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Original Article

The Alberta Pregnancy Outcomes and Nutrition (APrON) cohort study: rationale and methods

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Abstract

The Alberta Pregnancy Outcomes and Nutrition (APrON) study is an ongoing prospective cohort study that recruits pregnant women early in pregnancy and, as of 2012, is following up their infants to 3 years of age. It has currently enrolled approximately 5000 Canadians (2000 pregnant women, their offspring and many of their partners). The primary aims of the APrON study were to determine the relationships between maternal nutrient intake and status, before, during and after gestation, and (1) maternal mood; (2) birth and obstetric outcomes; and (3) infant neurodevelopment. We have collected comprehensive maternal nutrition, anthropometric, biological and mental health data at multiple points in the pregnancy and the post-partum period, as well as obstetrical, birth, health and neurodevelopmental outcomes of these pregnancies. The study continues to follow the infants through to 36 months of age. The current report describes the study design and methods, and findings of some pilot work. The APrON study is a significant resource with opportunities for collaboration.

Keywords: longitudinal cohort study, pregnancy, nutrition, mental health, birth outcomes, neurodevelopmental outcomes.

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'. . . it is our continuous exposure to foods throughout our lifetime that renders diet the most important environmental factor challenging our biological system'

– Mutch *et al*. 2005

Nutrition is fundamental to life, and its critical role in fetal development has been clearly demonstrated in decades of animal studies establishing the importance of individual nutrients (Abu-Saad & Fraser 2010). Although there is a vast literature on the relationship of nutrient insufficiencies to maternal and infant health in humans, the majority of this work has been done in the developing world (e.g. Lindstrom *et al*. 2011). Even in well-nourished populations, however, deficiencies of some individual nutrients have been reported and are the focus of educational and public

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health/policy campaigns (e.g. the role of folate in neural tube defects [Blencowe *et al*. 2010]).

Pregnancy is a time of increased demand for nutrients to meet both maternal and fetal needs, as well as a time when women are likely to change their diet, motivated by concern for the developing fetus (Crozier *et al*. 2009). The purpose of this manuscript was to describe a Canadian cohort study designed to examine the relationships between maternal nutrient intake, maternal mental health and neuro-cognitive development in infants. The current study extends the work of other large nutrition studies (Giddens *et al*. 2000; Denomme *et al*. 2005; Mouratidou *et al*. 2006; Klemmensen *et al*. 2009; Shand *et al*. 2010) by assessing nutrient intake and status at multiple time points in pregnancy in multiple nutritional biomarkers. This level of detail is novel, as the previously cited nutrition studies have generally used few data points [e.g. one or two 3–7-day food records (Giddens *et al*. 2000)], or a food frequency questionnaire (FFQ) administered at a single time point in pregnancy (Mouratidou *et al*. 2006; Klemmensen *et al*. 2009), or the measurement of single nutritional biomarkers such as serum 25-(OH)D3 (Shand *et al*. 2010) or n-3 polyunsaturated fatty acid (PUFA) status (Denomme *et al*. 2005).

Study aims

The Alberta Pregnancy Outcomes and Nutrition (APrON) study evolved out of concern for two increasingly prevalent societal problems: *mood disorders during and after pregnancy*, and *neurodevelopmental abnormalities in offspring*. As reviewed later, there is evidence that nutrition plays a crucial role in both areas. The *primary aims* of the APrON study were to determine the relationships between maternal nutrient intake and status, before, during and after gestation, and (1) maternal mood; (2) birth and obstetric outcomes; and (3) infant neurodevelopment. *Secondary aims* included determining the relationship between maternal thyroid status and neonatal and neurodevelopmental outcomes, and establishing nutrient predictors of infant behavioural patterns, including sleep, crying and temperament. Additionally, we documented many other variables such as maternal physical activity and weight gain during pregnancy, and infant feeding patterns. A DNA and serum biobank was also established to facilitate future studies on the role of genetic and epigenetic interactions.

The societal impact of mood disorders in the perinatal period

Mental health problems are on the rise worldwide: *depression* alone was the leading cause of disability in 2000, and the fourth leading contributor to the global burden of disease (World Health Organization 2011). *Perinatal depression* refers to both major and minor episodes of depression occurring during pregnancy ('antenatal' depression) and/or within the first 12 months after delivery ('post-partum' depression) (Gavin *et al*. 2005). The prevalence of major depression in the post-partum period in 1017 women in Holland ranged from 2% to 6% and subthreshold depression ranged from 6% to 13% (Pop *et al*. 2006). Similarly, in the Canadian Maternity Experiences Survey, 7.5% [95% confidence interval (CI): 6.8–8.2] of women exhibited high depression symptom scores [≥13 on the Edinburgh Postnatal Depression Scale (EPDS)], and a further 8.6% (95% CI: 7.9–9.3)

Key messages

- The APrON study is an ongoing prospective pregnancy and birth cohort. It will provide the most thorough longitudinal and multinutrient characterization yet available, of both maternal nutrient intake and nutrient status, both prior to and during pregnancy.
- The primary goal of the APrON study was to determine the relationships between maternal nutrient intake and status, before, during and after gestation, and (1) maternal mood (2) birth and obstetric outcomes and (3) infant neurodevelopment.
- Findings from this cohort will apply to many maternal and child health issues across populations.

scored moderately high (10–12 on the EPDS; Public Health Agency of Canada 2009). Depressed pregnant women are less likely to seek proper medical care during pregnancy and are more likely to engage in risk-taking activities such as alcohol and/or drug abuse (Bowen & Muhajarine 2006; Ross & Dennis 2009). For the newborn, maternal depression is strongly associated with reduced breastfeeding (Dennis *et al.* 2004).

