# HAPO FOLLOW-UP STUDY PROTOCOL

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## 1. INTRODUCTION

### HAPO FOLLOW-UP STUDY SPECIFIC AIMS

Offspring of mothers with pre-existing diabetes mellitus (DM) or gestational DM (GDM) have an increased risk of obesity in childhood (1, 2). Moreover, GDM is associated with an increased maternal risk of type 2 diabetes (3, 4). What has not been established is the risk of childhood obesity and metabolic disorders or maternal risk of disorders of glucose metabolism (diabetes, impaired fasting glucose, impaired glucose tolerance) and other cardiovascular risk factors (dyslipidemia, increased abdominal adiposity (girth), elevated blood pressure (BP)) along the continuum of glucose to levels diagnostic of diabetes. The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Follow-up Study will use its unique resource, a cohort of women and their offspring who were recruited into the HAPO Study in 2000-2006, to address these questions. The overall ***hypothesis*** of the HAPO Follow-up Study is:

> ***Hyperglycemia in pregnancy, less severe than overt DM, is independently associated with increased risk of adverse childhood and maternal outcomes 8-12 years later.***

The HAPO Study was an observational epidemiologic investigation aiming to clarify unanswered questions on associations between levels of glucose tolerance during pregnancy with risk of adverse outcomes and to derive internationally acceptable criteria for the diagnosis and classification of GDM (5). The underlying hypothesis was that hyperglycemia in pregnancy, less severe than overt DM, is independently associated with increased risk of adverse maternal, fetal and neonatal outcomes. The HAPO Study examined glucose tolerance in a large, multinational, multicultural, racially diverse cohort of women in the third trimester of gestation with medical caregivers and participants "blinded" to the status of glucose tolerance. Based upon the HAPO Study results, a consensus panel formulated the new International Association of Diabetes in Pregnancy Study Groups (IADPSG) criteria for the diagnosis of GDM (8).

In HAPO, higher levels of maternal glucose were independently associated with increased frequency of birthweight, cord serum C-peptide, and infant adiposity (infant body fat or sum of skinfolds) above the 90$^{th}$ percentile (6, 7). As shown primarily in the offspring of diabetic mothers, these neonatal outcomes are risk factors for obesity and metabolic disorders in childhood and later life (1, 2). The nature of the associations and magnitude of risk associated with increasing levels of maternal glucose below those diagnostic of diabetes, including mothers who would be newly diagnosed with GDM based upon the new IADPSG criteria, are not well characterized. A HAPO Follow-Up Study is uniquely positioned to address these important questions given the breadth of maternal and neonatal phenotype data collected across multiple ethnic groups. Because women meeting the new IADPSG definition of GDM were not treated for GDM in the HAPO Study, the HAPO Follow-Up Study also provides a unique opportunity to examine the association of GDM with obesity and metabolic disorders in childhood unconfounded by GDM treatment.

### *1.1 Hypotheses and Specific Aims*

The ***General Aim*** is to obtain data on measures of adiposity and glucose, as well as insulin sensitivity and secretion, lipid metabolism, BP and inflammation in 7,000 HAPO offspring aged 8-12 and their mothers from multiple ethnic/race groups from 10 of the 15 HAPO field centers.

The primary aims and hypotheses of the HAPO Follow-up Study are as follows:

***Primary Aim 1:*** To determine associations of maternal glucose levels during pregnancy with measures of adiposity in offspring at ages 8-12 years.

***Hypothesis 1:*** Maternal glucose levels during pregnancy, including those that would be classified as GDM by the new IADPSG criteria, are positively and independently associated with measures of adiposity in offspring (obesity, overweight, percent fat).

***Primary Aim 2:*** To determine associations of maternal glucose levels during pregnancy with risk of disorders of glucose metabolism in mothers 8-12 years later.

***Hypothesis 2:*** Higher levels of maternal glucose, including those that would be classified as GDM by the new IADPSG criteria, are positively and independently associated with later maternal risk of disorders of glucose metabolism (diabetes, impaired fasting glucose, impaired glucose tolerance).

Data collected as part of the General Aim will allow additional analyses to be performed to address secondary aims and hypotheses, which are as follows:

***Secondary Aim 1:*** To determine associations of maternal glucose levels during pregnancy with measures of glycemia as well as insulin sensitivity and secretion, lipids, BP and inflammation in offspring at ages 8-12 years.

***Secondary Hypothesis 1:*** Maternal glucose levels during pregnancy, including those classified as GDM by the new IADPSG criteria, are positively and independently associated with measures of glycemia, lipids, BP and inflammation in offspring. In addition, maternal glucose levels during pregnancy are negatively and independently associated with insulin sensitivity and secretion.

***Secondary Aim 2:*** To determine associations of maternal glucose levels during pregnancy with measures of cardiovascular risk in mothers 8-12 years later.

***Secondary Hypothesis 2:*** Higher levels of maternal glucose, including those that would be classified as GDM by the new IADPSG criteria, are positively and independently associated with maternal cardiovascular risk factors (dyslipidemia, increased abdominal adiposity (girth), higher BP).

**Secondary Aim 3:** To determine associations of measures of neonatal adiposity and hyperinsulinemia with measures of adiposity, as well as glycemia, insulin sensitivity and secretion, lipids, BP and inflammation in offspring at ages 8-12 years.

***Secondary Hypothesis 3:*** Sum of skinfolds and/or percent fat and cord C-peptide levels at birth are positively associated with measures of childhood adiposity, glycemia, lipids, BP and inflammation and negatively associated with insulin sensitivity and secretion, independent of maternal glycemia and BMI during pregnancy.

## 2. BACKGROUND

The intrauterine environment has a clear effect on fetal development with both maternal glucose levels and adiposity having independent effects on fetal size at birth (6, 9). More importantly, it is now becoming increasingly evident that these intrauterine exposures can have a longer term impact on growth and development with effects lasting into childhood and adulthood (1, 10). This association between chronic disease risk and birth weight is not limited to macrosomic offspring of hyperglycemic or obese mothers, as babies of low birth weight are also at increased risk of chronic diseases, including obesity, hypertension and type 2 diabetes, as adults (11-16). As a population-based, multi-ethnic study, the HAPO Follow-up Study is uniquely positioned to provide important new information about the relationship between maternal glucose levels and adiposity during pregnancy and the risk of increased adiposity, glucose, insulin, lipids, and blood pressure during childhood.

### *2.1 Long-term Implications of Maternal Glucose Levels and Adiposity during Pregnancy*

A broad array of studies have now established that both pre-existing and gestational DM (GDM) are associated with an increased risk of macrosomia followed by an increased risk of being overweight or obese in childhood. Elegant studies performed in the Pima Indians comparing the BMI of siblings born before and after maternal diagnosis of type 2 DM (T2DM) showed that offspring exposed to the diabetic milieu had an increased BMI compared to siblings not exposed to diabetes during development (17). Moreover, offspring of mothers with type 1 DM (T1DM), who do not necessarily have a hereditary predisposition to obesity, have an increased risk of being overweight or obese during childhood compared to offspring of non-diabetic mothers (18-20). Finally, being born to mothers with GDM is associated with an increased risk of overweight, adiposity, or obesity in both early and late childhood as well as adolescence (21-24). This association has not been observed in all studies, although treatment of women with GDM may have contributed to an absence of an association in some studies (25-27). Together, the above studies suggest that exposure to diabetes during development increases risk of childhood overweight or obesity. The HAPO Study has now demonstrated that higher levels of maternal glucose during pregnancy less than those diagnostic of overt diabetes are also related to increased risk of birth weight > 90$^{th}$ percentile (6). However, it is not known whether these same levels of maternal glycemia are related to increased risk of being overweight or obese in childhood.

### 2.1.1 Implications for the Child

#### *2.1.1.1 Implications of maternal glucose levels for offspring overweight and obesity:*

To date, studies of the impact of maternal glucose levels on childhood overweight and obesity have focused largely on the offspring of diabetic mothers, although some studies have examined the potential association of maternal glucose levels during pregnancy and outcomes in children of mothers with glucose levels below those diagnostic of diabetes. For example, Hillier et al showed a positive trend for increased risk of childhood obesity at age 5-7 years across the range of increasing maternal glucose values during screening, and this trend remained after adjustment for confounders (28). In Pima Indians, Pettitt et al found an overall linear association between maternal 2-hour glucose during pregnancy and obesity in the offspring (29). One small study showed a significant association between glucose (on a glucose challenge test) in the absence of diabetes or GDM during pregnancy and offspring BMI at age 3, independent of maternal prepregnancy BMI (30). In HAPO offspring from Belfast, UK, a significant association between maternal glucose at 28 weeks gestation and offspring measures of adiposity (BMI Z score and sum of skinfolds) at age 2 was not observed (31). This is consistent with the early normalization of size of offspring of diabetic mothers in the first 2 years of life (19). Other studies have examined the impact of treating GDM on childhood obesity. For example, in offspring of mothers with treated GDM the risk of childhood obesity was attenuated compared with the risks for groups with lesser degrees of hyperglycemia which was untreated (28). However, another small study which compared childhood BMI at age 4-5 years among offspring of mothers who were or were not treated for mild GDM did not find differences (32); although this study did not control for maternal BMI which differed between the groups. Together, these studies support the idea that maternal glucose at levels less than those diagnostic of diabetes impact childhood obesity, but they were small and/or had limitations.

#### *2.1.1.2 Implications of maternal glucose levels for other adverse outcomes in childhood:*

Beyond increased adiposity, maternal glucose levels during pregnancy are related to additional adverse outcomes in childhood. In Pima Indians, the level of maternal glucose during pregnancy, even among women who had normal glucose tolerance, was positively associated with risk of T2DM in offspring aged 10-14 after adjustment for confounders (33), whereas in a largely Caucasian population, exposure to maternal pre-existing diabetes or GDM increased risk of impaired glucose tolerance in offspring at a mean age of 12 years (34). Importantly, this risk was not confined to offspring that were large for gestational age at birth (34,35), and an association with fetal hyperinsulinemia has been suggested (34). Finally, in a multiethnic cohort of youth with and without T2DM, those with T2DM were more likely to have been exposed to diabetes in utero and exposure to diabetes in utero was independently associated with T2DM (36).

Others have demonstrated higher glucose, insulin and lipid levels during childhood in the offspring of mothers with T1DM (20), although exposure to pre-existing diabetes has not been shown in all studies to affect glucose tolerance during childhood (18). Intrauterine exposure to diabetes among the Pima Indians was also a significant determinant of higher hemoglobin A1c and systolic blood pressure during childhood, independent of adiposity and a genetic predisposition to T2DM (37). Increased systolic and mean arterial blood pressure at ages 10-14 were found in offspring of mothers in the Chicago area with either pre-existing diabetes or GDM (38). Increased systolic and diastolic blood pressure, along with lower HDL cholesterol, were also observed in Asian offspring of mothers with GDM (25). Finally, a small study of mothers and their children in India found that maternal GDM is associated with higher insulin concentrations in female offspring at age 5 and higher glucose, insulin, and blood pressure at age 9.5 independent of maternal adiposity (39).

Exposure to hyperglycemia in utero also impacts adult health. Adult offspring of mothers with T1DM have an increased risk of T2DM and the metabolic syndrome and exhibit decreased insulin secretion compared to controls (40-42). Together, the above data suggest that exposure to maternal hyperglycemia in utero impacts glucose and, possibly, lipid levels as well as blood pressure during childhood and subsequent risk of type 2 diabetes as adults.

#### *2.1.1.3 Implications of maternal obesity for the offspring:*

The hypothesis being addressed in the HAPO Follow-Up Study is that hyperglycemia in pregnancy, less severe than overt DM, is independently associated with increased risk of adverse childhood and maternal outcomes 8-12 years later. A confounding factor in addressing this hypothesis is maternal obesity, as it not only influences maternal glucose levels but is also an independent risk factor for being obese or overweight

during childhood. The British Birth Cohort Study found a strong independent association between maternal BMI (assessed when the offspring were 11) and offspring BMI at 11 and 45 years of age (43). The Motherwell birth cohort demonstrated that percentage of body fat, BMI, and fat mass at age 30 were significantly and independently related to maternal BMI early in pregnancy (44), while the Northern Finland Birth Cohort study showed that maternal prepregnancy overweight is an independent risk factor for offspring overweight and abdominal obesity at 16 years, although the risks were highest in offspring who were exposed both to prepregnancy overweight and GDM (26). An important confounder related to maternal overweight is maternal glucose levels, which have been addressed in a few studies. Catalano et al showed that maternal pregravid BMI, independent of maternal glucose status or birth weight, was the strongest predictor of childhood obesity (27), while another study in which follow-up did not extend beyond 2 years of age demonstrated association of childhood BMI with maternal prepregnant BMI independent of and not related to maternal glucose levels (45). Finally, in HAPO offspring from Belfast, maternal BMI was an independent predictor of offspring BMI Z score at age 2 (31).

As with maternal glucose levels, the risks associated with maternal obesity extend beyond childhood overweight. In youth from the US, exposure to maternal obesity in utero was independently associated with T2DM (36). A Brazilian birth cohort study showed that maternal prepregnancy weight and BMI were positively associated with systolic and diastolic BP at 11 years of age (46), while a small study showed that risk of metabolic syndrome by age 11 years was significantly associated with large for gestational age at birth and maternal obesity, independent of the presence or absence of GDM (47). Finally, a British cohort study showed that greater prepregnancy weight was associated with greater offspring adiposity and more adverse cardiovascular risk factors at age 9 years (48).

Given these effects of maternal BMI and the interrelationships of maternal BMI and glucose levels during pregnancy, it will be important to establish that observed associations of maternal glycemia with childhood overweight and obesity are independent of maternal BMI.

#### *2.1.1.4 Implications of birthweight:*

The major focus of the HAPO Follow-up Study is the association of maternal hyperglycemia with adverse childhood outcomes. Typically, at birth, offspring of mothers with higher levels of glucose have higher birth weight. However, birth weights in the HAPO Study spanned the full spectrum and included offspring with low as well as high birth weight. In that context, it is important to note that, like high birth weight babies, low birth weight babies also appear to be at increased risk of adverse childhood outcomes. Lower birth weight has been associated with higher systolic blood pressure, fasting plasma insulin, glucose, skinfolds, and cholesterol concentrations at age 8, although information related to maternal glucose levels during pregnancy was not considered (49). The association of low birth weight with higher BMI, increased fat mass, and central distribution of fat during childhood is most evident in those children who undergo early postnatal weight gain with upward weight centile crossing in the first years of life (50-53). Lower insulin secretion is also present in children who are thinner at birth regardless of their pattern of postnatal growth, suggesting that beta cell mass is programmed in utero (50).

