-feeding is associated with a decrease in serum leptin concentration in the child and subsequent increase in weight gain.

3. Background and Justification

3.1 Public Health Importance

Prevalence/incidence

In 2005, 73 percent of all U.S. born infants were breast fed (either exclusively or in combination with formula feeding) before initial hospital discharge. By 6 months of age, only 39 percent of infants were breast fed. There are marked differences by race in breast-feeding initiation, exclusivity, and continuation at 6 months of age (Centers for Disease Control and Prevention [CDC], 2005b). The prevalence of overweight among children is greater than 16 percent among children aged 6 years or older, and this prevalence has increased during the past 40 years (Hedley et al., 2004; Kuczmarski, Flegal, Campbell, & Johnson, 1994). While the obesity epidemic is occurring in boys and girls in all 50 states, specific subgroups (e.g., African American, Hispanic, American Indian) are disproportionately affected (Koplan, Liverman, Kraak, & Committee on Prevention of Obesity in Children and Youth, 2005; Strauss & Pollock, 2001). Being overweight as a child is a risk factor for other childhood diseases such as asthma (Oddy et al., 2004) and type 2 diabetes (Sinha et al., 2002). Childhood obesity is associated with adult obesity (Serdula et al., 1993; Parsons, Power, & Manor, 2003), type 2 diabetes, hypertension, and coronary artery disease (Freedman, Khan, Dietz, Srinivasan, & Berenson, 2001).

Economic and/or social burden

Because child obesity is a risk factor for adult obesity, child obesity contributes to the more than $132 billion annual cost of obesity in the United States (American Diabetes Association, 2003). Obese persons suffering from diabetes make up about half of all such persons but are responsible for nearly 60 percent of the cost (Finkelstein, Brown, Trogdon, Segel, & Ben Joseph, 2007). Estimates from the Medical Expenditure Panel Survey, the National Health Interview Survey, and National Health Accounts estimated costs at $50 billion to $80 billion in 1998 for overweight and obesity combined (Finkelstein, Fiebelkorn, & Wang, 2003). State Behavioral Risk Factor Surveillance Surveys estimate combined expenditures at $75 billion, with states ranging from $87 million for Wyoming to $7.7 billion for California. Among hospital discharges in the United States, the number of discharges associated with obesity-related diseases has increased dramatically during the past three decades. Childhood and adolescent obesity-associated annual hospital costs, adjusted for inflation, increased more than threefold from $35 million during 1979-1981 to $127 million during 1997-1999. Even more interesting, the percentage of total hospital costs that these numbers represent increased from 0.43 percent to 1.70 percent, respectively (Wang & Dietz, 2002).

3.2 Justification for a Large Prospective Longitudinal Study

Prior studies have either not included samples representative of the diverse U.S. population or have been underpowered to examine and compare the relationship between breast-feeding and childhood obesity across racial groups. Studies have been confounded by comparisons using exclusive breast-feeding or ever breast fed as the definition of breast-feeding, leading to samples with mixed breast/bottle feeding in one of the comparison groups. The relationship of breast-feeding to the development of childhood obesity has been studied primarily in cross-sectional or retrospective study designs with differing definitions of breast-feeding, overweight, and obesity. A prospective longitudinal study will investigate the complex relationship between infant feeding patterns and growth trajectories from infancy through adolescence while controlling for relevant environmental and genetic factors. The longitudinal data collection planned for the National Children's Study (NCS) should allow for clearer differentiation of the composition of early infant feeding and its relationship to obesity during childhood.

3.3 Scientific Merit

Current scientific understanding

The relationship between infant breast-feeding and subsequent overweight has been extensively examined. The results of meta-analyses and systematic reviews support a small protective dose response relationship between breast-feeding and childhood obesity (Harder, Bergmann, Kallischnigg, & Plagemann, 2005; Owen, Martin, Whincup, Davey Smith, et al., 2005; Arenz, Ruckerl, Koletzko, & van Kries, 2004) and this association appears to persist from infancy through adulthood (Dewey, 2003; Gillman et al., 2001). Whether this association is causal is not clear. The effect size is small and may be confounded by factors such as socioeconomic status, ethnicity, diet, and physical activity (Dewey, 2003).

Questions remain whether the protective effect, if any, is due to constituents of breast milk, metabolic programming, regulation/control of intake by mother and/or infant, or family lifestyle/environmental programming that are different for breast-fed and formula-fed infants (Dewey, 2003; Bergmann et al., 2003). Existing studies that relied on retrospectively reported breast-feeding histories that define breast-feeding as exclusive breast-feeding or any breast-feeding have not adequately addressed the impact of cumulative breast milk intake (duration plus breast milk as a percentage of

intake). Bogen, Hanusa, & Whitaker (2004) found exclusive breast-feeding for at least 16 weeks or 26 weeks with formula supplementation was associated with lower obesity at age 4 than formula feeding.

If breast-feeding does help prevent subsequent obesity, this would be one of the few protective preventive measures available (Dietz, 2001). The relationship of racial and ethnic differences in infant feeding practices (e.g., approximately 41 percent of white non-Hispanic infants are breast fed at age 6 months [15 percent exclusive breastfeeding], compared with 25 percent of black children [9 percent exclusive breast-feeding] and 42 percent of Hispanic children [14 percent exclusive breast-feeding]) (CDC, 2005b) to subsequent differences in childhood obesity requires more exploration (Grummer-Strawn, Mei, & CDC Nutrition Surveillance, 2004).