Recently, *pregnancy anxiety* – defined as anxiety during pregnancy that pertains to pregnancy, birth and subsequent parenting (Dunkel Schetter 2011) – has been identified as a distinct syndrome (Huizink *et al*. 2004). Three large prospective cohort studies have shown that pregnancy anxiety is significantly associated with preterm birth (Dole *et al*. 2004; Orr *et al*. 2007; Kramer *et al*. 2009). During their first 2 years of life, infants exposed to pregnancy anxiety may experience increased incidence of illness (Beijers *et al*. 2010), increased negative temperament (Blair *et al*. 2011), decreased cognitive performance (Davis & Sandman 2010), delayed motor development (DiPietro *et al*. 2006) and decreased attention regulation (Huizink *et al*. 2002). The combined impact of maternal *depression and anxiety* during pregnancy may alter maternal physiological reactivity to stress [e.g. cortisol (Evans *et al*. 2008; Giesbrecht *et al*. 2012)] with significant downstream implications for behavioural and emotional development of offspring (Van den Bergh & Marcoen 2004).

The societal impact of neurodevelopmental disorders

In recent decades, alarm has been raised about an apparent increase in neurodevelopmental disorders (Offord *et al*. 1987; Gillberg 1998). Regardless of whether changing diagnostic criteria have inflated national prevalence rates, the fact is that neurodevelopmental disorders are extremely common, affecting at least one out of six children (Boyle *et al*. 2011). The American National Center for Children in Poverty in 2010 concluded that fully 20% of school-aged children have a diagnosable developmental or mental disorder (Stagman & Cooper 2010).

Preterm birth, the rates of which have been increasing over the past two decades by as much as 20% in some developed countries (Stewart 2007), accounts for the vast majority of health complications and mortality in the neonatal period, and also for a range of developmental problems including motor and sensory impairment, neurocognitive impairment, lower academic achievement, and behavioural concerns such as attention deficit hyperactivity disorder (Institute of Health Economics 2007).

The relevance of nutrition to mental health and neurodevelopment

The association between nutrient deficiency during pregnancy and the post-natal development of mental illness in offspring has been well-documented in studies of the Dutch Hunger Winter and the Chinese Great Leap Forward Famine (Brown & Susser 2008). Recent population-level studies from both Europe and Australia have shown strong associations between vulnerability to mental disorders and the dietary pattern of nutrient-poor 'western' foods (processed, refined, fried and sugary) in comparison with a diet of vegetables, fruits, meat and whole grains (Akbaraly *et al*. 2009; Jacka *et al*. 2010). There is also evidence from within-subject analyses showing that dietary patterns correlate with quality of mental health (Davison & Kaplan 2012) and predict changes in mental health quality 2 years later (Jacka *et al*. 2011). Prospective studies support the notion that dietary patterns are a causal mechanism of mental health (Amminger *et al*. 2010; Jacka *et al*. 2011), as do a growing number of treatment studies in which use of a broad spectrum of nutrients ameliorated mood symptoms (Gately & Kaplan 2009; Rucklidge *et al*. 2010). There is also growing evidence that some nutrient deficiencies contribute to a woman's risk of maternal depression (Leung & Kaplan 2009).

Increasing interest in the association between maternal nutrition and child neurodevelopmental outcomes is evidenced by a series of publications over the past decade, many of which report inconsistent results following treatment with long-chain PUFA (LCPUFA) (Helland *et al*. 2001, 2003; Colombo *et al*. 2004; Makrides *et al*. 2010). Similarly, a systematic

review of the effects of pre-natal supplementation on child cognitive/behavioural development found inconsistent outcomes (Leung *et al*. 2011). Supplementation with single nutrients had null or negative effects on child development, while multi-nutrient supplements showed some positive effect on cognitive and motor development (Joos *et al*. 1983; McGrath *et al*. 2006; Li *et al*. 2009). A significant inverse association was demonstrated between autism spectrum disorder (ASD) prevalence and the American programme that includes food assistance, called Women, Infants and Children (WIC) (Shamberger 2011): children living in states with the highest WIC participation exhibited the lowest autism rates ($P < 0.02$ across all 50 states). It is also noteworthy that a clinical trial of a Mediterranean diet in pregnant women resulted in only one preterm birth in a sample of 141 women, compared with the 11 preterm births in 149 women in a control group who ate their usual diets (Khoury *et al*. 2005).

In summary, the combined evidence showing the association between dietary patterns and mental health, and the potential for influencing neurodevelopmental outcomes of offspring, emphasize the importance of further research that examines the effects of nutrition on perinatal maternal mental health and subsequent neurodevelopment of the offspring. The primary intention of the current paper was to describe how the APrON study has addressed the need to understand the links between nutrition, mental health and neurodevelopment.