Several studies have demonstrated a U-shaped association between birth weight and childhood and adult outcomes. A nationwide study in Taiwan showed a U-shaped association after adjustment for confounders between birth weight and childhood risk of type 2 diabetes (54). This has also been shown in Pima Indian children, independent of current weight and height (55). Harder et al in a meta-analysis showed a relation between birth weight and later-life risk of type 2 diabetes which is not linear but U-shaped (13). However, in a second meta-analysis the positive association between birth weight and type 2 diabetes risk at higher birth weights was not as evident in all of the populations studied, although clear U-shaped associations were evident in Pima Indians, Native North Americans and the Canadian general population (14). Most of the studies in these meta-analyses included some adjustment for confounders but few included maternal characteristics such as BMI or glucose levels.

The association between low birth weight and adverse metabolic outcomes and the presence of the U-shaped association in some populations described above will need to be considered in the proposed analyses to ensure that associations in low birth weight offspring do not confound identifying association of maternal hyperglycemia with adverse child hood outcomes.

#### *2.1.1.5 Implications of childhood obesity and glucose levels:*

As might be anticipated, childhood obesity has negative consequences for health status in both children and adults. First, prior to the onset of puberty, childhood obesity is associated with a proinflammatory and prothrombotic state before other comorbidities of the metabolic syndrome are present (56). Indeed, inflammatory markers are strongly associated with increased BMI in children as young as 3 years (57). A cross-sectional study of lean Chinese children and adolescents showed that BMI is strongly and independently associated with systolic blood pressure (58). Childhood obesity also has negative consequences for adult health. Childhood obesity tracks strongly into adulthood (59,60). In the Bogalusa Heart Study, children aged 5-17 with a BMI $\geq$ 95$^{th}$ percentile were significantly more likely to be obese as adults, after adjustment for childhood adiposity, gender, race, and age (61). Similarly, obese adolescents compared to overweight and normal weight adolescents are more likely to develop severe obesity as adults (hazard ratio 16.0) (62) and, thus, are at increased risk of the attendant negative health consequences of severe obesity. Finally, Franks et al. (63) demonstrated recently that adult Pima Indians in the highest quartile of BMI as children have double the rate of death from endogenous causes compared to adults in the lowest quartile of BMI as children. Thus, childhood obesity has negative consequences on the long term health status of individuals.

In addition to childhood overweight and obesity, outcomes of the HAPO Follow-Up Study include measures of glucose homeostasis, which are also associated with adult disease. The Bogalusa Heart Study demonstrated that children in the highest quintile of fasting glucose, insulin, and insulin sensitivity (as measured by HOMA-IR) tended to track in this quintile over a 17-yr period of follow-up, while glucose, insulin and HOMA-IR in the top decile predicted the development of prediabetes and T2DM (64). Studies in this same population also demonstrated that a fasting plasma glucose > 86 mg/dl in childhood was associated with a > 2-fold increase in risk for developing prediabetes and T2DM (65).

### 2.1.2 Implications for the Mother

#### *2.1.2.1 Implications of abnormal glucose levels during pregnancy:*

Previous studies have clearly established that women with gestational diabetes are at increased risk of developing T2DM (66,67). The O'Sullivan study that provided the original data for the diagnosis of GDM indicated that the risk of diabetes during 5-10 years of follow-up was related to the severity of glucose intolerance during pregnancy (66). What is less clear is the level of risk in women who will be diagnosed with GDM using the new IADPSG criteria (8). The results of prior studies suggest that these women will also be at increased risk. In studies with a limited follow-up period of 3 months postpartum, any degree of abnormal glucose homeostasis in pregnancy was an independent predictor of glucose intolerance postpartum (68). One small study showed that pregnant women with a single abnormal glucose value during an OGTT had an increased likelihood of developing disorders of glucose metabolism later in life, similar to GDM (69). Carr et al showed that women with modestly elevated glucose levels below the threshold for GDM had a higher risk of subsequent diabetes with an average of 9 years of follow-up; however, they only adjusted for age, parity, and preterm delivery and not for BMI or other potential confounders (70). The level of fasting glucose during pregnancy, even within the normal range, also demonstrated a continuous positive association with maternal insulin resistance 5 years later, although results were again not adjusted for potential confounders (71). Others have demonstrated that mild glucose intolerance during pregnancy is associated with increased likelihood of metabolic syndrome (72) and an increased risk of subsequent cardiovascular disease (73). Finally, in a study of Scottish women, the level of hemoglobin A1c during pregnancy was associated with a dose-related increase in the risk of cardiovascular disease and hypertension 20 years later (74).

## *2.2 Outcomes*

Given the work done to date as delineated above, the outcomes listed below will be examined.

### 2.2.1 Primary

The primary outcomes in the HAPO Follow-Up Study are:

- Child adiposity
- Maternal glucose intolerance

### 2.2.2 Secondary

Child secondary outcomes include:

- Glucose levels and intolerance
- Insulin sensitivity
- Insulin secretion
- Lipidemia
- Blood pressure
- Level of inflammation

Maternal secondary outcomes include:

- Measures of adiposity
- Lipidemia
- Blood pressure

## 3. DESIGN AND METHODS

### *3.1 Introduction*

The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study was an observational epidemiologic investigation aiming to clarify associations of levels of glucose intolerance during pregnancy and risk of adverse outcomes and to derive internationally acceptable criteria for the diagnosis and classification of gestational diabetes mellitus (GDM) (5). The underlying hypothesis was that hyperglycemia in pregnancy, less severe than overt diabetes, is independently associated with increased risk of adverse maternal, fetal and neonatal outcomes. The HAPO Study examined glucose tolerance in a large, multinational, multicultural, racially diverse cohort of women in the third trimester of gestation with medical caregivers and participants "blinded" to the status of glucose tolerance. A common protocol and data collection instruments, uniform training of personnel, and central laboratory for analyses were used. HAPO demonstrated continuous associations between increasing levels of maternal glucose and each primary and secondary study outcome. Based upon HAPO Study results, a consensus panel formulated new criteria for the diagnosis of GDM (8).

In HAPO, higher levels of maternal glucose were independently associated with increased frequency of birthweight, cord serum C-peptide, and infant adiposity (infant body fat or sum of skinfolds) above the 90$^{th}$ percentile (6, 7). As shown primarily in the offspring of diabetic mothers, these neonatal outcomes are risk factors for obesity and metabolic disorders in childhood and later life (1, 2). The nature of the associations and magnitude of risk associated with increasing levels of maternal glucose below those diagnostic of diabetes, including mothers who would be newly diagnosed with GDM based upon the new IADPSG criteria, are not well characterized. A HAPO Follow-Up Study of mothers and their offspring 8-12 years after participation in HAPO is uniquely positioned to address these important questions.

The HAPO Follow-up Study is to be accomplished with high quality standardized data collection on 7,000 women and their children from 10 of the original 15 HAPO field centers. The HAPO Follow-up Study includes a Clinical Coordinating Center (CCC), Data Coordinating Center (DCC), and Laboratory Coordinating Center (LCC) all located in Chicago and is funded by the US National Institutes of Health through a cooperative agreement.

Children, who will be 8-12 years old at the time of the follow-up study, will complete a 3-4 hour visit that includes collection of a urine sample for storage, questionnaires, measurement of height, weight, waist and mid-arm circumference, multiple skinfolds, body size and fat measures with the BOD POD, blood pressure, pubertal assessment, and completion of an OGTT with collection of blood for fasting measures of glucose, insulin/C-peptide, lipids, A1c and hsCRP, collection of a sample for longterm storage, and collection of blood samples at 30, 60, and 120 minutes for measurement of glucose and insulin/C-peptide, and collection of an additional blood sample for DNA (on those who provide consent). Mothers participating in the follow-up study will complete a 3-4 hour visit that includes collection of a urine sample for longterm storage, questionnaires, measurement of height, weight, waist and hip circumference, body size and fat measures with the BOD POD, blood pressure, and collection of fasting blood samples for glucose, insulin/C-peptide, lipids, A1c, collection of

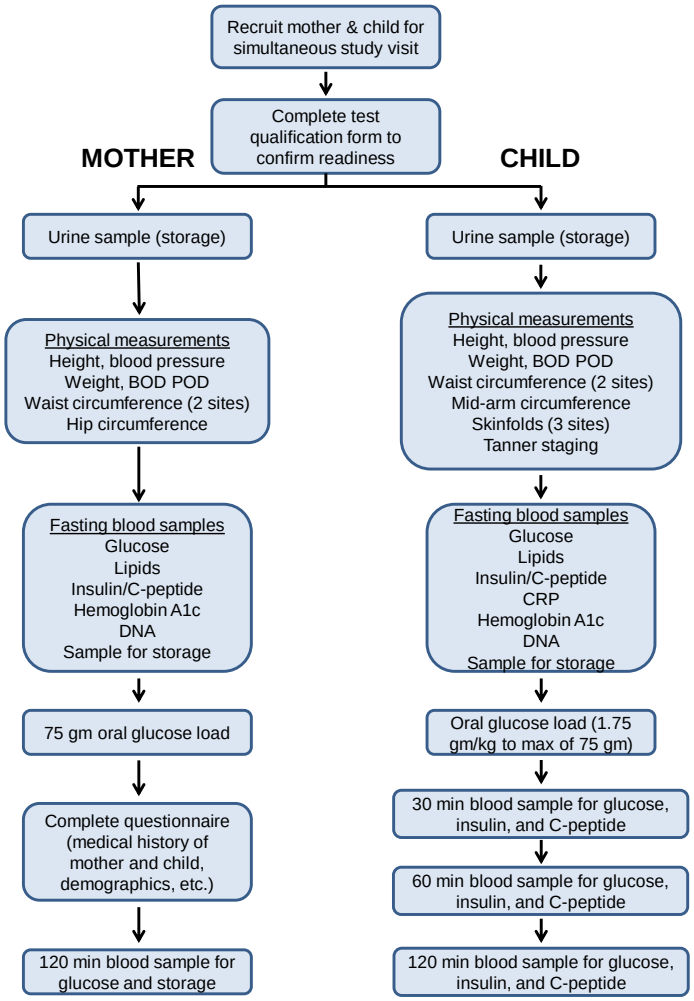


Fig. 1. Flow diagram of HAPO Follow-Up Study visits

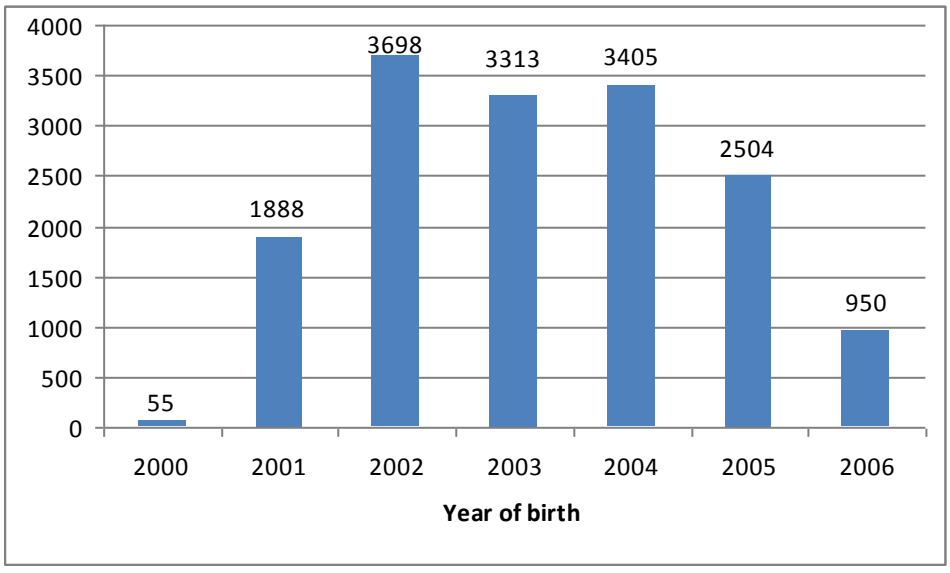


Fig. 2. Number of births per year

a sample for measurement of glucose at 120 minutes following a 75 g glucose load, collection of a sample for longterm storage at fasting and 120 minutes, and collection of an additional blood sample for DNA (on those who provide consent). Figure 1 provides an overview of the visits.

With 7,000 women and their children, the HAPO Follow-up study has high statistical power to detect associations between levels of glucose intolerance during pregnancy and risk of adverse child and maternal outcomes 8-12 years later. In addition, diversity of its population, its high quality standardized methods of data collection, including collection of data on potential confounding variables, its use of a Laboratory Coordinating Center for measurement of key metabolic variables (glucose, insulin/C-peptide, hemoglobin $A_{1C}$), and its analysis plan, all give high probability of valid assessment of its primary and secondary hypotheses.

### *3.2 HAPO Follow-Up Study Participants*

The distribution of the offspring date of birth for the blinded cohort at the 10 Follow-Up Study field centers is shown in Fig. 2. Follow-Up study visits will begin at the end of the summer of 2012 following training of field center staff. At that time, the majority of HAPO offspring will be 8-12 years of age. Recruitment will occur over a 3-year period and we will recruit the oldest children first followed by successively younger children. This will serve to reduce the age range of children at the time of follow-up.

The Follow-Up Study will recruit HAPO offspring and mothers across the range of maternal glucose levels represented in the blinded cohort from multiple ethnic/race groups from 10 of the 15 HAPO field centers (see Table 1). These 10 centers reflect a broad range of ethnic/racial groups and include 15,813 HAPO participants and their offspring. We will include 7,000 children and their mothers in the Follow-Up Study so that associations overall and within ethnic subgroups can be examined. We anticipate recruiting an average of 800 mothers and children from 8 of the 10 field centers and an average of 300 each from Chicago and Cleveland.

### *3.3 Standardization of Methods and Quality Control Procedures*

Uniformity of study methods and clinical and laboratory procedures are essential to the success of the HAPO Follow-Up Study. The plans for operationalizing the HAPO Follow-Up Study ensure that the field center investigators will have the capacity to carry out the study protocol and procedures in a standardized, consistent manner, enter collected data into the web-based REDCap data entry system and send samples to the Laboratory Coordinating Center in a timely, reliable and accurate manner.