There is conflicting evidence concerning the relationship between breast-feeding and insulin resistance. Breast-feeding has been reported as an independent protective factor against type 1 diabetes (Sadauskaite-Kuehne, Ludvigsson, Padaiga, Jasinskiene, & Samuelsson, 2004), but no relationship was found between breast-feeding and HOMA scores (homeostasis model assessment [Matthews et al., 1985]) as a measure of insulin resistance (Lawlor et al., 2005). In a systematic review of breast-feeding influence on the risk of type 2 diabetes, breast-feeding was associated with lower blood glucose and serum insulin concentrations in infancy and the decreased risk of type 2 diabetes in later life (Owen, Martin, Whincup, Smith, et al., 2005). It has been hypothesized that the beneficial effects of breast-feeding may be attributed to the long-chain polyunsaturated fatty acids in breast milk that suppress the production of proinflammatory cytokines, enhance the number of insulin receptors, and decrease insulin resistance (Das, 2002) or to the beneficial effects of adipokines on immune response (Weyermann et al., 2006).

Recent studies suggest the role of leptin found in breast milk is a bioactive substance involved in the regulation of energy intake positively affecting satiety and regulation of feeding frequency and amount. It may prime or set the neuroendocrine regulation of later feeding appetite and metabolism. Leptin has additional effects on immunomodulation, growth, development, and endocrine hormonal regulation (e.g., insulin). Human milk is a source of many nutrient and biological compounds (e.g., IGF-1, insulin, leptin) that regulate food intake, metabolism, and body composition (Locke, 2002; Savino, Costamagna, Prino, Oggero, & Silvestro, 2002). Leptin concentrations and leptin-to-weight ratios are higher in breast-fed than formula-fed infants. Serum leptin concentrations in infants are also positively correlated with maternal body mass indices (BMI) (Savino, Liguori, Oggero, Silvestro, & Miniero, 2006), which is correlated with obesity development (Dubois & Girard, 2006). The role of leptin in the complex nature of regulation of energy homeostatis and body weight requires continued investigation.

Breast-feeding has been associated with reduced systolic blood pressure in children (Lawlor et al., 2005; Martin, Gunnell, & Davey Smith, 2005) with lower cholesterol levels in adolescence (Owen et al., 2002), and insulin resistance in adulthood (Ravelli et al., 2000). It has been hypothesized that the advantages of breast-feeding for cardiovascular health may be due to slower growth in breast-fed than formula-fed babies. “The growth acceleration hypothesis” (Singhal & Lucas, 2004) suggests rapid weight gain in infancy adversely programs the metabolic syndrome, including childhood obesity (Ong et al., 2006; Dennison, Edmunds, Stratton, & Pruzek, 2006).

3.4 Potential for Innovative Research

Assessment of cumulative breast milk intake relative to total intake in the early months of life will help clarify the dose response relationship with childhood growth trajectories. Detailed analysis of feeding records and adiposity measures will examine differences in feeding and growth patterns for race/ethnicity groups. Analysis of sibling pairs will support investigation of gene-environment influences. Complementary analyses of feeding patterns, environmental factors, and biochemical markers will help uncover the complex interaction of variables associated with development of obesity in children.

Evaluation of the significance of early rapid growth for children of different gestational ages and weights will provide evidence for development of guidelines regarding postnatal weight management (Monteiro & Victora, 2005). Biochemical analysis of breast milk and child blood samples will extend the emerging science related to breast milk biochemistry and metabolic/endocrinologic effects on the growing child. The NCS offers the opportunity to distinguish the contributions of genes, gestation, and postnatal nutrition to childhood obesity (Jeffery et al., 2006).

3.5 Feasibility

Data collected through feeding diaries, maternal report, anthropometric assessments, and biologic samples will be used as data sources. Collection of data at multiple intervals during the first year of life will provide data needed to address the study hypotheses.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/maternal

- Breast milk feeding and duration, including exclusive and partial breast-feeding (Labbock & Krasovec, 1990), length of time of breast-feeding (as a surrogate for amount and a measure of self-regulation)
- Composition of breast milk, including leptin and adiponectin concentrations, and fatty acid composition (Ailhaud & Guesnet, 2004)

Primary/child

- Cumulative breast milk exposure in the first year of life relative to total milk/protein intake
- Timing of introduction of non-milk/solid foods
- Total energy intake

4.2 Methods

Primary/maternal

- Interview(s)
- Feeding diaries

Primary/child

- Breast milk sample(s)

4.3 Life Stage

Primary/maternal

- Birth and periodically through age 1

Primary/child

- Birth and periodically through age 1

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

- Insulin resistance
- Growth and obesity

5.2 Methods

- Insulin resistance: HgbA1C, lipoproteins, fasting glucose and insulin may be obtained in late childhood and adolescence. A glucose challenge test may be implemented in adolescence, though the timing and nature of that test has not been determined. The development of noninvasive measures of blood glucose, such as those based on infrared spectroscopy, measurement of interstitial fluid glucose, or the accumulation of advanced glycation end products in skin may provide additional serial measures of glucose levels as the study progresses.