Study design

APrON is a longitudinal cohort study of pregnant women and their children living in the central and southern regions of Alberta, Canada. The sampling frame included all pregnant women over the age of 16 living within Alberta's two largest metropolitan areas: Calgary (population 1.1 million) and Edmonton (population 0.8 million). In 2008, there were 50 164 live births in Alberta; of these, 18 633 were in Calgary and 14 866 in Edmonton (Government of Alberta 2010). Alberta is a western province of Canada (see Fig. 1) with a total population of 3.5 million.

Inclusion and exclusion criteria

Pregnant women >16 years, gestational age <27 weeks, and living in or near Calgary or Edmonton were eligible. Women were excluded if they were unable to answer questions in English or if they

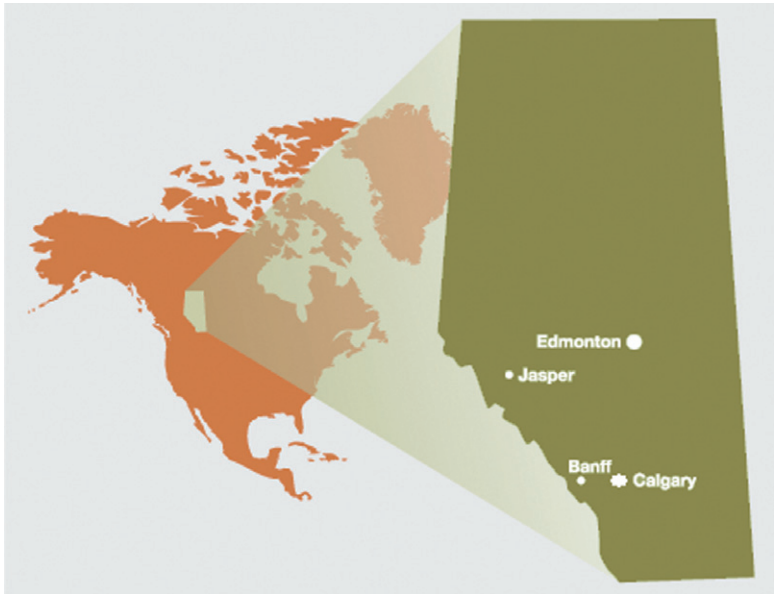

Fig. 1. Location of Alberta, Canada.

planned to move out of the region during the timeframe of the study. A decision was made *a priori* to divide APrON's participants into three cohorts, each of which could be reviewed and 'locked' for analyses separately, which allowed for interim analyses: the first 600 women and their families; the first 1200; and the final total sample (yet to be determined).

Recruitment

In Calgary, approximately half of the maternity care is provided in large family physician-led maternity clinics, and the other half in individual family medicine clinics or obstetrical clinics, whereas in Edmonton, maternity care is distributed widely across many individual family medicine and obstetrical clinics. Deliveries are also handled differently. In Calgary, about half of the deliveries are attended by family physicians, and in Edmonton, the majority of births are attended by obstetricians. Because of these differences, recruitment efforts were different in the two cities. Calgary recruitment included stationing research assistants in waiting rooms of high-volume maternity care and ultrasound clinics. In contrast, Edmonton recruitment was organized through a collaborative process with the Women and Children's Health Research Institute at the University of Alberta, distributing information across the city's many clinics. Other recruitment strategies used in both cities included posters in areas frequented by pregnant women (grocery stores, community centres, family physician offices), appearances by investigators on local television and radio shows, other media advertising (newspaper, banners along roads), prenatal education classes, pregnancy and baby fairs, and word of mouth.

After women expressed interest in the study, APrON research assistants contacted prospective participants to describe the study in more detail. Interested women were then scheduled for an initial clinic visit, and the first questionnaire packet was mailed to them. All participants provided informed consent prior to being included in the study. The project was approved by the University of Calgary Health Research Ethics Board and the University of Alberta Health Research Ethics Biomedical Panel.

Data collection

Women recruited at ≤13 weeks gestation were assessed once during each trimester; those recruited at 14–27 weeks gestation were assessed in the second and third trimesters only. Further assessment points at 3, 6, 12, 24 and 36 months post-partum with the variables monitored are illustrated in Fig. 2.

All pre-natal assessments consisted of a blood draw, anthropometric measurements (height, weight, skinfold measures), 24-h food recall and a urine sample. Questionnaires were used to assess pre-pregnancy dietary intake (described later), pre-pregnancy physical activity, current mental health, medical history and demographic information. At the first post-natal assessment, women provided a blood sample, anthropometric measures, a 24-h food recall and a random spot sample of breast milk (if lactating). Questionnaires were used to assess infant feeding practices, breastfeeding (where relevant) and mental health status. Blood was drawn from the infant, or a cheek swab (Medical Packaging Co., Camarillo, CA, USA) and a spot blood sample from a heel prick were obtained. DNA was isolated from the blood or buccal cells collected. Parent-report of infant health and behaviour was collected at all post-partum assessments. Paternal (biological) assessments consisted of DNA (cheek swab), health history and current mental health (during pregnancy and after birth).

Questionnaire assessments

Maternal questionnaires

At the first study visit, women provided sociodemographic information, including education, family income, family constellation, marital status, occupation, health history (including mental health and medications), obstetric history, medication use, street drug and alcohol use, and smoking history. Details that could change (e.g. marital status, medication use) were checked at each subsequent assessment point.

Maternal mental health

At each time point, symptoms of current depression [EPDS (Cox *et al*. 1987; Pop *et al*. 1992; Bergink *et al*.