Table 1. Proposed Study Sites and Eligible Participants

| **Field Center** | **Predominant Racial/Ethnic Group** | **Eligible** |
|---|---|---|
| Bellflower, CA | Hispanic | 1,774 |
| Barbados, West Indies | Black (Afro-Caribbean) | 1,827 |
| Chicago, IL | Caucasian | 688 |
| Cleveland, OH | Caucasian | 697 |
| Toronto, Canada | Caucasian[1] | 1,853 |
| Belfast, UK | Caucasian | 1,548 |
| Manchester, UK | Caucasian[2] | 2,086 |
| Petah Tiqva, Israel | Caucasian | 1,638 |
| Hong Kong, China | Asian/Chinese | 1,523 |
| Bangkok, Thailand | Asian/Thai | 2,179 |
| **Total** | | 15,813 |

[1]The Toronto center also enrolled a substantial minority of Asian participants from different countries of origin

[2]The Manchester center also enrolled a substantial minority of South Asian participants and a small minority of Afro-Caribbean origin.

The geographic distribution of the participating field centers assures ethnic/racial and socioeconomic diversity of the Study population and worldwide applicability of the findings; however, it introduces a number of challenges to obtaining the maximal possible uniformity of methods and procedures. These include language barriers, cultural differences and variability of clinical conditions, which have been taken into account in selection of the 10 field centers and study design.

The following steps are being taken in the HAPO Follow-Up Study to assure standardized procedures throughout:

- A common Protocol and Manual of Operations (MOO) are to guide all field work.
- Central training of field center personnel responsible for field work and oversight of local data entry are a requirement for field center participation.
- A single laboratory will measure key metabolic variables (glucose, insulin/C-peptide, lipid panel, hemoglobin $A_{1C}$); blood specimens must be shipped to the LCC under specified conditions for preservation.
- Standard equipment and supplies will be used, with delivery organized by the Coordinating Centers.
- Common centrally prepared data collection forms will be used. For foreign centers, where English is not the principal language, questionnaires administered to participants must be translated into the local language. Translated forms, prior to use, must be submitted to the DCC for back-translation to English, to check for uniformity of meaning.

Critical to study success are procedures to assess and enhance accuracy, i.e., quality control. For the HAPO Follow-Up Study, these include:

- Use of trained staff members to collect all data.
- Dry run of all procedures, except for the child OGTT (they will have to demonstrate calculation and preparation of the dose, and so on, but not do the actual blood sampling on the child), before the start

of actual data collection, with sufficient time for central review and any needed correction prior to start of field work; start of participant enrollment based on Executive and Steering Committee authorization.
- Use of blind duplicate samples to assess technical error of the laboratory, and thus provide external quality control of the laboratory.
- Entry on all forms of ID or name and initials of staff member performing specific procedure or task.
- System for checking accuracy of participant ID code on all forms and blood samples.
- Timely review and editing of all forms locally prior to data entry.
- Entry locally of all data forms using REDCap, including double entry of key data, via high speed internet connection and corresponding manual prepared by the DCC, with appropriate range, logic, and consistency checks.
- Defined procedures for the DCC and LCC for timely monitoring of data quality, including rapid edit procedures in the DCC of all data, with rapid feedback to field centers and the laboratory as necessary.
- Site visits during the Dry Run with site visit reports prepared for review by the Executive and Steering Committees.
- Additional site visits during field work as necessary.
- Retraining of field center staff as needed based on ongoing quality control procedures.

### 3.3.1 Language

English will be used as the language of the HAPO Follow-Up Study investigators including the research staff at the field centers. Standardized questionnaires (translated into local language, where appropriate) will be used to collect data from participants by interview with the research staff.

### 3.3.2 Manual of Operations (MOO)

A MOO has been developed by the Steering Committee, CCC, DCC and LCC to assure consistency and accuracy in use of all techniques (interviews, physical assessments, and laboratory procedures, including shipping of blood specimens to the LCC). Methods and procedures are laid out in great detail in the MOO and are not included in detail in this Protocol except in those instances where it is important to provide the rationale for selection of a given procedure or the timing of a particular test.

### 3.3.3 Standardized Central Training

We will conduct extensive central training of HAPO Follow-Up Study personnel in use of the MOO. Training will consist of lectures, slide presentations, and practical sessions, as well as individual consultations. Training will cover all aspects of the study, including: recruitment and informed consent; use of HAPO participant IDs and record keeping; child and maternal data collection; blood pressure, height, and weight measurement; anthropometric measurements; pubertal assessment; drawing, processing, storage, and shipping of blood specimens; data editing and entry; roles of field center PIs; and ethical issues. Training will take place centrally in face to face sessions conducted by the DCC, CCC, and LCC prior to beginning the enrollment of participants at the individual field centers. A package of training materials will also be prepared and distributed to each field center.

## *3.4 Recruitment and Enrollment*

An important objective of the HAPO Follow-up Study is to maximize the response rate among children and their mothers who are invited to participate. Among this cohort, exclusion criteria as determined from the original HAPO Study database will be:

- Delivered at < 37 weeks
- Had a fetal, neonatal or maternal death
- Had a HAPO baby with a major malformation

Potential participants will be contacted by the Research Nurse or other staff, the follow-up study explained and agreement for participation requested. Participants will be scheduled to have a clinic visit during the morning

after an overnight fast of at least 8 hours. At that visit standardized questionnaires will be administered by the Research Nurse or another trained and certified interviewer to ascertain demographic and other data, and height, weight, anthropometric measures and blood pressure will be measured and phlebotomy performed by trained and certified staff.

### 3.4.1 Special Circumstances

During recruitment some questions will be asked about the presence of circumstances that may exclude performing the OGTT but would still allow other data to be collected. Special circumstances include:

1. Mother reports that she and/or her child has a diagnosis of diabetes and is on treatment with oral medication or insulin. They will be asked to bring their medication to the visit. The individual with diabetes on medication will be told not to fast and samples that are collected at the fasting draw will be drawn. The OGTT will not be performed but the rest of the visit will be performed. If a diagnosis of diabetes is reported but there is no treatment, the individual will be asked to fast and the usual visit will be conducted.
2. Mother reports she is HIV positive or has Hepatitis B or C. In this circumstance, due to international shipping regulations, blood sampling will not be performed. Confirm with her that the child is not HIV positive and doesn't have Hepatitis B or C and that the child will participate. The mother will have the rest of the visit. The child will have the full visit.
3. Mother reports having undergone a bariatric surgery procedure. She will be asked whether she has been diagnosed with diabetes. She will have fasting samples collected but the OGTT will not be performed. She will have the rest of the visit.
4. Mother is currently pregnant or breastfeeding. If the mother agrees, the child will have the full visit and the mother will complete the Questionnaire. She will be asked to complete the visit at a later date.
5. The mother and/or child takes medication(s) regularly. They will be asked not to take the medication on the morning of the visit and to bring all medications to the visit. If any of the medications are oral anticonvulsants, glucocorticoids/corticosteroids, or atypical antipsychotics fasting samples will be collected but the OGTT will not be performed. The rest of the visit will be completed. If the medication is Metformin but a diagnosis of diabetes was not reported, the full visit, including the OGTT, will be performed and the mother will be asked to contact the physician's office to determine the reason for treatment with Metformin (diabetes, abnormal glucose, polycystic ovary syndrome, weight control, other).

## *3.5 Implementation of the Protocol*

### 3.5.1 Procedures

To assure uniformity of materials and technique, the glucose test doses will be purchased from a single source by the CCC and distributed to the field centers. Phlebotomy supplies and sample shipping and storage vials will also be distributed from a common, uniform CCC source.

#### *3.5.1.1 Child OGTT:*

After an overnight fast of >= 8 hours, venous blood will be collected for fasting PG and lipid, insulin/C-peptide, A1c, and hsCRP assays. An additional sample will be collected for longterm storage for potential future assays. Among those whose mother consents to it, a DNA sample will be collected. A glucose load (Trutol) of 1.75 gm/kg of body weight (not to exceed 75 gm total) will be given and samples for glucose and insulin/C-peptide assays collected at 30, 60 and 120 minutes after the glucose load. A total of 30 ml of blood will be drawn from an indwelling catheter. In addition, a urine sample will be collected and stored.

#### *3.5.1.2 Maternal OGTT:*

After an overnight fast of >= 8 hours, venous blood will be collected for fasting PG and lipid, insulin/C-peptide, and A1c assays. An additional sample will be collected for longterm storage. Among those who consented to it, a DNA sample will be collected. Those consenting to a full OGTT will drink 75gm of glucose in 296 ml of water (Trutol) over the course of 5 minutes unless the mother weighs < 42.6 kg, in which case she will receive 1.75 gm/kg. A sample for PG will be collected at 120 minutes after the glucose load. An additional sample for longterm storage for potential future assays will also be collected. A total of 40 ml of blood will be collected via needlestick. In addition, a urine sample will be collected and stored.

#### *3.5.1.3 Duplicate Samples:*

For purposes of quality control, the backup aliquot of all analysis samples will be sent to the laboratory for analysis on a randomly selected 5-10% of participants. Separate samples are not being collected for this due to the volume restrictions imposed by most IRBs/ethics boards for pediatric sampling.

### *3.6 Data to be Collected*

All data will be collected and examinations performed by trained personnel using the MOO and standardized procedures and forms. Physical measurements will be obtained at the time of the clinic visit. All other data will be ascertained from standardized questionnaires (translated into the local language, when appropriate) administered by a Research Nurse or other trained staff member prior to or at the time of the visit. The following represent the data to be ascertained in the HAPO Follow-Up Study:

Data that will be collected include:

- Identifiers: Mother and child's name, dates of birth, address and phone number; name, address and phone number, if available, of two contacts. To protect the privacy of HAPO Follow-Up Study participants, this information will be kept in a locked file (not transmitted to the DCC). If ancillary studies are performed at a later date, this information may be used locally to contact participants.
- Questionnaire (questions asked of the mother): visit date, maternal age, date of birth, marital status, employment status, years of education, presence of any major life stressors during the HAPO pregnancy, number of subsequent pregnancies and deliveries, any diagnosis of and medications for hypertension, diabetes, or dyslipidemia in self and biological father, first degree family history or diabetes and hypertension, menopausal status, current use of oral or other hormonal contraceptives, current smoking, alcohol consumption and medications, breast feeding duration and exclusivity for HAPO baby, height, weight, and birthweight of the child's biological father, if available. Information will also be collected about the child including socio-demographics (age, date of birth, gender), number of hours spent watching TV or playing computer games not requiring physical activity, number of hours sleep/night, current medications, medical conditions, that may have interfered with growth (chromosomal abnormalities, Down's syndrome, major endocrinopathies, cancer, rheumatoid arthritis, inflammatory bowel and GI diseases of malabsorption, renal disease), and menstrual history (for girls).

#### 3.6.1 Child Physical Measurements

Child physical measurements that will be performed:

- Blood presssure with an electronic device, height on a stadiometer, weight on the BOD POD calibrated scale, waist and mid-arm circumferences, multiple skinfolds (subscapular, suprailiac, triceps), pubertal assessment (Tanner stage for breast/areolar development for girls, measurement of testicular volume with a Prader orchidometer for boys) (75-77) and determination of fat and fat-free mass from air-displacement plethysmography (BOD POD).

#### 3.6.2 Maternal Physical Measurements

Physical measurements that will be performed on the mother:

- Blood pressure with an electronic device, height on a stadiometer, weight on the BOD POD calibrated scale, waist and hip circumferences, determination of fat and fat-free mass from air-displacement plethysmography (BOD POD).

#### 3.6.3 Existing HAPO Study Data

HAPO provides a rich database of phenotype information on the prenatal intrauterine environment and newborn traits that represent a unique resource. Data and/or resources that have been collected and will be available for analyses during the Follow-Up Study are outlined below.

*Data Collected During the HAPO Study*

NIH support for the HAPO Study was augmented by a grant from the American Diabetes Association that supported measurement of maternal fasting and 1-hour stimulated serum C-peptide and collection, processing, and storage of maternal and newborn (cord blood) DNA. Data that were collected by the HAPO Study include. Mothers: (i) Demographics (ascertained via standardized questionnaires): age, educational level, marital and employment status, ethnicity, first degree family history of diabetes and/or hypertension, frequency of smoking or alcohol use during pregnancy, prenatal weight, birthweight, father's height, weight, and birthweight. (ii) Physical measurements: weight, height, and blood pressure measured at the OGTT visit (28 weeks with a range of 24-32 weeks gestation). (iii) Blood samples: fasting, 1-, and 2-hour plasma glucose, A1c, fasting and 1-hour C-peptide, DNA on consenting women. (iv) Medical record abstraction: prenatal, labor and delivery, and postpartum course of care, pregnancy-induced hypertension, hospital readmission after delivery.
Offspring: (i) Blood samples: cord blood for glucose and C-peptide, 1-2 hour heel prick sample for plasma glucose, DNA of those whose mothers consented. (ii) Neonatal anthropometrics: weight, length, head circumference, skinfolds (flank, subscapular, triceps) measured within 72 hours of delivery. (iii) Medical record abstraction: neonatal course of care, adverse outcomes. (iv) 6-week follow-up questionnaire: to ascertain adverse outcomes following discharge.

*Storage of Existing Samples*
Samples collected during the HAPO Study are stored in -80 freezers in a temperature controlled, locked room, in the Northwestern University Department of Medicine Freezer Farm. These freezers contain a continuous monitoring system and are linked to a Sensaphone autodial system. This system will dial, in sequence, telephone numbers of designated HAPO investigators and staff when a malfunction is detected and continue to do so until an answer is obtained. All of the freezers are linked to a backup electricity generating supply in case of primary electricity supply failure.

## *3.7 Specific Outcomes*

### 3.7.1 Primary Outcomes

#### *3.7.1.1 Child adiposity:*

Primary measures of adiposity will be overweight and obesity which will be determined based on International Obesity Task Force cutpoints (78). Additional measures of adiposity will be determined based on percent fat (BOD POD) and waist circumference greater than the 85$^{th}$ percentile.

#### *3.7.1.2 Maternal glucose intolerance:*

Glucose intolerance defined as impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and diabetes mellitus based on a 2-hour 75g OGTT using ADA criteria (79).

### 3.7.2 Child Secondary Outcomes

#### *3.7.2.1 Glucose levels and intolerance:*

Continuous glucose levels and glucose intolerance defined as impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and diabetes mellitus based on a 2-hour 75g OGTT using ADA criteria (79).

#### *3.7.2.2 Insulin sensitivity and secretion:*

Insulin secretion will be estimated by calculating the insulinogenic index (insulin 30- insulin 0)/(glucose 30- glucose 0) as described by Phillips et al. (80). Insulin sensitivity will be estimated using the Homeostasis model (HOMA) by calculating HOMA-IR = {[fasting insulin (□U/ml)] x fasting glucose (mmol/l)]}/22.5 (81). Insulin sensitivity will be calculated as an index using the equation first described by Matsuda and DeFronzo (ISOGTT) where ISOGTT = 10,000/ the square root of [(FPG) x (FPI) x (G x I)] where FPG and FPI are fasting plasma glucose and fasting plasma insulin, respectively, and G and I are mean glucose and mean insulin from 0 to 120 minutes (82). Similar calculations will be done using C-peptide.