- Growth and obesity: IGF, adiposity (skin fold thickness and waist circumference), weight, weight for height, and head circumference. Instrumental measures of body composition, such as bioelectric impedance analysis, dual X-ray absorptiometry, or air displacement plethysmography (available as BodPod or PeaPod) may be implemented later in childhood or adolescence but not in early childhood.

- Other: Medical record reviews may provide information regarding clinical diagnoses of diabetes or obesity.

5.3 Life Stage

- Measures of growth and obesity: Starting at birth (or even before, if prenatal ultrasounds are considered) and periodically during the study.

- Measures of glucose metabolism: Blood draw starting at 36-month visit and periodically during the study.

6. Important Confounders, Mediators, and Effect Modifiers

- **Lipid profile:** Increased lipid levels are associated with an increased risk of insulin resistance (McGarry, 2002).

- **Glucokinase mutation:** Glucokinase mutation is associated with increased risk of type 2 diabetes, formerly known as adult-onset diabetes, among children (Kahn, Vicent, & Doria, 1996).

- **Hormone levels such as cortisol, growth hormone, insulin-like growth factors:** Elevated levels of these and other hormones are associated with obesity and insulin resistance in children (Reinehr & Andler, 2004).

- **Parent's body mass indices:** BMI and obesity are associated with certain genetic markers (Maes, Neale, Eaves, & Lindon, 1997). Maternal obesity is associated with obesity in offspring (Hedley et al., 2004) and an additive interaction between maternal prepregnancy BMI and lack of breast-feeding on childhood obesity risk has been reported (Li et al., 2005). In breast-fed infants, serum leptin concentration is positively associated with maternal BMI (Savino et al., 2006). Maternal weight gain in pregnancy should also be evaluated as a potential confounder. Paternal obesity is associated with childhood obesity and may reflect familial patterns of energy intake and expenditure (Scaglioni et al., 2000).

- **Family history of diabetes and obesity:** A family history of diabetes and obesity increases a child's risk of obesity (Maes et al., 1997; U.S. Department of Health and Human Services [HHS], 2005).

- **Lifestyle factors:** Less active lifestyles would increase the risk of obesity and insulin resistance (Hedley et al., 2004).

- **Nutrition:** Poor nutritional and high caloric diet would increase the risk of obesity and insulin resistance (Ogden et al., 2002). Protein intake is higher in bottle-fed babies and may increase the risk of obesity (Scaglioni et al., 2000; Haisma et al., 2003). Early introduction of complementary food is associated with greater infant weight gain (Baker, Michaelsen, Rasmussen, & Sorensen, 2004). Breastfeeding in the first year of life is associated with less restrictive maternal control of feeding and lower energy intake after infancy (Fisher, Birch, Smicilas-Wright, & Picciano, 2000; Taveras et al., 2004).

- **Socioeconomic status and demographics:** Children of lower economic status, ethnic and racial groups (particularly Native Americans, Hispanics, African Americans, and Asians) are at higher risk of obesity and insulin resistance (Liese et al., 2001).

- **Infant weight gain:** Amount (Dubois & Girard, 2006) and rapidity of weight gain in the first 6 months of life (Ravelli et al., 2000) are associated with the risk of childhood obesity.

- **Smoking during pregnancy:** This is associated with childhood obesity (Bergmann et al., 2003; Dubois & Girard, 2006; Liese et al., 2001; Adams, Harvey, & Prince, 2005; Hediger, Overpeck, Kuczmarski, & Ruan, 2001).

7. Power and Sample Size

Based on NHANES 2003-2004 data, 18.8 percent of children ages 6-11 are overweight (i.e., BMI for age at 95th percentile or higher based on CDC growth charts) (Ogden et al., 2006). For the outcome where 18.8 percent of the population is expected to be overweight at age 8 (approximate midpoint of the 6-11 age range) and 61 percent of the population are not breastfed at 6 months (CDC, 2005b), the smallest detectable relative risk for overweight (BMI greather than or equal to the 95th percentile) at age 8 would be 1.08.

The rate of breastfeeding differs by race. The percentage of infants who are breastfed at 6 months is approximately 41 percent for white non-Hispanic infants, 25 percent for black infants, and 42 percent for Hispanic infants (CDC, 2005b). Based on preliminary NCHS data for 2005 (Hamilton, Martin, & Ventura, 2007), 24 percent of births were classified as Hispanic, 14 percent as black non-

Hispanic, and 62 percent as white or other races. The smallest detectable relative risk for overweight at age 8 would be 1.10 for white non-Hispanic children, 1.14 for Hispanic children, and 1.20 for black children in the NCS.

Using 4 percent of 15-year-olds estimated to have metabolic syndrome (based on NHANES III data indicating 4-10 percent of 12-19-year-olds [Cook, Weitzman, Auinger, Nguyen, & Dietz, 2003; DeFerranti et al., 2004]), the smallest odds ratio that can be reliably detected is 1.19 for the entire study sample and 1.21, 1.32, and 1.50 for white non-Hispanic, Hispanic, and black subsamples, respectively.

Assuming a breast-feeding prevalence of 50 percent (in line with estimates from NHANES III) (Fisher et al., 2000), and using the power assumptions noted above, the smallest detectable relative risk for obesity would be 1.05 and for metabolic syndrome would be 1.3.