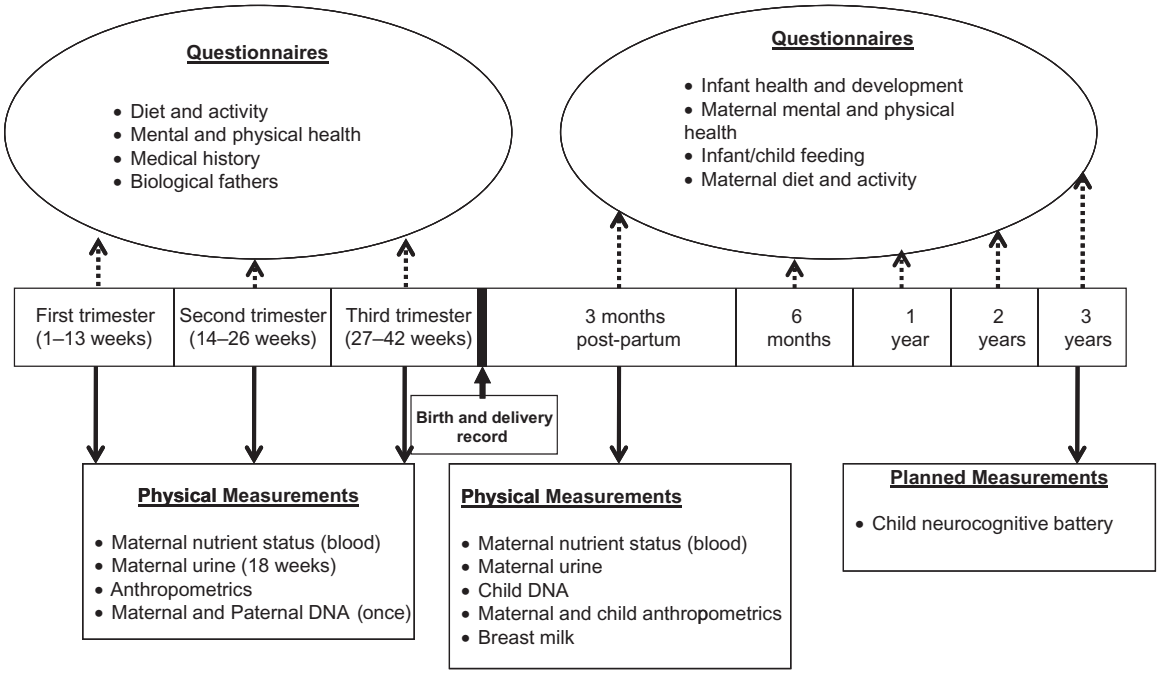

Fig. 2. Assessment timeline.

2011)] and anxiety [anxiety subscale of the Symptom Checklist-90-Revised (Derogatis 1994)] were measured. Stressful life events [modification of the Stressful Life Events Questionnaire (Bergman *et al*. 2007)] were measured at the time of intake into the study, in the third trimester, and at 3, 12, 24, and 36 months post-partum. In addition, at the second trimester and 3-month post-partum periods, the Psychiatric Diagnostic Screening Questionnaire (Zimmerman & Chelminski 2006) was used to screen for the most common Diagnostic and Statistical Manual of Mental Disorders IV Axis I disorders encountered in outpatient mental health settings. Women were asked questions about social support (Statistics Canada 1994) at the time of study intake, the second trimester, and 3, 12, 24, and 36 months post-partum. Finally, general health [with the 8-item Short Form Health Survey (SF-8) (Ware *et al*. 2001)] was measured at 3 and 6 months post-partum, as well as 1, 2, and 3 years post-partum.

Other maternal measures

Additional measures of pregnancy complications, nausea and vomiting, and physical activity (Baecke *et al*. 1982; Pols *et al*. 1995; Godin & Shephard 1997) were included at each time point during pregnancy. The maternal physical activity measures were also included in each post-pregnancy assessment. Finally, questions about food security modified from the Canadian Community Health Survey Cycle 2.2 (Health Canada 2004, 2007) were included once during pregnancy (third trimester) and at 6 months post-partum.

Paternal questionnaires

Once pre-natally and at 3 months post-partum, fathers were asked to provide sociodemographic information, medical history, current medical conditions and medications, street drug and alcohol use, smoking, mental health, social support, and physical activity. Paternal mental health and physical activity were assessed using the same measures described in the previous section.

Infant questionnaires

Mothers answered questions at 3, 6, 12, 24 and 36 months post-partum about infant feeding practices,

attitudes towards infant feeding [3 months post-partum only; Iowa Infant Feeding Attitude Scale (de la Mora *et al*. 1999)], infant health, infant sleeping patterns [Brief Infant Sleep Questionnaire (Sadeh 2004)], fussing and crying [Crying Patterns Questionnaire (St James-Roberts & Halil 1991; Wolke *et al*. 1994)], temperament [Infant Behavior Questionnaire – Revised (Gartstein & Rothbart 2003)], and adaptive behaviour [starting at 6 months Scales of Independent Behavior–Revised, Early Development Form (Bruininks et al. 1996)]. When infants were 2 years old, mothers also reported on early indicators of behaviour problems [Child Behavior Checklist–1.5-5 (Achenbach & Rescorla 2000)], including temper tantrums [Temper Tantrum Questionnaire; modified from (Potegal *et al*. 2003)] and executive function [Behavior Rating Inventory of Executive Function – Preschool (Gioia *et al*. 2003)]. Also at 2 years of age, mothers complete a Modified Checklist for Autism in Toddlers, which is a screener to assess risk for ASDs (Robins *et al*. 2001).