#### *3.7.2.3 Lipidemia:*

Total cholesterol, HDL cholesterol and triglycerides will be measured. LDL cholesterol will be calculated using the above values and the Friedewald equation (83), provided triglycerides are less than 400.

#### *3.7.2.4 Blood pressure:*

Systolic and diastolic blood pressure will each be measured three times using an automated device.

#### *3.7.2.5. Level of inflammation:*
A sample for high sensitivity C-reactive protein (hsCRP) will be collected.

### 3.7.3 Maternal Secondary Outcomes

#### *3.7.3.1 Overweight/obesity/adiposity:*
A BMI of 25 will be classified as overweight and a BMI of 30 will be classified as obese.

#### *3.7.3.2 Lipidemia:*
Total cholesterol, HDL cholesterol and triglycerides will be measured. LDL cholesterol will be calculated using the above values and the Friedewald equation, provided triglycerides are less than 400.

#### *3.7.3.3 Blood pressure:*
Systolic and diastolic blood pressure will each be measured three times using an automated device.

Child and maternal primary and secondary outcomes will be examined as both continuous variables and dichotomous or categorical variables.

## 4. SAFETY

### *4.1 Patient Safety*

### 4.1.1 Informed Consent
Although countries have different requirements for the institutional review board (IRB)/ethics committee and informed consent process, because the HAPO Follow-up Study is funded in part by the NIH, the Study is obligated to follow the policies and requirements of the Office for Protection from Research Risks (OPRR), an agency of the US government. Therefore, each time the protocol is revised or an ancillary study added, the change must be approved by the local IRB/ethics committee before it can be implemented. In addition, annual IRB/ethics committee review and approval is required. Each consent form will include the elements of informed consent as required by Title 45 in the Code of Federal Regulations. All mothers will be provided with details regarding the study and data will not be collected until the consent form has been signed. All participating children will sign an Assent Form where required by the local IRB/ethics committee.

### 4.1.2 Risks from Study Procedures
Potential participants will be informed that bruising at the site of blood sample collection is a potential risk of study participation. They will also be informed that they may feel claustrophobic in the BOD POD chamber but this is very rare. If this occurs, they can signal that they wish to stop the test. And, they will be informed that some people feel faint or lightheaded after the OGTT and, in very rare circumstances, vomit while drinking the Trutol. A snack will be provided after the OGTT and the participant will get up slowly to prevent any dizziness or fainting.

### 4.1.3 Alert Laboratory Values
When samples for glucose, A1c, lipids, and child hsCRP are assayed, the results will be provided to the field centers on a weekly basis for reporting back to the mothers. When the laboratory results are provided to the centers, those with a fasting plasma glucose $\geq$ 126 mg/dl (7 mmol/l) and/or a 2-hour plasma glucose $\geq$ 200 mg/dl (11.1 mmol/l) who are not known to have diabetes will be flagged as having a level diagnostic of diabetes. Other values that will be flagged as alert values will be LDL cholesterol $\geq$ 160 mg/dl (4.14 mmol/L), triglycerides $\geq$ 500 mg/dl (5.65 mmol/L), and A1c > 8.0%. Field centers will decide locally what specific values require urgent notification of the participant. Thresholds for blood pressure referral are dscribed in the MOO.

### *4.2 Privacy and Confidentiality*
Confidentiality of all data regarding individuals will be maintained. The Coordinating Centers will not receive names of HAPO Follow-Up Study participants. Only HAPO Follow-Up Study ID codes will be forwarded and

names will be removed from any forms sent to the DCC. Data on computers at the field centers will be safeguarded by passwords known only by authorized personnel, and participant names will not be entered.

### *4.3 Data Security*

#### 4.3.1 Northwestern University Bioinformatics Core (NUBIC) Security

To protect and monitor data collected by REDCap, a number of security measures have been implemented. A network firewall controls access to the application servers by only allowing traffic from known subnets, minimal set of data ports and minimal protocols. Thus, access is denied or closed by default. Behind this firewall is a two tiered system design. One server hosts the web server and application specific code. A second server hosts the database. Traffic between these two servers is again restricted by IP, port, and shared access key, and never leaves the private network. This ensures that only the trusted application server can talk to the database server and prevents unauthorized access to the database server from direct external attacks. To protect data in the case of corruption or system error, all data are backed up on a nightly basis with full redundancy.

The servers running NUBIC applications are currently Linux based. The operating system is CentOS, an open source version of Redhat Enterprise Linux (RHEL), which has excellent community support. CentOS is a widely used enterprise level Linux OS with a large user community. Patches are applied to the system when needed to address specific vulnerabilities or stability issues. The webserver is Apache which is the leading open-source web server. All application code is written in PHP and JavaScript. All data are stored in an open-source MySQL relational database server. To increase database-level security, access to the database is restricted on a per-application basis. This prevents, in the event of application level compromise, one application gaining access to another application's databases. For production database instances, NUBIC also implements table-level auditing to ensure compliance and an audit trail.

REDCap has application level security that restricts access to study data based on user roles and permissions. In addition to application level security, all access to REDCap is over SSL (https). This prevents any third party from deciphering any data sent between a user of REDCap and the system itself. This is the same level of encryption used on online banking, e-commerce, and other sensitive websites. All accounts for authenticated end users of the applications are tied with their Northwestern University NetID through a centralized identity management system that ensures strong passwords and frequent password changes.

#### 4.3.2 Data Coordinating Center Security

The Data Coordinating Center housed in the Department of Preventive Medicine uses microcomputers, connected via a local area network, for data storage. Data are stored in SAS data sets on hard disk. To preserve confidentiality of information, identification of individual participants is by means of the HAPO Study ID only and names will not be transmitted to the Data Coordinating Center. Efforts will be made to ensure that no data are lost. Data forms and microcomputers located at the Data Coordinating Center will be locked up outside working hours. To protect data from unauthorized use, files will be protected by giving each staff member a unique password known only to Coordinating Center staff.

Daily backup to the Department of Preventive Medicine's back-up servers will be done automatically every evening. There will also be weekly and monthly backups of the entire system to DVDs. Copies of these backups will be stored off-site. Prior to the processing of any new data from a field center (including data for that field center from the laboratory), all prior data from that center will be extracted, compressed, and stored on a DVD. In this way, data from each field center will be journaled prior to an update. If a serious error is discovered after new data are processed, it will be possible to restore the center's data to their original condition.

#### 4.3.3 Laboratory Coordinating Center Security

Laboratory samples will be scanned into BC Sample, a sample tracking software system which is housed at NUBIC (see **4.3.1** for NUBIC security details). Results of samples assayed in the hospital laboratory will be stored in the Northwestern Medicine Enterprise Data Warehouse (EDW) which is also housed at NUBIC. The EDW complies with hospital policies and procedures which are compliant with the Health Insurance and

Portability and Accountability Act (HIPAA). Examples of these policies include the use of a change management system, anti-virus software, off-site system backups, documented disaster recovery procedures, and regular and frequent system security batching. Additionally, the EDW is behind an institutional firewall that is monitored at all times for breeches.

## 5. STATISTICAL CONSIDERATIONS

### *5.1 Background*

The primary aims of the HAPO Follow-up Study are: 1) to determine associations of maternal glucose levels in the third trimester of pregnancy (fasting and in response to a 75g glucose load) with measures of adiposity in offspring at ages 8-12 years and 2) to determine associations of maternal glucose levels during pregnancy with risk of disorders of glucose metabolism in mothers 8-12 years later. These associations will be examined with adjustment for field center and potentially important confounders assessed during pregnancy, including maternal age, body mass index (BMI), height, mean arterial pressure, parity, smoking, drinking, any family history of diabetes, gestational age at the OGTT, (variables previously included in HAPO models), as well as such variables as child age, gender, and Tanner stage (75, 76). Models would also include any other potentially important confounders identified by the Steering Committee for inclusion in analyses. We do not expect that adjustment for ethnicity will be necessary, since in HAPO, ethnicity was not related to outcomes after adjustment for field center, given the strong overlap between ethnicity and field center.

The enrollment target for the HAPO Follow-up Study is 7,000 mothers and their offspring, with an average of 800 recruited from each of 8 field centers and an average of 300 each recruited from the Chicago and Cleveland field centers.

In determining the necessary sample size for the HAPO Follow-up Study, we have taken a relatively formal hypothesis testing approach to justification of the 7,000 participants proposed using a significance level of 0.05. An important goal of the follow-up study is to estimate associations along with their corresponding 95% confidence intervals of maternal glucose levels during pregnancy with measures of adiposity during childhood, and risk of disorders of glucose metabolism in mothers. To this end, we will perform a number of different analyses to gain insight and understanding of these associations. These different analyses are described below under 5.3 **Statistical Analysis Plan**.

### *5.2 Sample Size Requirement*

#### 5.2.1 Sample Size and Power for Primary Aims

Estimating the total sample size required for the HAPO Follow-up Study's two primary aims requires assumptions on the size of the associations that might reasonably be expected between maternal glucose during pregnancy and measures of adiposity in childhood, and between maternal glucose during pregnancy and subsequent disorders of glucose metabolism in mothers. Because it is not clear a priori whether or not the associations between maternal glucose levels and these and other follow-up study outcomes will be continuous and graded, we propose here that the follow-up study be powered to detect associations between GDM as defined by the new IADPSG criteria (8) and dichotomous outcomes related to childhood adiposity, e.g. child overweight, including obesity, and maternal disorders of glucose metabolism. The sample size required for these analyses depends on four things: (1) The proportion expected to have GDM in the HAPO Follow-up Study sample; (2) the proportion of children who are overweight at 8-12 years of age and the proportion of mothers with disordered glucose metabolism 8-12 years post-delivery; (3) the expected odds ratios for GDM (yes vs. no) in relation to these outcomes; and (4) the expected multiple correlation coefficient of the variable GDM with the other variables likely to be included in a fully adjusted model.

##### *5.2.1.1 Sample size and power for primary aim 1:*

The overall rate of GDM in the 10 field centers proposed for the follow-up study is 16%. Hence, in the power calculations that follow, we have assumed that the rate of GDM in those participating in the follow-up study will be 16%. Available data from the International Obesity Task Force (*http://www.iaso.org/site_media/uploads/Global_Childhood_Overweight_April_2011.pdf*) suggest that the rate of overweight including obesity in the HAPO Follow-up Study, based on the Task Force cutoffs for these

outcomes, is likely to be in the range of 20-25% or even higher. With respect to strength of the association that might be expected between GDM and childhood overweight and obesity, in a study of children 9-14 years of age, Gillman et al found odds ratios of 1.2 for mothers diagnosed with GDM during pregnancy for at risk for overweight and 1.4 for overweight with adjustment for age, gender, and Tanner stage (84). Additional adjustment for other variables including mother's current BMI reduced the odds ratio to 1.0 for at risk for overweight and 1.2 for overweight. Hillier et al examined the association of results for a 3-hour OGTT, following a positive glucose challenge test (> 140 mg/dl), and child's weight > 85$^{th}$ percentile and 95$^{th}$ percentile for 5-7 year old children (28). Women were classified as GDM based on Carpenter – Coustan (C&C) criteria and National Diabetes Data Group (NDDG) criteria, but only women positive by NDGG criteria were treated. Women with 1 abnormal result by either set of criteria or who had GDM only by C&C criteria were not treated. For women with 1 abnormal result by either criteria compared to women with a normal GCT, adjusted odds ratios were 1.37 and 1.30 for the child's weight > 85$^{th}$ percentile and > 95$^{th}$ percentile, respectively. For women with GDM by C&C criteria, the adjusted odds ratios were 1.89 and 1.82, respectively. Odds ratios for treated GDM were 1.29 and 1.38, respectively. These latter odds ratios suggest that studies that have looked at GDM and subsequent overweight and obesity in children may be potentially confounded by the effects of treatment.

Lawlor et al examined the association of existing diabetes, GDM, and glycosuria with BMI, waist circumference, and fat mass in children 9-11 years (85). Among 40 GDM women, associations attenuated toward the null with adjustment for maternal prepregnancy BMI, but independent associations remained for the 232 women with glycosuria. The adjusted odds ratio for general overweight/obesity when comparing women with at least two episodes of ++ glycosuria with those with no evidence of diabetes or glycosuria was 1.35 and that for central obesity (top 10% of waist circumference) was 1.31.

Pettitt et al examined the associations of maternal glucose levels with child overweight and obesity at 22 – 30 months in the Belfast sample of the main HAPO Study (31). In unadjusted analyses, while overweight and obesity generally increased across fasting, 1-hour, and 2-hour plasma glucose levels, only the association between 1-hour glucose and child overweight was statistically significant. Because the number of women in the highest category of each glucose measure was relatively small, we combined the women in the two highest categories for each measure and computed unadjusted odds ratios relative to the lowest category. For FPG, the odds of child overweight and obesity for women with FPG $\geq$ 90, relative to FPG < 75 were 1.89 and 1.51, respectively; for 1-hr PG $\geq$ 172, relative to 1-hr PG $\leq$ 105, 1.85 and 1.11; and for 2-hr PG $\geq$ 140 relative to 2-hr PG $\leq$ 90, 1.44 and 1.07.

While the results of these studies do not provide a clear and convincing picture on the size of the odds ratio that might be expected on the association of GDM with the outcomes of child overweight and obesity, they do suggest that the sample size should be selected to have adequate power to detect odds ratios of 1.30 and greater. For univariate logistic regression, the sample size required to detect an odds ratio of 1.30 with 90% power is 6,513 for an outcome frequency of 20% in non-GDM women and 5,632 for an outcome frequency of 25% in non-GDM women. To obtain sample sizes for multiple logistic regression analyses, these numbers need to be adjusted upward by $1/(1 - R^2)$ where $R^2$ is the square of the multiple correlation coefficient of the binary variable – GDM (yes vs. no) -- with the other variables likely to be included in the model (86). Based on the current HAPO data, with adjustment only for field center for those field centers to be included in the follow-up study, the sample size would need to be increased by about 1.7% or to 6,623 and 5,727, respectively, in order to maintain power for an odds ratio of 1.30. With additional adjustment for variables such as maternal age, BMI, height, mean arterial pressure, parity, smoking, drinking, any family history of diabetes, gestational age at the OGTT, neonatal gender (variables previously included in HAPO models), the sample size would need to be increased by 8.5% or to 7,066 and 6,111, respectively. Inclusion of additional variables in the model could indicate a need to increase the sample size by as much as 10%, in which case the necessary sample sizes would be 7,164 and 6,195, respectively. Thus, 7,000 women and their offspring appear to be a reasonable target for recruitment in the HAPO Follow-up Study.