These sample power calculations were based on simple bivariate comparisons without consideration of interactions, multiple measures, or the clustered survey design, all of which will decrease the power associated with each example.

8. Other Design Issues

- Blood studies, especially fasting, in younger children, will require attention. Obtaining consent for the use of DNA may be an issue.
- Addressing this hypothesis based on obesity and insulin resistance measures at later life stages may be adversely impacted by attrition of study subjects.

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FIBER, WHOLE GRAINS, HIGH GLYCEMIC INDEX AND OBESITY AND INSULIN RESISTANCE

1. Meta Hypothesis

During childhood, consumption of a high glycemic load diet is associated with obesity and subsequent insulin resistance.

2. Specific Hypotheses

1. Consumption of diets with high glycemic load is associated with childhood overweight and obesity independent of total energy consumption and expenditure.

2. In addition to its contribution to a diet's glycemic index, dietary soluble fiber modifies any relationship between glycemic load and obesity or inflammation.

3. Genetically determined underexpression of the GLUT4 gene, in either the GLUT4 gene itself or other genes influencing GLUT4 expression (e.g., D2, CASQ1), strengthens any association between glycemic load and subsequent obesity or insulin resistance.

4. Consumption of a high glycemic load diet is associated with elevated systemic markers of oxidative stress (e.g., isoprostanes) and inflammation (e.g., CRP, IL-6).

5. Polymorphisms in the GST family of genes resulting in decreased glutathione-S-transferase activity strengthen the association between glycemic load and oxidative stress and inflammation.

6. Elevated systemic markers of oxidative stress and inflammation associated with dietary glycemic load in childhood are also associated with risk of insulin resistance in adolescence, independent of overweight or obesity.

3. Background and Justification

3.1 Public Health Importance

Prevalence/incidence

In the United States, obesity among adults and overweight among children has steadily increased during the past three decades (Ogden et al., 2006; Ogden et al., 2004; Flegal & Troiano, 2000). Approximately 4 percent of children ages 6-11 were overweight in the early 1970s compared with 19 percent in 2003-2004. Being overweight during childhood is related to obesity in adulthood (Serdula et al., 1993) and the attendant health risks, including type 2 diabetes mellitus (Freedman, Khan, Dietz, Srinivasan, & Berenson, 2001; Sinha et al., 2002). Prevalence estimates of pediatric type 2 diabetes in the United States are limited and varied due to the diverse populations and methods used in the studies and the limitations of small sample sizes (Fagot-Campagna, 2000; Fagot-Campagna, Saadine, Flegal, & Beckles, 2001). However, among young U.S. adults age 20-39 in 1999-2002, the combined incidence of diagnosed and undiagnosed (based on a fasting blood glucose) diabetes was 2.3 percent. This represented an increase in diabetes of approximately 50 percent since 1988-1994 (Cowie et al., 2006).

3.2 Justification for a Large Prospective Longitudinal Study

The National Children's Study (NCS) is positioned to advance the understanding of the relationship between dietary glycemic load, obesity, and type 2 diabetes. The majority of studies referenced above were based on an adult population and were either cross-sectional or had relatively limited follow-up. The potential influence of childhood diets on both contemporary and subsequent pathophysiology is unknown. This is true for elucidation of the potential etiology of type 2 diabetes. A longitudinal study is necessary to capture the temporal sequence of glycemic load, overweight or obesity, oxidative stress and inflammation, and insulin resistance or diabetes. Understanding those relationships will enable targeting of interventions early in life that will help prevent the development of obesity, type 2 diabetes, and associated cardiovascular pathologies later in life.

3.3 Scientific Merit

Understanding the underlying causes driving the national trends in childhood obesity, diabetes, and related pathology is crucial to guide prevention and treatment. An obvious explanation for the increase in obesity is an increase in the imbalance between energy intake and energy expenditure. Other factors associated with diet may contribute to the increase in obesity and diabetes in important, though more subtle, ways. One of these factors may be the increasing consumption of processed foods and the differences in the metabolic characteristics of those foods compared with previous diets. In particular, the increased bioavailability of carbohydrates found in processed foods has the potential to contribute to obesity, to type 2 diabetes indirectly through obesity, and perhaps directly to type 2 diabetes independent of fat accumulation.

One commonly used measure of a food's glucose availability is the glycemic index, a comparison of the rise in blood glucose after consumption of a specified amount of carbohydrate-containing food to the rise of glucose after the consumption of the same amount of a "control" carbohydrate (generally white bread or white rice). A glycemic index for a mixed meal can be calculated by taking the weighted average of the glycemic index of each carbohydrate-containing food consumed during the meal. The glycemic load of a serving of food is the product of the glycemic index and the available carbohydrate in that serving, and therefore may provide a better measure of a diet's metabolic impact. Other dietary components, most notably soluble (or viscous) fiber, directly contribute to a food's glycemic load or may influence carbohydrate metabolism and subsequent effects which will be discussed in Section 6 of this hypothesis.

Foods with a relatively high glycemic index, by definition, result in relatively high blood glucose levels in the immediate postprandial period. The metabolic influence of a high glycemic index meal transcends the direct effects on postprandial blood glucose and insulin release. The exaggerated insulin response to the initial glucose surge results in an overcorrection and leads to a relative hypoglycemia shortly after the meal. In contrast, consumption of a low glycemic index meal invokes an attenuated insulin response and relatively stable serum glucose levels and low fatty acids (Frost & Dornhorst, 2005; Ludwig, 2002).