Maternal dietary interview

At each pregnancy visit, women were asked to describe in detail the quantity and type of both food and dietary supplements (e.g. pre-natal multivitamins) consumed in the previous 24-h period (midnight to midnight), as well as details about average water consumption and supplement intake. For the first 600 participants, the food recall interviews were conducted by research assistants with an educational background in nutrition using a 'Multiple Pass Method' (Conway *et al*. 2003). Food models helped women estimate portion sizes, and probes included details such as cooking methods, location and time of eating, and food brand names. All information was reviewed back to the women to ensure information was correctly recorded.

Beginning in August 2010, 24-h food recalls were completed using the validated online Food Behaviour Questionnaire developed at the University of Waterloo (Hanning *et al*. 2003, 2007, 2009; Minaker *et al*. 2006). This online 24-h food recall lists over 800 foods and also used a multiple-pass–based methodology. Pictures of foods are on the online questionnaire with pictorial depictions of portion sizes. At the end of completing the online questionnaire, women are provided with a summary of what they ate according to food groups in Canada's Food Guide to Healthy Eating (Health Canada 2007).

Maternal body measurements

At each pregnancy visit, and at 3 months post-partum, body measurements (anthropometrics) were completed to measure maternal weight, height, skin fold thicknesses and circumferences. Trained research assistants completed all measurements (inter-rater and intra-rater reliabilities were at least 15% and 12%, respectively). For the measurements, women wore light clothing. Weight was measured to the nearest 0.01 kg (Healthometer Professional 752KL, Pelstar LLC, Bridgeview, IL, USA) and height was measured to the nearest 0.1 cm using a digital stadiometer (HM200P Portstad Portable Stadiometer, Charder, Seattle, WA, USA). Upper arm, hip and thigh circumferences were measured using the protocol outlined by Lohman (Lohman *et al*. 1988); waist circumference was measured in all women <16 weeks gestation. Skin fold thicknesses (tricep, bicep, subscapula, suprailiac, tricep and thigh) were assessed using standard methods (Lohman *et al*. 1988) and were measured to the nearest 0.1 mm using Lange calipers (Beta Technologies, Inc., Cambridge, MD, USA). Body fat mass was calculated using equations adapted for pregnancy (van Raaij *et al*. 1988).

Biological samples

Maternal

Maternal blood was collected at each clinic visit by a certified phlebotomist. Women were also interviewed at that time about what they had consumed in the past 3 h, to determine whether the sample was taken while the woman was in a fasting state (fasting was not a requirement). Serum, plasma [with ethylenediaminetetraacetic acid (EDTA)], buffy coat and red blood cells were processed immediately after collection and aliquoted and stored in microcentrifuge tubes at –80°C for future analysis. DNA was extracted from

blood specimens using the Gentra Puregene Blood DNA purification kits on the Autopure LS Automated Nucleic Acid Purification Instrument according to manufacturer's recommendation and purified DNA samples stored at –4°C. Urine samples were collected at the second trimester and at 3 months post-partum clinic visits. Two cryovials of each sample were stored at –70°C for future assessment of iodine excretion to reflect iodine intake through diet and for metabolomics analysis.

Four drops of breast milk were collected onto chromatography paper from lactating mothers at 3 months post-partum. Each breast milk sample was stored in a plastic bag at –70°C for direct methylation of gas chromatography analysis of fatty acids (described later).

Paternal

A DNA sample (banked for future studies of nutrient/gene and genetic links to infant development) were collected by rolling two sterile buccal swabs along the cheek (Medical Packaging Co.), by pressing firmly on the inside of the cheek, approximately five times on one side, making certain to move the brush over the entire cheek. It was repeated with a second sterile brush on the other cheek. The brushes air dried for 10–15 min at room temperature and were then placed into a labelled plastic bag and transported to the lab for isolation. DNA was extracted from buccal specimens using the Gentra Buccal DNA purification kits also on the Autopure LS Automated Nucleic Acid Purification Instrument, and samples were stored at –4°C.

Infant

An infant blood sample was collected at 3 months post-partum by a certified phlebotomist. Plasma (EDTA) was separated immediately and aliquoted and stored in microcentrifuge tubes at –80°C for future analysis. When a venupuncture sample was not possible, a drop of blood was collected on filter paper (as described for breast milk) and stored at –80°C for fatty acid assessment. A cheek swab was collected from the infant using the method described for the parental sample mentioned earlier.