With respect to other measures of adiposity to be examined as part of the primary aim for children, because maternal glucose levels might be expected to be more strongly related to percent fat based on use of the BOD POD, and to central adiposity based on waist circumference, than to overweight and obesity based on child BMI, the selected sample size should have adequate power to detect slightly larger odds ratios, e.g. 1.35 and greater, for outcomes based on percent fat and waist circumference. Because power depends on the

proportion of children with the outcome, we propose to define outcomes for percent fat and waist circumference as greater than the 85$^{th}$ percentile. For a sample of 7,000 and a total outcome frequency of 15%, power is 90% to detect an odds ratio of 1.35, assuming a correction for multiple correlation with other variables in the model of 10%. If we were to instead define the outcomes for percent fat and waist circumference as > 90$^{th}$ percentile, a sample of 7,000 provides only 80% power for an odds ratio of 1.35, although 88% power for an odds ratio of 1.40.

In addition to examining the association of GDM with overweight and obesity based on child BMI and percent fat and waist circumference > 85$^{th}$ percentile, we will also examine the association of each glucose measure as a continuous variable with these outcomes. For these analyses, power and sample size estimates need to take into account the multiple correlation coefficient of each glucose measure with the other variables likely to be included in the model. With adjustment for those variables previously included in HAPO models, the necessary increases in sample size over that required for univariate logistic regression would be 24.0% for FPG, 18.4% for 1-hr PG, and 16.2% for 2-hr PG. With a total sample size of 7,000, there is at least 90% power to detect an odds ratio of 1.13 for glucose higher by one standard deviation for an outcome frequency of 15% if the sample size adjustment for multiple correlation is 25% or less. For an outcome frequency of 20%, the detectable odds ratio is 1.12, and for an outcome frequency of 25%, the detectable odds ratio is 1.11.

We will also examine the association of GDM with measures of adiposity as continuous variables. There is 90% power to detect a difference in mean child BMI, percent fat, or waist circumference between GDM and non-GDM women of 0.105 standard deviations of BMI, percent fat, or waist circumference for a sample size of 7,000. For example, if the standard deviation of child BMI is 4.0, then power is 90% to detect a mean difference of 0.42 kg/m$^2$ in child BMI between children of women with and without GDM.

Finally, we will also examine the association of each glucose measure as a continuous variable with continuous measures of adiposity in linear regression analyses. With a sample size of 7,000, power is 90% to detect a partial correlation of 0.039 between glucose and these outcomes.

***5.2.1.2 Sample size and power for primary aim 2:***

With respect to the association between GDM and disturbances of glucose metabolism in mothers, Carr et al (70) conducted a retrospective cohort study of first pregnancies delivered in Washington state between 1985 and 2002 and observed a 1% incidence of diabetes over a median follow-up of 8.8 years. In this study, women with a positive GCT (> 140 mg/dl) were given a 3-hour OGTT. In women given the OGTT, the rate of diabetes was 2.8% in women who had one abnormal value by C&C criteria, and 1.4% in women with no abnormal value. In addition, the incidence of diabetes increased progressively across GCT quartiles with 1.81% of the women in the highest quartile developing diabetes compared to 0.44% in the lowest quartile, and 0.75% in the three lowest quartiles. In a small study in Sicily (69) with an average follow-up of 6.9 years, among 66 women with one abnormal value by C&C criteria who were given a 3-hour OGTT if a GCT was $\geq$ 135 mg/dl, the rate of diabetes was 4.5%, the rate of impaired fasting glucose (IFG) ($\geq$ 110 mg/dl and < 126 mg/dl) was 16.7%, and the rate of impaired glucose tolerance (IGT) (2-hour glucose $\geq$ 140 mg/dl and < 200 mg/dl) was also 16.7%. The overall rate of glucose disturbances in this group was 28.7%. Control women had a normal OGTT but a GCT $\geq$ 135 mg/dl. Among these women, the rate of diabetes was 1.8%, the rate of IGF 1.8%, and the rate of IGT 7.1%, with an overall rate of 9.7%. These rates (28.7% vs. 9.7%) correspond to an odds ratio of 3.75.

Because the data from the Sicilian study suggest that the rate of glucose disturbances in non-GDM mothers may be around 10% (or a bit less since their control group had a 1-hour GCT $\geq$ 135 mg/dl), with large odds ratios for HAPO GDM women likely, i.e. 3.0 or greater, we will focus on the incidence of diabetes for sample size estimates for disturbances of glucose metabolism given the substantially smaller number of women we would expect to develop diabetes. If we conservatively assume that 1% of non-GDM women will develop diabetes over 8-12 years of follow-up, and 2.5% of GDM women will develop diabetes (an odds ratio of 2.54), the required sample size is 6,156 for 90% power. An increase of 10% for other variables included in the model results in a required sample size of 6,772. A higher incidence of diabetes in the non-GDM women with a similar odds ratio for GDM vs. non-GDM would require a substantially smaller sample, e.g. 4.5% vs. 1.8% would require a sample size of 3,709 for multivariate analyses for 90% power.

For analyses involving glucose measures as continuous variables, if the overall rate of diabetes is 1.24% (1% for non-GDM and 2.5% for GDM), with a sample size of 7,000, there is at least 90% power to detect an odds ratio 1.49 for diabetes for glucose higher by one standard deviation if the sample size adjustment for multiple correlation is 25% or less. If the overall rate of diabetes is 2.23% (1.8% for non-GDM and 4.5% for GDM), the detectable odds ratio with at least 90% power is 1.34.

### 5.2.2 Sample Size and Power for Secondary Aims

#### *5.2.2.1 Sample size and power for secondary aims 1 and 2:*

The secondary aims of the HAPO Follow-up Study relating to maternal glucose levels during pregnancy are: 1) to determine associations with measures of glucose, insulin sensitivity and secretion, lipids, BP, and inflammation in offspring at ages 8-12 years; and 2) to determine associations with measures of cardiovascular risk in mothers 8-12 years later.

In what follows we will describe sample size requirements for the following four types of analyses planned for these two secondary aims:

(1) Examination of the association of GDM based on the new IADPSG criteria (2) with outcomes dichotomized as present or absent;
(2) Examination of the association of each glucose measure as a continuous variable with outcomes dichotomized as present or absent;
(3) Examination of the association of GDM with continuous outcomes; and
(4) Examination of the association of each glucose measure as a continuous variable with continuous outcomes.

Table 1 gives the detectable odds ratios for GDM (yes vs. no) for outcome frequencies of 10% to 25% for those without GDM for power of 80%, assuming a frequency of GDM in the follow-up study of 16% and a multiple correlation of GDM with other variables in the model that results in a sample size adjustment of 10%. Detectable odds ratios are provided for the total sample of 7,000, gender and ethnic-specific subgroups and a single field center of 800 participants. We expect approximately equal numbers of boys and girls in the 7,000, and 3,150 Caucasians, 1,050 blacks, 700 Hispanics, and 2,100 Asians to be included. Table 1 can also be used to determine detectable differences for subgroup analyses for Primary Aim #1.

**Table 1.** Detectable odds ratios for GDM vs. No GDM for outcome frequencies of 10% to 25% in those without GDM for 80% power in multivariate logistic regression analyses assuming a frequency of 16% with GDM and a correction for multiple correlation with other model variables of 10%

| Group | No GDM Outcome Frequency | | | |
|---|---|---|---|---|
| | **10%** | **15%** | **20%** | **25%** |
| All | 1.35 | 1.29 | 1.26 | 1.24 |
| *Subgroups* | | | | |
| Gender | 1.50 | 1.42 | 1.38 | 1.35 |
| Caucasians | 1.54 | 1.45 | 1.40 | 1.37 |
| Blacks | 2.01 | 1.84 | 1.76 | 1.70 |
| Hispanics | 2.29 | 2.08 | 1.96 | 1.90 |
| Asians | 1.68 | 1.56 | 1.50 | 1.47 |
| Single FC | 2.19 | 2.00 | 1.89 | 1.83 |

For analyses of the association of each glucose measure as a continuous variable with outcomes dichotomized as present or absent, Table 2 gives the detectable odds ratios for maternal OGTT glucose measures higher by one SD for outcome frequencies of 10% to 25% for power of 80% for multiple logistic regression analyses and a correction for multiple correlation of 25%. For 1-hour and 2-hour glucose for which the correction for multiple correlation is less than 20%, power with these sample sizes is greater than 80%.

**Table 2.** Detectable odds ratios for maternal OGTT glucose measures higher by 1 SD for outcome frequencies of 10% to 25% for 80% power assuming a correction for multiple correlation of 25%.

| Group | Outcome Frequency | | | |
|---|---|---|---|---|
| | **10%** | **15%** | **20%** | **25%** |
| All | 1.13 | 1.11 | 1.10 | 1.09 |
| *Subgroups* | | | | |
| Gender | 1.19 | 1.16 | 1.14 | 1.13 |
| Caucasians | 1.20 | 1.17 | 1.15 | 1.14 |
| Blacks | 1.38 | 1.31 | 1.27 | 1.25 |
| Hispanics | 1.48 | 1.39 | 1.34 | 1.31 |
| Asians | 1.26 | 1.21 | 1.19 | 1.17 |
| Single FC | 1.45 | 1.36 | 1.32 | 1.29 |

For analyses examining the association of GDM with continuous outcomes, power is 80% to detect a mean difference in the outcome for women with GDM vs. all other women as a proportion of the standard deviation (SD) of 0.092 for the total sample of 7,000, 0.130 for subgroup analyses of boys and girls, and 0.136 for Caucasians, 0.236 for Blacks, 0.289 for Hispanics, 0.167 for Asians, and 0.270 for a single field center with 800 participants. For example, if the standard deviation of child systolic blood pressure is 15, then power is 80% to detect a difference of 1.38 mmHg between children of women with and without GDM, and 1.95 mm Hg for separate analyses in boys and girls, etc.

For analyses of the association of each glucose measure as a continuous variable with continuous outcomes, the detectable partial correlation assuming up to 20 covariates in the regression model is 0.092 for the total sample of 7,000, 0.048 for subgroup analyses of boys and girls, and 0.050 for Caucasians, 0.088 for Blacks, 0.107 for Hispanics, 0.062 for Asians, and 0.10 for a single field center with 800 participants.

***5.2.2.2 Sample size and power for secondary aim 3:***

The third secondary aim of the HAPO Follow-up Study is to determine associations of measures of neonatal adiposity and hyperinsulinemia with measures of adiposity, glucose, insulin sensitivity and secretion, lipids, BP, and inflammation in offspring at ages 8-12 years. For analyses involving neonatal percent fat or hyperinsulinemia, data are expected to be available on 86.7% of HAPO follow-up children, or 6,070 of the children. The percentages with birthweight > 90$^{th}$ percentile, percent body fat > 90$^{th}$ percentile, and cord C-peptide > 90$^{th}$ percentile among the 10 field centers participating in the HAPO follow-up study were 9.60%, 9.86%, and 8.35% respectively. Hence, in the follow-up study we would expect 672 children to have had birthweight > 90$^{th}$ percentile, 599 to have had percent fat > 90$^{th}$ percentile, and 507 to have had cord C-peptide > 90$^{th}$ percentile.

In examining the associations of these three neonatal variables with dichotomous outcomes in the HAPO Follow-up Study, sample sizes required for univariate analyses need to be adjusted upward by 4% for birthweight > 90$^{th}$ percentile, 3% for percent fat > 90$^{th}$ percentile, and 5% for cord C-peptide > 90$^{th}$ percentile to account for the multiple correlation of these measures with other variables expected to be included in multivariate models, including maternal body mass index and maternal fasting glucose from the OGTT during pregnancy. Table 3 below gives the detectable odds ratios for birthweight > 90$^{th}$ percentile (yes vs. no), percent fat > 90$^{th}$ percentile (yes vs. no), and cord c-peptide > 90$^{th}$ percentile (yes vs. no) for outcome frequencies of 10% to 25% for those neonates $\leq$ 90$^{th}$ percentiles for these characteristics for power of 0.80 assuming frequencies of these characteristics of 9.6%, 9.86%, and 8.35%, respectively, and multiple correlations with other variables in the model that result in sample size adjustments of 4%, 3%, and 5%, respectively.

**Table 3.** Detectable odds ratios for birthweight, percent fat and cord c-peptide > 90$^{th}$ percentiles for outcome frequencies of 10% to 25% in those $\leq$ 90$^{th}$ percentiles for 80% power in multivariate logistic regression analyses

| **Characteristic > 90$^{th}$ Percentile** | **Outcome Frequency in Neonates $\leq$ 90$^{th}$ Percentile** | | | |
|---|---|---|---|---|
| | **10%** | **15%** | **20%** | **25%** |
| Birthweight | 1.42 | 1.36 | 1.32 | 1.29 |
| Percent Fat | 1.45 | 1.38 | 1.34 | 1.31 |
| Cord C-peptide | 1.49 | 1.41 | 1.37 | 1.34 |

For analyses of associations of birthweight, percent fat, and cord c-peptide as continuous variables with outcomes dichotomized as present or absent, Table 4 gives the detectable odds ratios of for each of these measures higher by one SD for outcome frequencies of 10% to 25% for power of 80% for multiple logistic regression analyses. These sample sizes have a correction for multiple correlation of 50% for birthweight, 28% for percent fat, and 10% for cord C-peptide. The high correction for multiple correlation for birthweight reflects the large differences in birthweight across field centers as well as the impact of gestational age at delivery on birthweight.

**Table 4.** Detectable odds ratios for neonatal birthweight, percent fat and cord c-peptide for outcome frequencies of 10% to 25% for 80% power in multivariate logistic regression analyses

| Neonatal Measure | Outcome Frequency | | | |
|---|---|---|---|---|
| | **10%** | **15%** | **20%** | **25%** |
| Birthweight | 1.15 | 1.12 | 1.11 | 1.10 |
| Percent Fat | 1.15 | 1.12 | 1.11 | 1.10 |
| Cord C-peptide | 1.13 | 1.11 | 1.10 | 1.09 |

For analyses of birthweight, percent fat, and cord c-peptide > 90$^{th}$ percentile with continuous outcomes, with a total sample size of 7,000 power is 80% to detect a mean difference as a proportion of the SD in those above and below the 90$^{th}$ percentile of 0.114 for birthweight, 0.121 for percent fat, and 0.130 for cord C-peptide.