In rats, consumption of isocaloric high glycemic and low glycemic loads results in increased weight and increased adiposity among animals fed a high glycemic index diet (Pawlak, Kushner, & Ludwig, 2004). In addition, evidence suggests that, compared to an isocaloric low glycemic index meal, consumption of a high glycemic index meal results in less satiety and leads to increased caloric intake following that meal, at least in obese adolescents (Ludwig et al., 1999). Thus, in comparison to diets with a low glycemic load, high glycemic load diets seem to result in increased weight gain and fat mass on a calorie-for-calorie basis and may stimulate increased caloric intake and poses a double menace.

In addition to the relationships with weight and adiposity, high glycemic load diets have been associated with increased insulin resistance and, possibly, type 2 diabetes. The results of large-scale epidemiologic studies examining the association between glycemic index or load and diabetes are inconsistent (Liese et al., 2005; Schulze et al., 2004, for instance). However, experimental evidence in animals and humans suggests a relationship between high glycemic load, insulin resistance, and (among rats) disruption of pancreatic islet structure (Frost & Dornhorst, 2005; Ludwig, 2002). A number of potential mechanisms that may underlay a relationship between glycemic index and insulin resistance have been proposed, such as the direct effect of fatty acids on insulin metabolism or the down-regulation of insulin receptors in a high-insulin environment. Another possible explanation that may be related to the etiology of insulin resistance and the subsequent pathophysiology associated with diabetes is the association of high glycemic load with evidence of oxidative stress and inflammation.

A body of literature has examined the relationship between diabetes and systemic markers of both oxidative stress and inflammation. The underlying rationale for most of those studies is that hyperglycemia results in oxidative damage and stress. The physiologic response to that stress is inflammation, which is implicated in the numerous vascular pathologies associated with diabetes (Hu et al., 2006; Monnier, 2006; Qi & Hu, 2007; Sorenson, Raben, Stender, & Astrup, 2005; Esposito et al., 2002). However, some authors have suggested the oxidative stress and inflammation associated with diabetes is not a result of the condition but a potential precursor and cause of diabetes or insulin resistance (Duncan & Ines Schmidt, 2006; Browning & Jebb, 2006).

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/child

- Dietary intake
- Glycemic index and load

4.2 Methods

Assessment of glycemic index and glycemic load in the NCS will be dependent on three factors: accurate measurement of dietary intake, translation of foods into a glycemic index and load, and a sufficient number of dietary assessments throughout childhood to capture temporal changes in diet. The third factor is the simplest to assure. Dietary assessments, in the form of food frequency questionnaires and dietary recalls, will occur repeatedly throughout infancy and childhood. Early in life, they will be completed as proxy questionnaires by the mother or other caregiver. As the children get older, they will report on their own diet. Exact tools have not been specified for those collections, and their content and mode of administration will likely evolve between now and the time they are instituted.

Limitations in the accuracy of dietary intake by interview measures, whether food frequency, recall, or diary, are well-recognized (Trabulsi & Schoeller, 2001; Livingstone et al., 1992). There are systematic errors in the reporting of total energy intake and specific macro- and micro-nutrient intake. For the purposes of this hypothesis, the reporting of consumption of specific foods will enable calculation of a glycemic index, even if the misreporting of amounts of foods compromises accurate calculation of glycemic load (Hui & Nelson, 2006). Additional methods of dietary assessment in the NCS, such as the collection of duplicate diets of the children's meals or the digital photography of certain meals, is under consideration.

Calculation of glycemic index and glycemic load from the reported dietary intake involves merging the dietary data with a published database containing the glycemic measures (Foster-Powell, Holt, & Brand-Miller, 2002). While not all foods may be found within a specific database, databases can be combined to achieve reasonable coverage. An inherent difficulty in assigning glycemic index or load to an individual's diet is the variability between people and within a person in response to a carbohydrate-containing food. The glycemic indices in published databases are generally based on average response to a particular food in a study population. However, not all people will have the same metabolic response to the same food, and an individual's response to a certain food may not be consistent through time (Frost & Dornhorst, 2005).

Overall, while the ability to calculate a precise glycemic index for each individual's diet is limited due to difficulties in accurately assessing diet and then assigning glycemic values, the longitudinal nature and frequent assessments included in the NCS should allow for sufficient discrimination to enable accurate categorization for epidemiologic analysis.

4.3 Life Stage

- From birth and periodically throughout course of Study

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

5.2 Methods

- Physical growth (height, weight, circumferential measures, etc.) and body composition
- Assessment of glucose and insulin metabolism (biological/blood samples, e.g., HgbA1c, blood glucose levels, and fasting blood glucose levels in older children, when appropriate)

5.3 Life Stage

- Measures of growth and adiposity: Starting at birth (or even before, if prenatal ultrasounds are available to analyze for such data) and periodically during the Study.
- Measures of glucose metabolism: Blood draw starting at 36-month visit and periodically throughout the Study.