Processing and assays

All analyses, unless otherwise stated, were performed in the APrON core laboratory at the University of Alberta. To meet the initial nutrition status objectives of APrON, the following nutrients were assessed:

Red blood cell (RBC) folate concentration

Directly after blood sampling, a haemolysate was prepared by diluting 0.1 mL blood (with EDTA) in 0.9 mL (1:10) freshly prepared 1.0% v/v ascorbic acid. The tube was mixed by inversion or vortexing followed by incubating at 37°C for 30 ± 5 min. For analysis, a 1:2 dilution of the haemolysate with AxSYM Folate RBC Protein Diluent was prepared and analysed using the AXSYM® analyser (Abbott, Mississauga, ON, Canada) according to manufacturer's instructions. RBC concentration was determined as (AxSYM Folate (nmol L^{-1}) × 20 × 100)/% haematocrit. A commercial standard and reference sample (Abbot) were included in each batch of samples analysed on a particular day to ensure standardization. Twenty random samples were repeatedly analysed to determine the day-to-day variation in analysis, and this was determined to be less than 5% variation. The RBC folate measurement using the AxSYM analyser was compared with the microbiological method (Newman & Tsai 1986) using 100 randomly selected Time A samples. The RBC folate values obtained with the AxSYM analyser were positively correlated with those using the microbiological method (Spearman $r = 0.212$, $P < 0.05$). Mean RBC folate concentration was observed to be higher using AxSYM as compared with the microbiological method (508.10 ± 228.35 vs. 247.37 ± 89.83 ng mL^{-1}). The clinical ranges set for assessing status using values obtained using an ion capture method (AxSYM) will be used to assess folate status in the cohort.

Plasma folate and B12 concentrations

Plasma folate (105 μL) and holotranscobalamin (active B12) (173 μL) concentrations were deter-

mined using the AXSYM® analyser (Abbott) as per manufacturer's instructions. Commercial standards and a reference sample (Abbott) were included with each batch of samples analysed on a particular day. Ten random samples were repeatedly analysed to determine the day-to-day variation in analysis, and this was determined to be less than 5%. For samples that fell above the standard curve, a 1:1 (folate) or 1:5 (B12) dilution with the low control was performed and the analysis repeated.

Vitamin B6

The concentration of pyridoxal phosphate was determined using a high-performance liquid chromatography (HPLC) assay kit (Eagle Biosciences Inc., Nashua, NH, USA). Briefly, 200 μL of plasma, vitamin B6 calibrators and controls were deproteinized and derivitized according to manufacturer's instructions. A 20-μL aliquot of supernatant was injected onto a B6-specific column (IC2100rp, Eagle Biosciences Inc.) and quantified by HPLC using a fluorescent detector (Agilent 1200s, Agilent, Mississauga, ON, Canada). The final plasma concentration in the controls and samples was determined using the calibrator as a reference: concentration = [peak area sample/(control × concentration of calibrator)]/peak area of calibrator. The same lot number was used to analyse all the samples from the first cohort of APrON. A control high and low sample, included in the kit (Eagle Biosciences Inc.) was analysed for each batch of samples to ensure consistency in the final calculation of the plasma concentration. Twenty random samples were repeatedly analysed to determine the day-to-day variation in analysis and this was determined to be less than 5%.

Iron

Maternal blood haemoglobin concentration was assessed immediately on 10 μL of freshly drawn blood using a portable analyser (Hemocue 201®, Hemocue, Cypress, CA, USA), according to manufacturer's instructions. Haematocrit was assessed immediately on 10 μL of freshly drawn whole blood using a portable analyser (StatSpin CritSpin®, IRIS, ThermoFisher, Edmonton AB, Canada) according to manufacturer's instructions. Normal haemoglobin concentration was defined as 110 g L^{-1}–150 g L^{-1}. If a haemoglobin or haematocrit measure was found outside the normal range, a second test was conducted and the mean of the two measures was used to represent haemoglobin concentration or haematocrit. A sample of 100 women (collected at each of the time points) was analysed on a CBC machine (Beckman Coulter Model: AC.T 5 diff CP, Miami, FL, USA) and compared with the whole blood haemoglobulins and haematocrits (from the same women) analysed at the collection site. These were found to not differ significantly. Serum ferritin was analysed using 150 μL of thawed sample in the AXSYM® analyser (Abbott) according to manufacturer's instructions. Ten random samples were repeatedly analysed to determine the day-to-day variation in ferritin analysis and this was determined to be less than 5% variation.

Essential fatty acids

The concentration and relative percent of phospholipid fatty acids was determined on 300 μL of serum at each time point that a blood sample was collected. Briefly, 10 μg (100 μL of 10 mg/100 mL) C15:0 phospholipid (phosphatidylcholine) standard (Avanti Polar Lipids Inc., Alabaster, USA) was added to the sample prior to extraction of lipid using a modified Folch method (Folch *et al*. 1957) as previously described (Field *et al*. 1988). Separation of phospholipids from other major lipid classes and methylation was performed by thin-layer chromatography (Field *et al*. 1988). The phospholipid band was visualized, scraped and 10 μg C17:0 (100 μL of 10 mg/100 mL) triglyceride standard was added to the silica prior to methylation with BF_3 (Sigma-Aldrich, Mississauga, ON, Canada). Fatty acids were separated by automated gas liquid chromatography (Agilent GC model 7890a, Agilent) using a (100 × 0.25 column, Varian, Mississauga, ON, Canada as previously described (Cruz-Hernandez *et al*. 2004). Fatty acid status was calculated as a % of total plasma phospholipids and as a concentration (after correction with the C15:0 phospholipid standard).