For analyses of birthweight, percent fat, and cord C-peptide as continuous measures with continuous outcomes, with a total sample size of 7,000, power is 80% to detect a partial correlation of 0.034 for birthweight and 0.037 for percent fat and cord C-peptide.

### *5.3 Statistical Analysis Plan*

Analyses that will be conducted during the HAPO Follow-up Study include analyses involving descriptive statistics; analyses to compare participants in the follow-up study with those who choose not to participate, or cannot be contacted; analyses on associations of maternal glucose levels with child and maternal outcomes; and analyses on associations of neonatal birthweight, percent fat, and cord c-peptide with child outcomes. The analyses planned for each of these areas is described below.

#### 5.3.1 Descriptive Statistics

Descriptive statistics summarizing both dependent and independent variables will be generated for each field center prior to conducting formal analyses of outcome. For continuous variables, these will include means, medians, standard deviations, and important percentiles, e.g., 5th, 25th, 75th, and 95th, and for dichotomous variables, counts and percentages.

#### 5.3.2 Analyses Comparing Original HAPO Participants with HAPO Follow-Up Study Participants

Participants from the original HAPO study who are eligible for the HAPO Follow-up Study but do not participate will be compared to HAPO Follow-up Study participants on important study variables including maternal glucose levels during pregnancy, proportion with GDM by the new IADPSG criteria, maternal age, BMI, height, mean arterial blood pressure, parity, smoking, drinking, any family history of diabetes, gestational age at the OGTT, neonatal gender, birth weight, etc. Means, medians, standard deviations and important percentiles will be calculated for continuous variables and means will be compared for the two groups using two sample t-tests. Counts and percentages will be calculated for dichotomous variables and will be compared for the two groups using chi-square tests. All summaries and comparisons will be made for the entire group and within field center. If there are substantial differences in any of these variables, this will affect generalizability of HAPO Follow-up Study findings to the original set of HAPO participants (see Section 6.3.4).

In addition, on an ongoing basis, we will monitor the maternal glucose levels of women who agree to participate in the Follow-up Study compared to those who do not. If the Follow-up cohort looks to be largely different from those who cannot be contacted or do not agree to participate, then we will modify the sampling strategy to recruit across the spectrum of glucose levels, particularly those mothers who would have been diagnosed with GDM by the new IADPSG criteria if those women are underrepresented in the Follow-Up cohort.

#### 5.3.3 Analyses of Associations of Maternal Glucose Levels with Child and Maternal Outcomes

For analyses of associations of maternal glucose levels during pregnancy with primary and secondary outcome measures, there will be four different types of analyses, including:

(1) Examination of the association of gestational diabetes mellitus (GDM) based on the new IADPSG criteria (2) with outcomes dichotomized as present or absent, e.g. child overweight, including obesity, percent fat and waist circumference > 85$^{th}$ percentile, disorders of glucose metabolism in mothers, high blood pressure/hypertension, hyperlipidemia;
(2) Examination of the association of each glucose measure as a continuous variable with those outcomes dichotomized as present or absent;
(3) Examination of the association of GDM with continuous outcomes, e.g. child BMI, percent body fat, waist circumference, sum of skinfolds, insulin sensitivity, glucose, blood pressure, lipids and maternal glucose, BMI, percent fat, waist circumference, blood pressure, lipids;
(4) Examination of the association of each glucose measure as a continuous variable with continuous outcomes.

For analyses involving associations of each glucose measure as a continuous variable with dichotomous outcomes, we will also conduct preliminary descriptive analyses. These analyses will involve dividing fasting, 1-hr, and 2-hr plasma glucose into those categories that were used to examine associations of maternal glucose levels with adverse pregnancy outcomes in the primary HAPO Study (1). However, because of expected small numbers in the highest category of each glucose measure, i.e. 1% overall, we will combine the two highest categories for the follow-up study. Table 5 gives the categories for each glucose measure, as well as the percentage of women in each category for the 10 field centers that will be included in the follow-up study.

**Table 5**. Percentages of Women Expected in Categories of Fasting, 1-hr, and 2-hr Plasma Glucose in the HAPO Follow-up Study

| **Fasting Glucose** | | **1-Hour Glucose** | | **2-Hour Glucose** | |
|---|---|---|---|---|---|
| Category | % | Category | % | Category | % |
| < 75 | 16.3 | ≤ 105 | 18.0 | ≤ 90 | 18.1 |
| 75 – 79 | 32.2 | 106 – 132 | 32.6 | 91 – 108 | 32.9 |
| 80 – 84 | 27.0 | 133 – 155 | 26.1 | 109 – 125 | 25.1 |
| 85 – 89 | 12.3 | 156 – 171 | 11.8 | 126 – 139 | 13.0 |
| 90 – 94 | 8.3 | 172 – 193 | 7.8 | 140 – 157 | 7.2 |
| ≥ 95 | 3.8 | ≥ 194 | 3.7 | ≥ 158 | 3.7 |

The primary purpose of these analyses will be to assess the shape of the association of each glucose measure with each outcome. For example, does the association appear continuous and graded, and thus support the use of a linear term for glucose in the regression models? Does there appear to be a threshold effect? Does there appear to be a non-linear association between glucose and the outcome that requires more complex models, e.g. the inclusion of a squared term in glucose?

Analyses for the associations of GDM and maternal glucose levels with dichotomous outcome measures will utilize logistic regression, and analyses for continuous outcome measures will be based on linear regression. For each type of analysis, a series of models will be fit. The first model will adjust only for field center, with subsequent models adding variables measured at follow-up that may be related to the outcome, e.g. child age, gender, and Tanner stage for analyses involving child anthropometric measurements, variables measured during pregnancy, e.g. maternal age, body mass index (BMI), height, mean arterial pressure, parity, smoking, drinking, any family history of diabetes, gestational age at the OGTT (variables previously included in HAPO

models), and finally other variables measured at the follow-up exam for the child and mother, e.g. mother's current BMI for models involving child anthropometric measurements.

In models that utilize glucose as a continuous measure, we will also include squared terms in glucose to assess whether or not associations are linear. We will also add squared terms in important confounders, e.g. maternal BMI and maternal age, to assess whether or not important confounders are appropriately modeled. We will also test for interactions between glucose as a continuous variable, and potentially important confounders, including field center, maternal BMI, maternal age, child gender, etc.

Because of the international nature of the HAPO Study, for analyses involving child overweight and obesity based on BMI, we will define overweight and obesity based on the cutoffs recommended by the International Obesity Task Force (10). For measures of adiposity that do not have predefined cutpoints, e.g. percent fat based on the BOD POD or waist circumference, we will use the 85$^{th}$ percentile, taking into account gender and field center.

For analyses involving disorders of glucose metabolism in mothers, diabetes will be defined as a fasting glucose $\geq$ 126 mg/dl, a 2-hour glucose $\geq$ 200 mg/dl, or use of medication for diabetes. An impaired fasting glucose will be defined as 100 – 125 mg/dl, and impaired glucose tolerance as a 2-hour glucose of 140 – 199 mg/dl.

Because a study of a quantitative trait may be seriously compromised when the trait is subject to the effects of treatment, for lipid measurements and blood pressure, which may be treated in the follow-up study, we will conduct additional analyses using censored normal regression, where those on treatment will be considered as censored observations (11). The results of these analyses will be compared to those based on the observed lipid and blood pressure values.

### 5.3.4 Other Analyses

At the completion of recruitment, if there are substantial differences in participants from the original HAPO study who are eligible for the HAPO Follow-Up Study but do not participate compared to those who do, then inverse probability weighted regression will be used to account for potential selection/recruitment bias into the HAPO Follow-Up Study. Parameter estimates from the inverse probability weighted models will be compared to those from un-weighted models and examined for changes in the magnitude of associations. It is expected that each field center may demonstrate differences in selection into the follow-up study; hence the inverse probability weights will be field-center-specific. The weighted regression results will aid in interpretation of HAPO Follow-Up Study results for all original HAPO participants in the event of apparent selection/recruitment bias.

Given the standardized data collection and report procedures, missing data should be minimal. However, for variables with more than just a few missing observations, analyses will be conducted using multiple imputation methods for the missing values and parameter estimates will be compared with those derived from complete observations only (12, 13). These analyses will assist in recognition of potentially informative patterns of missing data.

## 6. ADMINISTRATIVE STRUCTURE

### *6.1 Executive and Steering Committees*

The Executive Committee will be comprised of the heads of the Clinical Coordinating Center (CCC), Data Coordinating Center (DCC), Laboratory Coordinating Center (LCC), the Project Manager, a field center PI, and the appropriate NIDDK program staff. This committee will have weekly conference calls to monitor study progress including dry run results at each field center, field center readiness to begin recruitment, recruitment, DCC data quality reports, oversight of laboratory performance and results, preliminary review of ancillary study proposals and presentations and publications, and preparation of materials for review by the Steering Committee.

The members of the Steering Committee are the heads of the HAPO Follow-up Study CCC, DCC, LCC, the Project Manager, the head of the HAPO Study DCC, the principal investigator from each of the field centers (who will include pediatric endocrinologists or pediatricians) and the NIDDK project scientist and program official. Renowned experts in clinical and genetic aspects of growth and adiposity of children and also in the measurement of maternal and childhood adiposity will be consulted, as needed.

Throughout the period of the project, the Steering Committee will meet annually and will conduct frequent conference calls and/or videoconferences to provide oversight and ensure implementation and execution of the project. Initially, the focus will be on training of field center staff and the implementation of the protocol. The focus will then be on recruitment and operational issues related to the study. Following that, the Steering Committee will engage in analysis of data, interpretation and presentation/publication. The Steering Committee will also establish subcommittees as needed to assist it in its duties, including an Ancillary Studies subcommittee. All data collected for the HAPO Follow-Up Study will be the property of the Study as a whole rather than any individual(s). Policies and procedures that were established for publication and presentation of HAPO Study data will be utilized for the HAPO Follow-Up Study.

### *6.2 Observational Study Monitoring Board*

An external Observational Study Monitoring Board (OSMB) will be appointed by NIDDK. The responsibilities of the OSMB include: 1. periodic review of overall study progress to ensure that the rate of recruitment of participants and the quality of data collection and entry are sufficient to fulfill the objectives of the study; 2. provide input to NIDDK if the OSMB has concerns about progress of the study that might require its early termination; and 3. review of any other issues for which the Principal Investigator, NIDDK staff, and/or the OSMB request review.

### *6.3 Subcommittees*

#### 6.3.1 Ancillary Studies Committee

The Ancillary Studies Committee will review all proposed ancillary studies and make recommendations to the Steering Committee to approve, revise, or disapprove each proposed study. The Ancillary Studies Committee formulates detailed guidelines for submission of proposals for ancillary studies and distributes them to field center investigators. The chair and co-chair of this committee will be selected from the Steering Committee.

#### 6.3.2 Other Subcommittees

The Steering Committee will set up other subcommittees as needed to manage presentations and publications, recruitment, and so on.

### *6.4 Study Components*

The project as a whole is organized into four tightly linked component parts: clinical study sites (field centers); Laboratory Coordinating Center (LCC) responsible for management of samples and oversight of measurement of key metabolic variables (glucose, C-peptide or insulin, lipids and hemoglobin $A_{1C}$); a Clinical Coordinating Center (CCC) responsible for directly overseeing the activities of the field centers and integrating the activities of all components of the study; as well as a Data Coordinating Center (DCC) responsible for a comprehensive data base for the entire study and for monitoring and maintaining quality control of all aspects of the study.

#### 6.4.1 Field Centers (FC)

There are 10 field centers in the HAPO Follow-Up Study. Each field center has a PI. Functions of each field center are recruitment, collection of questionnaire, physical and biological data, sample processing and shipment, and data entry. The field center staff person responsible for performing each of these functions will be trained centrally at a training meeting (the person responsible for data entry may be trained at the training meeting or locally) (only 1 person per function will be trained centrally and 1 person may perform more than 1 function). If other staff members are taking part in the study they are to be trained locally in the appropriate tasks by the field center PI or Research Nurse.

### 6.4.2 Clinical Coordinating Center (CCC)

Under the direction of the HAPO Follow-Up Study PI, the CCC has as its primary responsibility the successful execution of the Study protocol and procedures so that the primary hypotheses can be addressed in a definitive manner. The Study PI and CCC also have major responsibility for the fiscal integrity of the project. Funding of the individual field centers is provided through subcontracts with Northwestern University. Training in the standardized application of the clinical procedures, collection of specimens and use of the data forms will be essential for a successful study. This will be a joint effort of the CCC, DCC, and LCC. Implementation of the HAPO Follow-Up Study protocol and procedures at the field centers will be supervised jointly by the CCC and DCC. In addition to close communication by E-mail and conference calls, the PI (or his designee) will make a visit to each field center as part of the Dry Run site visit which will take place before the enrollment of participants is started. Additional site visits will be conducted by the PI, if necessary, to assure optimal performance at a specific field center. Under the supervision of the PI and Steering Committee, the CCC will integrate the activities of all components of the HAPO Follow-Up Study.

The CCC will also be responsible for ensuring that consent forms contain all elements included in the initial templates, that changes are made as needed, and that continuing review occurs at each center.

### 6.4.3 Data Coordinating Center (DCC) Functions and Responsibilities

- Setup the secure, web-based REDCap (Research Electronic Data Capture) data entry system and prepare the Manual of Operations chapter on for field center entry of study data using REDCap.
- Allocate staff IDs for each field center to be associated with REDCap entry.
- Distribute Recruiting Register, Dry Run material, documentation and forms, and up-date them when necessary
- Amending the Manual of Operations as needed, distributing changes to the field centers, assuring that all are aware of changes in procedures.
- Train field center personnel in use of the REDCap system.
- Back-translate HAPO forms and questionnaires translated for local use.
- Receive necessary documentation regarding equipment and staff training from each field center prior to start of data collection.
- Hold regular staff meetings to discuss progress and policy.
- Receive and review completed Dry Run data forms, with entered data, and provide rapid feedback to each field center to allow timely start of field work.
- Communicate with each field center before and during data collection, and clarify problem areas, maintaining a log of all such communications, for periodic review by senior CCC and DCC staff.
- Maintain SAS datasets for each center.
- Review entered data for important errors and omissions.
- Inform field centers of errors, omissions and inconsistencies in the data and request clarification and corrections, maintaining a file of all such requests, including receipt of clarifications and corrections.
- Monitor progress so that the exact status of each center is known as to number of participants enrolled; number with complete data entry, and number of blood specimens shipped and processed.
- Prepare a weekly report of number of forms (with number of participants) downloaded from the REDCap database, checked for accuracy, and edited and verified when necessary.
- Receive documentation monthly or biweekly from each cente.
- Prepare a monthly summary of recruitment and data collected at each center, including calculation of participation rates, for review by the Steering Committee.
- Track blood and urine samples received at the Laboratory Coordinating Center using BC Sample software.
- Retrieve blood sample codes and HAPO IDs from BC Sample for batch ordering of laboratory assays.
- Receive computerized data from the laboratory and merge data with the field center computer file.
- Carry out quality control procedures to monitor the technical error of the laboratory (5% of mothers and children will have the backup aliquots of their samples sent blind for analysis)

- Prepare reports to be shared with the CCC, Executive and Steering Committees on study progress, problems, and quality control results.
- Hold regular meetings to discuss data collection progress and policy.
- Perform data analysis in preparation for the main HAPO Follow-Up Study publications.
- Suggest and perform subsidiary data analyses, in collaboration with other HAPO investigators, including analyses for ancillary studies and secondary outcomes.