6. Important Confounders, Mediators, and Effect Modifiers

- **Dietary fiber:** Reduced dietary fiber intake, particularly soluble (or viscous) fiber, has, like high glycemic index, been associated with obesity and type 2 diabetes (Johnson, 2005). Some of this association is likely due to the relationship between soluble fiber and glycemic index, a carbohydrate-containing food with a relatively large amount of soluble fiber will have a lower glycemic index than a food with similar carbohydrate composition but less fiber. However, there is also evidence that soluble fiber may have additional influence on both lipid and glucose metabolism through inhibition of reabsorption of bile salts and the fermentation of undigested

carbohydrate in the colon. Assessment of dietary fiber intake is subject to the same limitations as glycemic index, as discussed in Section 4. The specification of soluble fiber content, rather than total fiber content, of specific foods is difficult given currently available databases.

- **Genetic influences and gene-environment interactions:** The genetic contributions to obesity and diabetes are manifold. Two potential areas of interest for this hypothesis may be the influence of the glucose transporter family of genes on the development of obesity and insulin resistance, and the effect of reduced activity of the Glutathione-S-Transferase (GST) family of genes on glycemia-related inflammation.

- **Glucose transport:** Cellular access to and metabolism of glucose is largely regulated by the GLUT family of genes. Decreased activity of GLUT4, the insulin-regulated glucose transporter, is associated with insulin resistance in animal models. The regulation of GLUT4 is complex, depending on glucose and insulin levels, as well the activity of other genes. To date, no specific polymorphisms of the GLUT4 gene have been identified that are directly associated with decreased function (Friedel et al., 2002). However, polymorphisms in other genes, such as those related to thyroid hormone or calcium metabolism, have been identified that result in decreased expression of GLUT4 and thus are associated with insulin resistance (Canani et al., 2005; Fu et al., 2004).

- **GST:** The GST family of genes is responsible for the conjugation of glutathione to potentially toxic compounds including reactive oxidative byproducts. Decreased activity of the GST enzymes is thus associated with an increase in oxidative stress-related inflammation. Well-described polymorphisms resulting in decreased activity of GST have been related to asthma and some cancers. Those polymorphisms also may be associated with an increased risk of glycemia-induced inflammation and subsequent diabetes or insulin resistance (Bolt & Thier, 2006).

- Other potential confounders:

  - Parental body habitus.

  - Parental or family history of insulin resistance or type 2 diabetes.

  - Exercise or energy expenditure, and

  - Demographic factors such as socio-economic status, race, and ethnicity.

7. Power and Sample Size

The prevalence of overweight children among children in the United States is approximately 15 percent, providing an opportunity to explore subpopulation differences in the relationship between diet and body composition. While the prevalence of insulin resistance among U.S. children is essentially unknown, there appear to be dramatic differences in subpopulations, with estimates from 0.1 to 5 percent (Fagot-Campagna et al., 2000). In early adulthood the prevalence increases to approximately 2 percent overall with variation by racial or ethnic subgroup.

Similarly, the distribution of dietary glycemic index or glycemic load among U.S. children has not been well described. However, if one assumes a 20 percent exposure rate of high glycemic index (the upper quintile compared with the rest of the population), a prevalence of insulin resistance of 0.50 percent in the "low" glycemic index population, and an alpha error of 0.05 percent and beta error of 0.20 percent, the full study population of 100,000 can be used to uncover a relative risk of approximately 1.4 without accounting for the clustered sample or multiple measurements.

8. Other Design Issues

- Blood studies, especially fasting, in any minor or younger children will require attention. Obtaining consent for the use of DNA may be an issue.
- Addressing this hypothesis based on obesity and insulin resistance measures at later life stages may be adversely impacted by attrition of study subjects.

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GENETICS, ENVIRONMENTAL EXPOSURES, AND TYPE I DIABETES

1. Meta Hypothesis

The development of beta cell autoantibodies and subsequent type 1 diabetes is causally associated with the interaction between genetic susceptibility, early exposure to viral infections, and early exposure to cow's milk protein or other dietary components.

2. Specific Hypotheses

1. The development of beta cell autoantibodies and subsequent type 1 diabetes is causally associated with:

    1.1 Genetic susceptibility (e.g., HLA D3 or D4 haplotypes) (Concannon et al., 1998; Pugliese, 1999; Rewers et al., 1996; Caillat-Zucman et al., 1992);

    1.2 Delayed exposure to Coxsakie (enterovirus) or other viral infections until later childhood or adolescence (Viskari et al., 2004; Skarsvik et al., 2006; Laugesen & Elliott, 2003);

    1.3 Early exposure to cow's milk protein (the timing and quantity of A1 β-casein consumption) or other dietary components (Schatz, Rogers, & Brouhard, 1996); and

    1.4 Interactions between these factors (Harris, 1995; Charatan, 2002; WHO Multinational Project for Childhood Diabetes Group, 1991; Green & Patterson, 2001; Kondrashova et al., 2005).

2. Insulin-producing β-cells, in the event of inhibited interferon production, are highly susceptible to acute infection by Coxsackie virus, resulting in type 1 diabetes.