Fatty acids in breast milk and infant blood (3 months of age)

Blood/breast milk samples were spotted on chromatography filter paper (Whatman Filter Paper, 3MM CHR, 2.0 cm × 100 cm cut into 1/2" squares, ThermoFisher) and stored at –80 °C until processed. The paper was processed by direct methylation using BF_3, hexane and heat as previously described (Fratesi *et al*. 2009). The relative percent of fatty acids in the sample was determined by gas liquid chromatography using the same column and under the conditions described earlier for maternal blood.

Thyroid function

Serum and urine samples are being banked for future assessments of thyroid stimulating hormone, free thyroxine and antibodies to thyroid peroxidase.

Medical records

Pre-natal and labour and delivery medical records were reviewed for demographics, obstetrical and medical history, use of fertility treatments, pre-natal vitamins, smoking, alcohol, street drugs, medications, weight and height (and body mass index), fundal height, breastfeeding intentions, ultrasound details, blood work and screens, and genetic screening. Antenatal and intrapartum risk assessments were extracted from the records, as well as labour and delivery information, including: newborn health, birthweight and gestational age at birth, head circumference, Apgar scores, and labour and delivery details (duration, pain management, induction, medications, anaesthesia, complications). For infants who were admitted to the neonatal intensive care unit, we also collected more detailed information regarding medical condition, including diagnosis and management.

Data management and processing

Questionnaires and anthropometric data were manually entered into Microsoft Access or Excel, using fixed data entry menu options. Sociodemographic data were double-entered and discrepancies were resolved by reference to original questionnaires. Ten-per cent of the data for all other variables were double-checked. Comparisons between the initial data entry and second data re-entry resulted in >99% accuracy rate. The 24-h food recalls that were completed face-to-face were entered into a programme called Food Processor.

Statistical power and proposed data analyses

The primary analysis will assess whether maternal depression and anxiety at 12 weeks post-partum are related to nutrient inadequacy during pregnancy. Depression will be defined as scores at or above 10 on the EPDS, and anxiety will be defined as *T*-scores at or above 63 on the SCL-90. Nutrient inadequacy is defined as not meeting *Dietary Reference Intake* recommendations for at least one of three nutrients (iron, essential fatty acids, folate), which have been estimated to occur in about 67% of pregnant women studied (Denomme *et al*. 2005; Sherwood *et al*. 2006; Leung & Kaplan 2009). Based upon the systematic review from Gavin *et al*. (2005), we estimate the risk of depression in our entire cohort will be 10–13%. We assume that mothers with adequate nutrient intake will represent the lowest risk, and have accordingly used 10% for the purpose of calculating statistical power. Based on the current sample size of 2000 mothers, the study is adequately powered (80% at a two-sided 5% significance level) to detect a 4.4% absolute increase in the risk of depression (relative risk = 1.44) among mothers with nutrient inadequacy. Given that the occurrence of anxiety is highly correlated with the occurrence of depression, the sample size and power calculations given earlier also apply to the anxiety outcome variable.

The primary analysis will be to assess the association between nutrient intake (maternal self-report), nutrient status (assayed from blood) and the variables related to the three major outcome questions: maternal mental health, obstetric and birth outcomes, and child development. As the study is prospective, multivariable log-binomial regression will be used to analyse binary outcome variables, such as maternal depression and anxiety at 12 weeks post-partum,

premature birth, and low birthweight at delivery. Assessment of variable effect modification and potential confounding, such as weight gain, physical activity and maternal thyroid function during pregnancy, will be incorporated as part of the modelling process. For the 3-year neurodevelopmental outcomes, infant feeding practice and maternal mental health will also be included in the regression model as a potential confounder. Recognizing that some of the psychological outcome variables are measured on discrete scales before and after delivery, multivariable linear regression analysis will be used to assess change between post-partum and pre-delivery scores, in relation to pre-delivery predictor variables. Finally, random effects models and generalized estimating equations will be used to account for within-participant repeated measures across time, when assessing the relationship between time-varying measures of outcome and predictor variables.

Results

Recruitment and retention

Recruitment began in May 2009 and the first cohort of 600 women was enrolled by March 2010. The distribution of participants [82% (492/600) from Calgary and 18% (108/600) from Edmonton] resulted in the decision to focus recruitment efforts on enrolling women in Calgary. Information regarding successful recruitment strategies and how they are influenced by health care delivery systems is being analysed for a future paper. At the time of this writing, about 2000 pregnant women have been recruited, 1200 fathers have participated and about 1600 babies have been born. Efforts are underway to ensure the retention of these women and to enrol additional women to reach an anticipated sample of 5500 individuals (including parents and children). Consideration is also being given to a second wave of recruitment.

The completion rate for self-report questionnaires from the first cohort of 600 women through the 12-week post-partum clinic visit (across four study visits) is approximately 85%. It is noteworthy that each of the assessment occasions up to and including the 12-week post-partum assessment require participant attendance at one of the study clinics for an in-person visit. When a clinic visit does not accompany the mailed questionnaires (completed at home and returned by postage paid envelope), completion rates are lower at around 65%. We are currently preparing to use an Internet data entry system (see description in future plans below) that will offer greater convenience to participants via web-based questionnaires that they can complete from their homes. It is expected that this convenience will improve completion rates of our self-report questionnaires.