### 6.4.4 Laboratory Coordinating Center (LCC)

The Laboratory Coordinating Center is responsible for sample receipt, tracking, and transfer of samples to the laboratory for analysis or for longterm storage. The LCC will provide day to day oversight of the laboratory workflow (scheduling and performing assays and reporting results). Central supplies (phlebotomy tubes, storage vials, shipping containers, etc) will be purchased in bulk for shipment to each field center, and sets of numbered and blank labels printed for each field center.

Close communication between the CCC and/or DCC staff and the LCC will be maintained for review of laboratory and quality control procedures, including backup of laboratory data and transfer of data. Computerized laboratory data when forwarded to the DCC will be merged into the database.

### 6.4.5 Joint Responsibilities of the Coordinating Centers

- Organize and help conduct the centralized training session to train key field center personnel in HAPO Follow-up Study methods and procedures, to hold individual consultations with local PIs, and to ensure that all documentation has been received and is up-to-date.
- Arrange for the bulk purchase of follow-up study supplies and materials.
- Ensure that all necessary supplies and equipment are shipped to each field center in a timely manner for start of field work.
- Communicate regularly with all centers, in the form of a Newsletter, reports, etc.
- Coordinate site visits to each field center during the Dry Run and subsequently during field work as necessary.
- Prepare reports of site visits for review by the Executive and Steering Committees.
- Oversee laboratory procedures, including internal and external quality control, with periodic meetings with laboratory staff, as needed.
- Ensure that blood specimens are shipped as required to the LCC, and monitor arrival, completeness, and integrity of all blood samples shipped.
- Periodically review finances, staffing levels, and progress of the study to enable a high level of supervision and quality control to be maintained.
- Inform the Steering Committee and funding agencies of the progress of the study.
- Prepare the main final papers for publication, and advise on other publications, including those involving ancillary studies and secondary outcomes.

## 7. DATA PROCESSING, MANAGEMENT, AND QUALITY CONTROL

### *7.1 Field Centers*

### 7.1.1 Introduction

The HAPO Follow-up Study Protocol includes provision for site visits to each field center by Coordinating Center staff during the Dry Run and during ongoing field work as required based on field center performance.

Procedures for maintaining the quality of data collected in the field are laid out in the Manual of Operations and will be stressed at Central Training. Particular emphasis is placed on **local** field center review and editing of all forms prior to double data entry using REDCap (Research Electronic Data Capture), a secure web-based software package for data entry and management (90).

Each field center is identified by **name** and unique 1-character field **center code**, determined during the main HAPO Study. The center code is incorporated as the first character (e.g., A, B, C, etc.) of each ID number listed on pre-printed labels.

### 7.1.2 Preliminary Information

Before data collection for the follow-up study begins, each field center will be asked to provide preliminary information to the Data Coordinating Center. A checklist will be filled out for each field center documenting the following:

- Access to the following equipment: 1) a centrifuge (refrigerated, if possible); 2) a -20$^0$C or colder freezer; 3) a desktop or notebook computer with high-speed Internet access for data entry of completed forms into the HAPO Follow-up Study REDCap data base; and 4) a photocopier, preferably a multi-function copier/scanner capable of converting documents to PDF files that can then be e-mailed to the Data Coordinating Center.
- Access to dry ice and air freight delivery to Chicago that can be guaranteed within 48-72 hours.
- Completion and return by the field center PI of a Staff Member Training Form for each of his or her staff, trained after Central Training, indicating those aspects of the HAPO Follow-up Study for which each staff member has been trained. HAPO staff ID numbers are assigned by the DCC, and a computer check of HAPO staff ID number against study tasks (as recorded on the Staff Member Training Forms) will be carried out.

### 7.1.3 Data Forms

The Protocol, Manual of Operations, and data forms are being prepared in English. For field centers with a primary language other than English, or field centers that have > 10-15% participants who prefer conversing in a language other than English and where local HAPO Follow-up Study staff are fluent in that language, local translations of questionnaires administered to participants are to be made and sent to Chicago for approval. Back-translation of these questionnaires will be made without reference to the original. Comparison will then be made with the original English version, and discrepancies referred back to the field center for clarification. To minimize errors in data entry, it is particularly important that translated versions of questionnaires maintain the structure of the English originals, and efforts need to be made to ensure that questions line up correctly with answer boxes on the right-hand side of the forms. Data forms that are not administered to participants will not be translated. To maintain standardization of forms, it will not be permissible for field centers to use an interpreter to translate forms for use with participants who do not converse in the language(s) of the forms.

Field centers must provide print copies of the forms for routine use during data collection. Each form page contains space for a participant ID label. Pages of labels for each HAPO participant ID number are supplied centrally by the Data Coordinating Center to each field center. When a woman and her child agree to participate in the follow-up study and an appointment has been made, form sets for their ID number with labels attached should be prepared. Prepared form sets should be stored in the polypropylene file folders provided by the DCC. Labels for the aliquot tubes should also be stored in the folder.

### 7.1.4 Equipment and Supplies

The Clinical Coordinating Center will provide phlebotomy tubes to each field center for the follow-up OGTT and other blood samples, as well as bottles of glucose for the OGTT. Each field center will also be provided with storage vials and freezer boxes for storage of blood and urine specimens, and with shipping boxes. The Clinical Coordinating Center will also provide field centers with an electronic machine for measurement of blood pressure, and with the BOD POD which will be used to perform child and maternal anthropometric measurements. In addition, the Clinical Coordinating Center will provide the necessary equipment for measurement of skinfolds and waist circumference.

The Data Coordinating Center will provide the following to each center: 1) Manual of Operations, Protocol, data forms, and Recruiting Register for identifying eligible follow-up study participants; 2) training materials; 3) pre-printed labels for data collection forms; 4) pre-printed labels for phlebotomy tubes, storage vials, and freezer boxes; 5) file folders for data forms and labels.

### 7.1.5 Dry Run

Each field center must carry out a Dry Run (test run) of all study procedures, except the child OGTT, prior to start of actual fieldwork. The Dry Run will be conducted within 4-8 weeks after the completion of Central Training, or sooner if feasible. Field centers are expected to practice HAPO Follow-up Study procedures during the interim, and to train any additional local staff who will be participating in the follow-up study.

During the Dry Run, HAPO Follow-up Study research staff will complete data collection procedures on 4-6 women and children (who will not be HAPO participants but, rather, local volunteers). Each field center will be site visited during the Dry Run by Coordinating Center personnel, with a site visit report prepared for review by the Executive Committee. Reports will also be shared with the Steering Committee and the Data and Safety Monitoring Committee. Special Dry Run shipping labels will be prepared by the Data Coordinating Center for use by the field center during the Dry Run. All Dry Run documents and entered data will be transferred to the Data Coordinating Center for quick review. Fieldwork will begin following the Dry Run after all procedures have been certified as acceptable.

The readiness of a field center to begin follow-up study field work will be determined by the Executive Committee, with input from the Clinical Coordinating Center, the Data Coordinating Center, which will review all computerized data, and the Laboratory Coordinating Center which receives Dry Run blood samples, as well as the observers present at the Dry Run.

Only staff members certified as ready, after the above review, are to carry out HAPO Follow-up Study procedures.

### 7.1.6 Documentation of Recruitment

It is essential that accurate records be kept of participant recruitment for the follow-up study, including information on refusals and exclusions, and that study IDs be correctly utilized, i.e. that participants be given the same ID they were given in the original HAPO study. A Recruiting Register detailing recruitment contacts with original HAPO Study participants will be prepared centrally for day-to-day use in each field center. The DCC will preprint the Recruiting Register with the HAPO IDs of women who are eligible to be invited to participate. The ordering of the IDs will be based on the birth date of the child, in order to ensure that mothers with the oldest children are contacted first. All women known to meet initial inclusion criteria for the follow-up study will be included (term delivery $\geq$ 37 weeks gestation, no known fetal or neonatal death, no known major malformation). In order to ensure that names are correctly matched with HAPO IDs, the DCC will also preprint the birth dates of both the mother and child, based on the original HAPO data set, so that field center staff can verify that they are contacting the correct person during completion of the Screening Form. Use of the Recruiting Register is described in the HAPO Follow-up Study Manual of Operations in the Chapter on Recruitment. Data on recruitment will be used to help the Data Coordinating Center monitor follow-up study participation rates for each field center over the course of field work and at the end of field work allow comparison of original HAPO Study data between participants and non-participants in each field center, as well as comparison of participation rates across centers.

### 7.1.7 Data Entry

Data entry will be done at each field center, using REDCap, a secure web-based software package for data entry and management, developed for use as part of the CTSA – Clinical & Translational Science Awards (1). Field center staff will enter completed forms through a high speed Internet connection. During data entry data will be subject to range and logic checks. A chapter with instructions for data entry using REDCap is included in the Manual of Operations. Instruction on data entry procedures will be included at Central Training. Entered data will be imported from the HAPO Follow-up Study REDCap data base in SAS data sets to the DCC weekly. Key data, including all questionnaire, physical measurement, and test qualification forms, will be entered twice to minimize data entry errors.

### 7.1.8 Shipment of Specimens to the Laboratory Coordinating Center

Each field center is to make monthly shipments of blood and urine samples to the Laboratory Coordinating Center. Instruction in procedures for shipment will be included at Central Training. In addition, as part of the Dry Run site visit, the field center PI and site visitor(s) will also review procedures for shipment of blood samples. The proposed route for shipment of samples from each center is to be agreed on with Chicago in advance of the first shipment. Shipments will generally be made on a Sunday or Monday, so that they do not arrive in Chicago on a weekend. At the start of fieldwork, a shipping schedule for each field center will be agreed on with the Project Manager.

Both the Clinical and Laboratory Coordinating Centers are to be informed of shipping details (date, time, courier, tracking number, etc.) by FAX or e-mail. When shipping boxes are packed, lists of tube and cryovial IDs for each storage box included in the shipment are to be included with the shipment. A copy of each shipping list should be kept locally and a copy kept for forwarding to the Data Coordinating Center.

### 7.1.9 Communications

The Coordinating Centers will maintain close contact with each field center throughout the study. This will take the form of regular general communications (e.g., updates to the MOO, newsletters, etc.), and direct contact at different stages of the Study locally. Separate files and a "field center notebook" will be kept to record details of communications with each center.

Lines of communication will be established in advance of data collection, so that field errors can be detected and corrected as quickly as possible. In all cases, Clinical or Data Coordinating Center staff are to be on hand to answer queries arising in the field. Urgent problems will be dealt with by telephone, FAX, or e-mail. Other queries will also be handled by e-mail and a standard form for this purpose will be prepared. The Laboratory or Clinical Coordinating Center will also advise each field center by e-mail on whether shipments of specimens have been received, and whether backup specimens are required.

After completion of data collection, a request will be made for answers to any outstanding queries. Once all data have been merged into the database in Chicago, checked and verified, a summary report of data received will be prepared and sent to the field center PI.

### 7.1.10 Retraining

The Steering Committee together with the Coordinating Centers will develop criteria that will be used to determine whether a staff member(s) in a particular field center needs to be retrained. For field centers in which two persons have been trained and certified for HAPO Follow-up Study data collection, e.g., a PI and a Research Nurse, or two Research Nurses, measurements (child skinfolds, waist and midarm circumference and mother waist and hip circumference) may be made by these two persons on 5% or 10% of participants, with the request that forms and measurements be completed independently by each person. Criteria for retraining will also involve the number of missing data items for questionnaires.

On an ongoing basis mean values for continuous measurements will also be compared by HAPO Follow-up Study staff member within and between field centers, e.g., child anthropometric measurements, maternal height and weight, maternal and child blood pressure, etc. Values will also be monitored over time for each staff member within a field center, by comparing means by month for that person, to assess consistency of measurement over time. While differences within and between field centers, and within the same HAPO Follow-up Study staff member, may be real, criteria will nonetheless be developed for identifying potential problems. Steps that will be taken to deal with problems identified during this process include not permitting a staff member to perform particular components of data collection if there is a second staff member available who is also trained and certified in those areas; or suspension of recruitment pending retraining, either at the local center, through a site visit from Coordinating Center staff, or through travel by the staff member to the Coordinating Centers in Chicago.

## *7.2 Laboratory Coordinating Center*

### 7.2.1 Overview

Central supplies (phlebotomy tubes, storage vials, freezer boxes, etc.) will be purchased in bulk for shipment to each field center, and sets of numbered and blank labels printed for each field center. The three Coordinating Centers will work closely together to ensure that central supplies are delivered to field centers on time.

Regular meetings among the staff of the three Coordinating Centers will be held to review laboratory and quality control procedures, including backup of laboratory data. Other items of interest will also be discussed, including transfer of data, shipping, and finances. Plots of internal quality control samples will be periodically shared with the Executive Committee for review.

A close working relationship will be established among the three Coordinating Centers, with detailed minutes kept of all meetings.

### 7.2.2 Notification of Local Shipments

Both the Clinical and Laboratory Coordinating Center are to be advised of incoming shipments from field centers. Once the shipment has been received and the boxes opened, an inventory of aliquots is to be taken and compared with the storage box shipping list from the field center, and an assessment made of the state of the samples (whether thawed, whether tubes are cracked, whether labels are intact, etc.), with appropriate feedback to the field center given. Forms documenting the date of arrival of each shipment, the IDs of samples received and the state of the samples are to be kept. In the exceptional case, where shipments have thawed or gone astray, alternative routes of shipping will be reviewed with the field center PI.

