Etiologic roles of vitamin D, folates, and long chain n-3 fatty acids in preeclampsia, gestational diabetes, preterm delivery, and intrauterine growth retardation: a biomarker based study

**Aims**

Aim 1. To develop material-efficient and valid procedures for biochemical assessment of status for vitamin D, folates, and long chain n-3 fatty acids in DNBC, and to validate these measurements, by
   a. Comparing biomarker levels with self-reported intakes from foods and supplements.
   b. Specifically for vitamin D, examining seasonal variation in biomarker levels.

Aim 2. To examine the etiologic roles of vitamin D, folates, and long chain n-3 fatty acids in preeclampsia, gestational diabetes, preterm delivery, and intrauterine growth retardation, by
   a. Examining to what extent biomarker levels for these nutrients determine risks of these complications
   b. Examining whether biomarkers measured in the first trimester or the second trimester are most important in determining risk.

Aim 3. Specifically in order to shed further light on the possible etiologic roles of vitamin D, to examine seasonal variations in preeclampsia, gestational diabetes, preterm delivery, intrauterine growth retardation and related outcomes, while taking into account estimated intakes of vitamin D and calcium and other nutrients.

**Background**

Recent research suggests that vitamin D (1-3), folates (1;4;5) and long chain n-3 fatty acids (1;6) may play important etiologic roles in common pregnancy complications such as preeclampsia, gestational diabetes, preterm delivery and intrauterine growth retardation.

Few studies have been able to examine these relationships prospectively, and even fewer to discern between the impacts that these exposures may have during different critical time windows of pregnancy. Use of biomarkers to reflect nutrient exposures and nutrient status (7) is a field that is developing fast, and it is getting increasingly possible to use minute amounts of biomaterials for such purposes (e.g. (8)). Such methods are particularly useful in large cohort studies that have collected biomaterials concurrently (9), enabling the conduct of nested (and de facto prospectively designed) case-control studies. The Danish National Birth Cohort gives a unique opportunity to examine the relationships between biomarker levels assessed in first (blood sample taken in gestation weeks 8-10) and second trimester (gestation weeks 24-25) of pregnancy and risks of the subsequent development of pregnancy complications. Discerning between these critical time windows has potential
implications both for our understanding of the pathogenesis of the complications in question, and for developing efficient preventative measures against these complications.

**Work plan**

**Selection of samples for analysis**

Ad Aim 1:

*Validation study, development of biomarkers procedures*

We will select 500 women based on reported intakes of the 3 nutrient groups. The precise selection procedure will depend on intake distributions. For each woman, we wish to use up to 800 ul from 1st and 2nd maternal plasma samples as well as from the umbilical cord sample.

Ad Aim 2:

*Case-control study*

From the pool of mother-child pairs in DNBC for whom relevant co-variates are present, and where there are samples available from all three occasions, we will select 200 women with preeclampsia, 200 with gestational diabetes, 200 with preterm delivery, and 200 infants with intrauterine growth retardation. In addition, 500 will be selected randomly from the same pool of mother-child pairs, who will be used as controls. Our preliminary estimate is that 150 ul will be needed for each of the three types of biomarker analysis. From each woman, therefore, we wish to be able to use up to 450 ul from the 1st and 2nd maternal plasma samples (and for the control group, from the umbilical cord sample). Please note, however, that we in the initial phase will develop procedures for handling samples and undertaking the biochemical/chemical analyses, such that uses of biomaterials will be minimised; developing such procedures is an important overall goal of the project.

Ad Aim 3:

*Seasonal variations study*

This study will be undertaken on the basis of the entire sample of women/children in DNBC.

**Case definitions**

We will use all available information in the DNBC databases to obtain the most valid diagnoses of preeclampsia, gestational diabetes, preterm delivery, and intrauterine growth retardation. For each of the four outcomes studied, the numbers needed (200) will be substantially smaller than numbers of women with the diagnosis.

**Statistical analysis**

To address Aim 1, we will use standard statistical graphical and analytic methods. To address Aim 2, we will use logistic regression, taking available relevant covariates into account such as maternal parity, height, pre-pregnant BMI, smoking, and other lifestyle factors. To address Aim 3, we will develop and apply relevant statistical methods that can depict seasonal variations in outcomes, while taking estimated intakes of vitamin D and calcium and covariates into account.

**Collaborative setting**

Preliminary agreements have been made with the following labs:

*Vitamin D2/3 assessment:*

Department of Clinical Biochemistry and Immunology, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen S (contacts: David Hougaard and Arieh Cohen).
25-OH-vitamin D3 and 25-OH-vitamin D2 will be measured by HPLC-tandem spectrometry by means of commercially available kits.

**Folate assessment:**
Vitamin and Metabolism Laboratory, Jean Mayer USDA HNRCA at Tufts University, 711 Washington Street, Boston, MA 02111-1524 (contact: Jacob Selhub). Folates concentrations will be determined with the use of affinity-HPLC methods, as described (10).

**Fatty acid assessment:**
Department of Nutrition, Harvard School of Public Health, 655 Huntington Ave, Boston, MA 02115 (contact: Hannia Campos). Fatty acid concentrations in whole plasma will be analyzed by gas-liquid chromatography, as described (11).

**Financial plan**

The process to address the first aim will be initiated right away using money from a grant given by The Danish Council for Strategic Research. We will raise additional funds to finance the further development and conduct of the project, including expenses related to biochemical analyses, statistical analyses, and the writing of articles.

**Time plan**

Sept 2010 to May 2011: Initial study to develop procedures and to submit grant applications.

June 2011 to January 2012: Biochemical analyses of samples from cases and controls (provided we are successful with applications to finance the analyses).

February 2012 to February 2013: Data cleaning, data analysis, writing of papers.

Tentative working titles of papers:
1. Biomarker levels of vitamin D, folate and n-3 fatty acids in early and mid-pregnancy as determinants of preeclampsia
2. Biomarker levels of vitamin D, folate and n-3 fatty acids in early and mid-pregnancy as determinants of gestational diabetes
3. Biomarker levels of vitamin D, folate and n-3 fatty acids in early and mid-pregnancy as determinants of preterm delivery
4. Biomarker levels of vitamin D, folate and n-3 fatty acids in early and mid-pregnancy as determinants of intrauterine growth retardation
5. Validity of biomarkers used to reflect status of vitamin D, folate and n-3 fatty acids in the Danish National Birth Cohort: Comparison against self-reported intakes
6. Impact of season and vitamin D intake on risks of preeclampsia, gestational diabetes, preterm delivery and intra-uterine growth retardation

**Future perspectives: test of concept**

If the approaches that we develop prove feasible and productive, we intend to apply them to other outcomes identified both in the mother and the child. As mentioned, developing material-efficient and valid methods for uses of the biomaterials is an important overall goal of the project. We also envisage that results from the biomarker analyses will be useful as one step in applying principles of Mendelian randomisation to explore etiologic roles of vitamin D, folates, and long chain n-3 fatty acids in relation to pregnancy complications and other outcomes in the mother and child: we hope to be able to pursue that perspective in a future application.
Reference List