3. Background and Justification

3.1 Public Health Importance

Prevalence/incidence

Of all chronic diseases affecting children, type 1 diabetes is one of the most common, with prevalence rates at approximately one in every 5,000 children in the United States (SEARCH for Diabetes in Youth Study Group, 2006). The incidence of the disorder is increasing worldwide at an estimated rate of 3 percent per year (Onkamo, Vaananen, Karvonen, & Tuomilehto, 1999), particularly among those younger than 5 and ethnic minorities. About 12.6 percent of the National Health and Nutrition Examination Survey (NHANES) sample had high fasting glucose or medication use indicating treatment for abnormal glucose metabolism. Figures were highest in Mexican Americans and higher in African Americans than whites, according to NHANES data from 1988-94 (Ford, Giles, & Dietz, 2002). Estimates of prevalence of impair glucose tolerance among children, by race, have been shown to be increasing, and are similar to the distribution of impaired glucose tolerance among adults. (Sinha et al, 2002)

Economic and social burden

Studies describing the economic costs of diabetes often consider the direct or medical costs of the disease, but less frequently examine the indirect costs, such as the value assigned to morbidity, disability, and premature mortality associated with type 1 diabetes.

Important medical costs relate to the daily management of the disease and the treatment of late-stage complications. The annual costs of type 1 diabetes typically range between $1,500 per person for the standard insulin regimens to nearly $6,000 for insulin pump protocols (Ford, Giles, & Dietz, 2002). Out-of-pocket health care costs for families with type 1 diabetes in the United States typically exceed $1,000 per year (Marrosu et al., 2004). These costs increase substantially after long-term complications develop. In 2002, the direct medical and indirect costs attributed to diabetes were estimated at $132 billion (Hogan, Dall, & Nikolov, 2003).

3.2 Justification for a Large Prospective Longitudinal Study

While Type 1 diabetes is a rare disease within the entire U.S. populations ages 21 years and younger with an incidence of about 20 per 100,000 per year, among chronic diseases affecting children, it is one of the most prevalent (CDC, 2005).. To study the etiology of such a rare condition, a large sample of high-risk individuals is needed. The National Children's Study (NCS) as planned with 100,000 births recruited and followed to age 21 years and storage of biological specimens, will likely include at least 100 incident cases, allowing nested case-control studies (and potentially case-cohort adjunct studies) to test the proposed hypotheses regarding gene-environment interactions in the etiology of type 1 diabetes. The database generated by the Study will provide an invaluable resource for hypothesis testing and developing new treatments and strategies for preventing type 1 diabetes. In addition, the nationally representative sample to be recruited for the Study will allow exploration of geographic and ethnic differences within the United States in the prevalence and etiology of type 1 diabetes.

3.3 Scientific Merit

Theory supporting hypothesis

The etiology of type 1 diabetes remains unclear. Epidemiologic patterns, including the dramatic geographic variations in type 1 diabetes incidence rates, variation in risk by ethnicity, the peak age at onset of puberty, and the more frequent diagnosis of the disease during the winter suggest viruses, nutrition, and socioeconomic factors are involved (Harris, 1995). Potential environmental risk factors have been investigated in numerous populations, but the studies yielded conflicting results. This has been due, in part, to a failure to account for disease susceptibility genes. Although genome screens found evidence of linkage to more than 15 potential genes, the primary locus of susceptibility (IDDM1) is located in the HLA region of chromosome 6 (Concannon et al., 1998; Mein et al., 1998). Estimated relative (odds ratios range from 10-100) and absolute risks (approximately 3-6 percent through age 20) associated with high-risk HLA genotypes have been studied in many populations (Laugesen & Elliott, 2003).

Current scientific understanding

Although lifelong insulin therapy is the only treatment for type 1 diabetes, there are currently several large clinical trials designed to evaluate a variety of approaches for primary (i.e., avoidance of cow's milk formula) and secondary disease prevention (i.e., high doses of nicotinamide, oral/nasal insulin) (Schatz et al., 1996). Newborns, children, and young adults who have a first-degree relative with type 1 diabetes are being screened for early preclinical markers (i.e., beta cell autoantibodies) and high-

risk HLA susceptibility alleles. Those who are positive for these preclinical markers are eligible for randomization. However, approximately 90 percent of individuals who develop type 1 diabetes have no family history of the disease and are not eligible for these trials (Rewers et al., 1996). For the public health impact of any of these interventions to be realized, they must be based on the general population and not high-risk family members. As a result, several natural history studies are following newborns who have high-risk HLA susceptibility alleles (Rewers et al., 1996; Charatan, 2002). Only half of the children who eventually develop type 1 diabetes carry these genes. Thus, approximately half of the remaining future incident cases will occur among those who screen negative for high-risk HLA genotypes. These individuals are being excluded from follow-up.

Since 2000, a group of European investigators have explored the hypothesis that type 1 diabetes has an epidemiology similar to polio because the populations most severely impacted by the chronic disease are children. There has been an increase in the incidence of type 1 diabetes throughout Europe in recent decades of a magnitude that can only be explained by environmental changes or gene-environment interaction (Green & Patterson, 2001). The multinational group has shown there is an inverse relationship between the background rate of enterovirus infections in a population and the incidence of type 1 diabetes (Viskari et al., 2004) and a six-fold gradient in the incidence of type 1 diabetes between Finland and the immediately adjacent Karelia area of Russia. The Finland and Karelia populations have a similar frequency of chromosome 6 high-risk HLA haplotypes (Kondrashova et al., 2005). The same group has also demonstrated there is no difference in the frequency of beta-cell auto-antibodies between Finnish and Karelian school children (Kondrashova et al., 2007).