The shipment containing the first set of blood samples from each field center is to be analyzed quickly by the Laboratory Coordinating Center and a report prepared identifying any problems in labeling or in use of IDs. If necessary, the field center will be contacted urgently for a review of local procedures.

### 7.2.3 Quality Control Procedures

An extensive system of internal quality control for the HAPO Follow-up Study will be developed by the Laboratory Coordinating Center in collaboration with the Clinical and Data Coordinating Centers. Regular review of the quality control procedures will be carried out during meetings with Laboratory Coordinating Center staff.

- Participant IDs: Aliquot tubes will be sent to the Laboratory Coordinating Center with Bar-code labels containing the original HAPO participant ID and aliquot code. These labels will be read at the Laboratory Coordinating Center using a Bar-code reader for direct computer entry of the ID and sample result. Use of Bar-code labels will minimize any problems that could arise from transcription of aliquot IDs or sample results onto laboratory working sheets. The computer list will be forwarded to the Data Coordinating Center for comparison against the shipping list to ensure that the labels have been read correctly and that all specimens listed were in fact sent. Any discrepancies will be investigated and, if necessary, checked with the field center. Confirmation of the full 8-character aliquot ID will allow the laboratory data to be merged with the mother's or child's other data at the Data Coordinating Center, and allow the Data Coordinating Center to identify the specific measurement.
- Duplicate samples: Analyses of duplicate samples will be reviewed in Chicago and any gross discrepancies checked first for mislabeling in the laboratory and then for mislabeling in the field. A formal analysis of laboratory technical error will be undertaken once computerized laboratory data are retrieved by the Data Coordinating Center.

### 7.2.4 Retrieval of Laboratory Data by the Data Coordinating Center

Definitive data from the laboratory will be directly accessed by the DCC using the Enterprise Data Warehouse (EDW) at Northwestern University. The EDW is a single integrated database of all clinical and research data for studies housed by the Northwestern research community.

### *7.3 Data Coordinating Center Procedures*

#### 7.3.1 Planning

Based on the proposed number of field centers in the HAPO Follow-up Study, the general workload of the Data Coordinating Center will be relatively predictable over the course of data collection. Nonetheless, the workload will vary from week to week, depending on a variety of factors beyond the control of the Data Coordinating Center, e.g., a delay in the start-up of a center, the state of the postal services, etc. Efforts will be made to plan ahead and keep abreast of the workload. Timetables will be regularly produced giving expected starting dates of field centers, with anticipated data flow over the coming months. A summary will also be made each week of number of data forms imported from the HAPO Follow-up Study REDCap data base, edited, entered into the SAS data files, checked, and verified.

#### 7.3.2 Review of Dry Run

All Dry Run documents for each field center and entered data are to be received at the Data Coordinating Center for review. Entered data from the Dry Run will be checked against data forms and data forms will be checked for consistency with study procedures. The field center file and checklist will also be inspected to see whether all preliminary information has been received, e.g., current consent form, translated forms, etc. A complete record of errors, inaccuracies, omissions and data editing or entry errors on the Dry Run and a note of any missing documentation will be recorded in the field center notebook, which will be passed to the appropriate Coordinating Center staff person. The field center PI and/or Research Nurse will be contacted to discuss the Dry Run and any outstanding problems or queries. In all cases, a FAX or e-mail message will be sent.

#### 7.3.3 Processing Field Center Data

With each weekly import of data to the Data Coordinating Center from the REDCap data base, an initial computer check will be carried out to confirm that no systematic errors are being made in data collection. Checking programs will also test whether the data are internally consistent. A list of unknown or missing fields will also be produced. Every effort will be made to ensure that imported data have this checking procedure carried out within 48-72 hours. If major problems are identified, the field center will be contacted by telephone, FAX, or e-mail. In urgent cases, where the Manual of Operations appears not to have been followed or has been misunderstood, Data Coordinating Center staff will contact the field center PI and/or Research Nurse by both telephone and e-mail, with the e-mail providing written documentation of the problem. A log of all such communications will be maintained for periodic review by senior Coordinating Center staff.

##### *7.3.3.1 Communication of errors to field centers:*

Field center errors will be detected at various stages of the review procedure. In general, errors will be of two types:

- Major (systematic) errors: These arise from misinterpretation of the Protocol or Manual of Operations, and result in incorrect recording of data. They will be discovered at the Dry Run, or during the computer checks. In all cases, contact with the field center is to be made immediately by telephone and e-mail, so that corrective action can be taken.
- Minor errors: These occur when information is missing, incorrectly entered, out of range, etc. They will generally be found during data entry or by the data checking programs at the Data Coordinating Center. Errors discovered by the checking programs at the Data Coordinating Center will be reported to the field center by FAX or e-mail using a Problem Report by form and participant ID for review and correction by field center staff. Pages of these Problem Reports are to be filed in a loose leaf binder following entry of the correct values into the data system. However, for these reports a photocopy is also to be forwarded to the Data Coordinating Center by e-mail as a PDF for filing in the field center's notebook at the Data Coordinating Center. A computer transaction file of errors, omissions, inconsistencies, or other problems will be maintained. The transaction file will be updated whenever an error report with corrections is received from the field center. Summary reports will also be prepared for review by senior staff on the number of requests made for corrections by the data checking programs, the number satisfactorily resolved, the number still outstanding, etc.

#### *7.3.3.2 Correction of errors:*

Field center staff are to enter corrections locally into the HAPO Follow-up Study REDCap data base using the Problem Reports generated by the Data Coordinating Center. These reports contain the form name or names, participant ID, question numbers, the value or values entered, and space for writing in the correct values. Only the correct values are entered into the data system. Copies of completed Problem Reports for errors found during data checking at the Data Coordinating Center will be sent by e-mail as PDF files by the field center bi-weekly. When corrections are received from the field center, the transaction file will be updated to indicate that the correction has been made.

#### *7.3.3.3 Monitoring field center performance:*

Analyses to monitor field center performance will include monthly tabulations by field center of the number of mothers and children enrolled, and current rate of recruitment compared with that required to achieve each field center's recruitment goal; number of mothers and children completing the follow-up exam, number of data forms entered into REDCap (Research Electronic Data Capture), the number of forms downloaded to the Data Coordinating Center from REDCap and processed since the last report, number that generated edit messages, current edit message rate per form, number of forms with missing data, and number of unanswered edit queries.

### 7.3.4 Processing Data from the Laboratory Coordinating Center

At the Laboratory Coordinating Center, computer entry of aliquot IDs and sample results will be automated through use of Bar-code labels containing the participant ID and aliquot code on all tubes and cryovials shipped to the Laboratory Coordinating Center. When laboratory data are transferred to the Data Coordinating Center, they will be processed through checking programs for range and consistency checks and then merged with the corresponding participant data in SAS data files, using the first 5 characters of the participant ID and 3-digit aliquot code as identifier. An automated record keeping system will be developed that will produce a monthly summary of the number of sample results received, and the number of participants for whom laboratory data have been received and merged into the data base.

For day-to-day running of the study, additional data will also be received from the Laboratory Coordinating Center:

- Results on first backup sample shipment: Backup blood samples pre-identified for use as duplicate samples from the first shipment of backup samples from each field center will be analyzed very soon after receipt at the Laboratory Coordinating Center. If gross discrepancies between the original and backup samples are apparent, the samples will be re-analyzed to determine whether mislabeling of the specimens has occurred, either in the field or in the Laboratory Coordinating Center.
- Inventory of blood samples: Lists of blood samples received, giving date of arrival, state of arrival, etc., are to be stored on computer and transferred to the Data Coordinating Center as shipments arrive. Comparison of IDs is to be made with those in data transmissions, and any discrepancies investigated.

#### *7.3.4.1 Monitoring Laboratory Coordinating Center performance:*

Analyses to monitor Laboratory Coordinating Center performance will include monthly tabulations of the number of samples received at the Laboratory Coordinating Center; the number thawed, lost, or unusable; number of samples requiring reanalysis, including reasons for reanalysis; backlog of samples remaining to be analyzed; summary of any events affecting laboratory operation, e.g., power outages; review of internal and external quality control reports; and calculation of mean differences, correlations, and technical errors from submission of duplicate backup samples. We will also tabulate the number of women and children with data received from the laboratory at the Data Coordinating Center.

### 7.3.5 Data Entry System

Data will be recorded on custom designed paper forms and then keyed on a continuous basis into the REDCap data entry and data management system. Data will be routinely imported by the Data Coordinating Center in SAS data sets for quality assurance monitoring, long-term storage, organization and, ultimately, analysis.

The forms used in this study will be written in English, with those administered to the participant then translated into the local language at each field center as needed. However, it will not be possible to translate REDCap data entry screens for translated questionnaires because of the many different character sets involved. Thus, the screens will remain in English regardless of the language of the form. To minimize confusion, the forms will be divided into a text portion on the left, and a data portion on the right. The layout of the text portion will be free to vary with the translation of the form from field center to field center, but the layout of the right hand portion will be the same on every version of the form and will use the same numerical coding rules and item numbering as the data entry screens.

## 8. STUDY TIMELINE

The timeline for the HAPO Follow-Up Study is outlined in the table below.

| Year of Study | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Training & Certification | 1-6 mos | | | | |
| Initiate Recruitment & Study Visits | 7-12 mos | | | | |
| Ongoing Recruitment & Study Visits | | X | X | 1-6 mos | |
| Close of Data Collection, Data Cleanup | | | | 7-12 mos | |
| Data Analysis & Manuscript Preparation | | | | | X |

This timeline would start at the time of funding. The earliest date that funding would begin would be April 1, 2012.

## 9. POLICIES

It is understood by all collaborating investigators that the data collected in the HAPO Follow-up Study are the property of the Study as a whole rather than any individual(s). This study is certain to catalyze great interest in examining associations between maternal glucose levels during pregnancy and secondary outcomes or characteristics of the population(s) as well as many ancillary studies.

### *9.1 Informed Consent*

Although countries have different requirements for the institutional review board (IRB) and informed consent process, because the HAPO Follow-up Study is funded in part by the NIH, the Study is obligated to follow the policies and requirements of the Office for Protection from Research Risks (OPRR), an agency of the US government. Therefore, each time the protocol is revised or an ancillary study added, the change must be approved by the local IRB before it can be implemented. In addition, annual IRB review and approval is required. Each consent form will include the elements of informed consent as required by Title 45 in the Code of Federal Regulations.

### *9.2 Training*

All field center personnel participating in the HAPO Follow-up Study must be trained in HAPO Follow-up Study procedures, either during Central Training or by the field center PI or Research Nurse following Central Training. Only trained field center staff will be allowed to participate in data collection for the HAPO Study.

### *9.3 Privacy of Records*

Data collected from individual participants are to be entered into REDCap (Research Electronic Data Capture), a secure web-based software package for data entry and management, without name and identified only by the original HAPO ID. Blood samples sent to the LCC are also to be so identified. Only local field center investigators and HAPO staff are to have the names of participants. The need for protecting confidentiality of these names will be stressed during Central Training as well as in the Manual of Operations.

### *9.4 Field Center Data Access*

At the end of data collection, cleaned and corrected data from individual field centers will be made available to local investigators. Papers based on these data are not to be published prior to the main reports of the HAPO Follow-Up Study.

### *9.5 Publications and Presentations*

#### 9.5.1 Main Final Paper(s)

These are to be prepared under the supervision of the Coordinating Centers and Steering Committee. They will also be circulated to all field center investigators. Field center investigators may be members of the writing group for any paper. Authorship is to be the HAPO Follow-up Study Cooperative Research Group, with acknowledgement of Principal Investigators, Coordinating Centers, Steering Committee, and NIH Staff.

#### 9.5.2 Other Study-Wide Papers

Other papers using combined study data are to be drafted by ad hoc groups of local and/or central investigators appointed by the Steering Committee. The topics of such papers may be suggested by the Coordinating Centers, Steering Committee, or local investigators. Such papers are to be authored by the investigators drafting the paper on behalf of the HAPO Follow-up Study Cooperative Research Group, with acknowledgement also of Principal Investigators, etc. These draft papers are to be reviewed by the Steering Committee.

#### 9.5.3 Ancillary Study Papers

Collaborating investigators in ancillary studies are to prepare papers in cooperation with the CCC and DCC. The final text must be jointly approved by the Steering Committee before it is submitted for presentation and/or publication. Authorship is to be the investigators concerned, on behalf of the HAPO Follow-up Study Cooperative Research Group.

#### 9.5.4 Local Papers

At the end of data collection, cleaned and corrected data from individual field centers are to be made available to local investigators. Local papers are not to be published prior to publication of the main reports of the HAPO Follow-up Study Cooperative Research Group. Local papers must also be submitted to the Steering Committee for approval.

### *9.6 Ancillary Studies*

The Steering Committee will establish an Ancillary Studies Subcommittee to evaluate requests for such studies for their scientific merit as well as potential impact on the conduct of the primary study. Ancillary studies that could in any way compromise the primary study will not be approved. After the primary analyses are completed and the results are published, secondary analysis of certain local or regional outcomes or trends will be considered and carried out in collaboration with the DCC upon recommendation and approval by the Steering Committee.

Ancillary studies proposed by individual field centers must first be approved by the local Institutional Review Board. They are then to be forwarded to the Ancillary Studies Committee for review and approval to assure that they do not interfere with the design and conduct of the HAPO Follow-up Study. Documents certifying approval by the local Institutional Review Board are also to be forwarded to the CCC, together with the proposal for the ancillary study.

Ancillary studies are to provide an additional local consent form, describing in detail procedures to be performed, and possible risks and benefits to the participant. These documents and the completed HAPO form requesting approval must be submitted and approved by the Ancillary Studies Committee prior to the start of data collection for the ancillary study.

Presentation and publication of the results of an ancillary study are subject to the same guidelines as apply to all other presentations and publications of the HAPO Follow-up Study, i.e., prior review and approval by the Steering Committee, before submission of an abstract for a meeting, before presentation of an oral report or poster to a meeting, and before submission to a journal.

### *9.7 Data Sharing*

Northwestern University is committed to the open and timely dissemination of research outcomes. Participants in the HAPO Follow-Up Study will be asked to provide informed consent for the sharing of their data (including biospecimens) with other investigators. For those participants providing informed consent for data sharing, we will make de-identified study biospecimens and data available following publication of all primary results from the study to the general community by providing it to the NIDDK for their biospecimen and data repositories. The Coordinating Centers have experience with preparing data, biospecimens, and documentation for archiving and will work with repository personnel to provide these as required. Data will be provided to NIDDK in SAS datasets or other format compatible with their repository, along with copies of data collection forms and documentation of original and derived data.

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