Findings reported by the group are what would be predicted by the polio model -populations with the same genetic background are more or less at risk for type 1 diabetes depending on the age at which Coxsackie B (enterovirus) infections occur.

The studies to date have used ecological or case-control designs. The opportunity to carry out longitudinal studies would provide real promise for further exploration of this hypothesis, which if supported, would suggest opportunities for prevention.

3.4 Potential for Innovative Research

The study offers an unprecedented opportunity to complement the investigations of high-risk children by evaluating different disease susceptibility genes and environmental exposures. For example, there is evidence to suggest the contribution of HLA susceptibility alleles varies by age (Kondrashova et al., 2007). The largest relative and absolute risk estimates for children who carry high-risk genotypes are strongest for children younger than 5. The contribution of the same genotypes is much less dramatic among children with an older age at onset (older than 10). This suggests that around the time of puberty, loci other than those in the HLA region and/or environmental exposures may play a more important etiologic role. Thus, the evaluation of gene-environmental interactions in the development of type 1 diabetes must be evaluated among children with and without high-risk HLA susceptibility alleles.

3.5 Feasibility

Testing of the proposed hypotheses will require systematic collection and storage of information on family history of type 1 diabetes, child and family genetics, infant and child nutritional histories, longitudinal medical history and biomarkers of viral infections, and biomarkers of immune responses and onset of insulin resistance and diabetes mellitus. Data required to test this hypothesis and suggested specific hypotheses are included in the currently proposed Study protocol.

4. Exposure Measures

4.1 Individuals Targeted for Measurement

Primary/child

- Measures of diabetes susceptibility:
  - Genetic markers (e.g., insulin gene VNTR-insulin-dependent diabetes mellitus gene)
  - Family history of type 1 diabetes and other autoimmune disorders
- Environmental exposures:
  - Viruses (particularly Coxsackie virus B4)
  - Infant/childhood nutrition (particularly cow's milk and A1 β-casein content)
  - Pesticides or other chemical exposures

4.2 Methods

Primary/child

- Blood test
- Questionnaire
- Periodic environmental sampling
- Medical record review

4.3 Life Stage

Primary/child

- Prenatal and years 1, 2, 5, 10, 15, and 20

5. Outcome Measures

5.1 Outcomes Targeted for Measurement in Child

Primary/child

- Beta cell and other autoantibodies
- Manifestation of type 1 diabetes (e.g., average blood sugar level [HgbA1C], lipid profile, serum insulin levels, cortisol)

5.2 Methods

- Blood sample
- Physical exam
- Medical record review
- Questionnaire

5.3 Life Stage

- Periodically throughout participation

6. Important Confounders, Mediators, and Effect Modifiers

- **Unknown environmental factors:** Increase in risk associated with type 1 diabetes not sufficiently explained by genetic susceptibilities; most likely significant contribution by environmental factors (Hogan et al., 2003).
- **Family, medical history factors:** Several genetic loci implicated in risk for T1D, e.g., CTLA4 exon 1 A49G polymorphism; genotype higher in patients (12.6 percent) than controls (4.2 percent); may be family association ($p = 0.0229$) (Levin et al., 2004; Pitkaniemi, Onkama, Tuomilehto, & Arjas, 2004; Ziegler & Ziegler, 1989; Murao et al., 2004).
- **Obesity:** Lower risk for transient ischemic dilation (TID) associated with obesity even among genetically susceptible children (Zalloua et al., 2004).

7. Power and Sample Size

With an incidence of about 20 per 100,000 per year in U.S. populations ages 21 and younger, the study sample will likely yield at least 100 incident cases of type 1 diabetes. This number would yield more than 90 percent power, using a nested case-control design, to detect odds ratios of 2.5 or greater for exposures that affect approximately 10 percent of the general population. Less frequent exposures, such as the HLA haplotypes associated with type 1 diabetes, which may occur in only 2 percent of the population, may not be identified as significant predictors of type 1 diabetes unless their association is at least four- or five-fold.

The sample power calculations are based on simple bivariate comparisons without consideration of interactions, multiple measures, or the clustered survey design, all of which will decrease the power associated with each example. Most especially, the interaction between specific genotypic factors and multiple environmental exposures will need to be considered when estimating the lowest detectable odds ratio.

8. Other Design Issues

- **Ethical/burden considerations:** There may be ethical considerations associated with genetic tests, but such considerations underlie the overall study and would not be unique to this specific hypothesis. Collecting blood samples to obtain genetic information on parents and grandparents would make the study design complex.

For nongenetic factors, the Study will need to have a formal strategy and process to effectively communicate the results of physiological and biochemical measures to the child's parents and to a responsible health care provider. The Study also will need to have a formal strategy and process to effectively communicate the results of environmental monitoring to the child's parents along with appropriate and feasible recommendations regarding the correction of any unhealthful environmental findings, as state and/or federal laws require.

- **Cost/complexity of data collection:** Case/parent trios may be considered as a possible sampling unit.

- **Cost of sample analysis:** The use of exposure- and outcome-dependent sampling from stored or archived samples would provide efficiency (Green, Casabonne, & Newton, 2004).

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