Pilot research

The FFQ used at the first visit to assess dietary intake for the 12 months prior to pregnancy was based on the Diet History Questionnaire adapted for a Canadian population (Csizmadi *et al*. 2007), and was further adapted for pregnant women (Thomas 2011). The validity of the adapted FFQ was assessed using two approaches. First, responses of pregnant ($n = 91$) and non-pregnant ($n = 101$) women were compared. Pregnant women were asked to recall dietary intake patterns for the year prior to pregnancy and, for non-pregnant women, the past year. Because women in each group were reporting on intake patterns during a year when they were not pregnant, no differences in intake were expected. Independent *t*-tests were used to compare mean intakes. In addition to energy and macronutrients, key micronutrients chosen for comparison were iron, folate, vitamin B_6, vitamin B_{12}, calcium and vitamin D. The comparison between groups (pregnant FFQ vs. non-pregnant FFQ) showed no differences in mean intakes for energy, carbohydrate, fat, protein or any key micronutrient except calcium, which was significantly higher in the pregnant group ($P = 0.026$). The generally null differences between the pregnant and non-pregnant groups support the validity of the FFQ for use with pregnant women.

For a second approach, dietary intake in non-pregnant women only was measured by FFQ and compared with intake measured by a 24-h recall (24HR). Paired sample *t*-tests were used to compare

mean intakes. The comparison between tools (FFQ vs. 24HR) in the non-pregnant group showed no differences in mean intakes for energy, or macronutrients. However, mean intakes were significantly different for folate, vitamin B6, vitamin B12, vitamin D and iron; there was no difference for calcium.

On the bases of the analysis conducted, we concluded that the adapted FFQ is a reasonable tool for assessment of pre-pregnancy dietary intake for energy, macronutrients and calcium. However, estimates of iron, folate and other key micronutrients assessed by the FFQ should be interpreted with caution.

Subsample studies

The prospective collection of this large amount of information provides a rich data source, lending itself to many collaborations. Several additional questions about maternal mental health, nutrition and child outcomes have received focussed attention in subsamples of APrON participants. We highlight two of those subsample studies here.

The role of stress on fetal programming

A series of studies on the psychobiology of stress during pregnancy has enrolled 380 APrON participants who provide diurnal suites of saliva and momentary ratings of psychological distress. The aim of these studies was to understand the mechanisms by which maternal stress during pregnancy can affect infant stress reactivity. These studies employ an ecological momentary assessment strategy to capture the vicissitudes of mood, stress and the psychobiology of stress across multiple days and across trimesters of pregnancy. To date, this research has shown that cortisol is a plausible mechanism for transmitting the maternal stress signal to the fetus (Giesbrecht *et al*. 2012) and has demonstrated the usefulness of basal salivary alpha-amylase as a marker for emotional arousal in pregnant women (Giesbrecht *et al*. in press). Infant outcomes (currently being collected) are behavioural, neuroendocrine and cardiac reactivity to stress measured at 3- and 6-months post-partum.

Vitamin D

A second substudy focuses on cord blood from 100 APrON women. The objective of the study was to determine the feasibility of obtaining maternal and cord blood samples during delivery at two centres in Calgary and to correlate the level of 25(OH) D in maternal blood and cord blood. Secondary objectives were (1) to determine the best way to approach women from APrON to participate in these substudies involving the collection of cord blood; (2) to evaluate the reasons that women refuse blood work during labour; and (3) to establish the best way to remind the participant, her delivery provider and her nurse to obtain the study samples.

Future plans and collaborations

At the time of this writing, the first APrON babies are just turning 2 years of age. One future plan is that when they reach the age of 3 years, standardized one-on-one assessments will be used to evaluate neurodevelopmental function. Intellectual ability will be assessed with the *Wechsler Preschool and Primary Scale of Intelligence – Third Edition (Canadian)* (Wechsler 2002). Children will complete the four core subtests (vocabulary, information, block design and object assembly) plus the supplemental subtest, picture naming, from which four composite scores, verbal intelligence quotient (IQ), performance IQ, full-scale IQ, and general language composite are derived. Neuropsychological functioning will be assessed with the *NEPSY-II* (Korkman *et al*. 2007), which assesses six functional domains: memory and learning, executive functioning/attention, social perception, language, sensorimotor functioning and visuospatial processing. Finally, motor functioning will be assessed with the Movement Assessment Battery for Children – Second Edition (MABC-II) (Henderson *et al*. 2007). The MABC-II assesses movement skills in three domains: manual dexterity, balance and aiming and catching. In addition, a number of initiatives are underway to use the banked samples and information we have collected.

A second plan for the future was mentioned earlier: APrON is about to embark on the development of

an internet data entry system to improve the convenience of questionnaires for our participants. The Electronic Patient Reported Outcomes system has been developed at the University of Alberta, one of our APrON sites. It will provide secure servers and de-identified data in a manner that will meet the highest standards of privacy and confidentiality. A randomized controlled trial comparing the Internet system with paper mail-out questionnaires is planned, to evaluate the impact of each of participant retention.

Other future planning involves efforts to develop national and international collaborations to leverage the initial investment required to establish this cohort. Two projects in particular are noteworthy as they expand considerably upon the original design of the APrON study. The first is a local collaboration with scientists at the University of Alberta to examine the effects of perinatal toxicant exposure on neurodevelopmental outcomes. This project will focus on bisphenol A and phthalates, ubiquitous environmental contaminants with known neurodevelopmental effects. A second project in collaboration with individuals at the University of Adelaide, Australia will examine the effects of maternal nutrition and mental health on telomere maintenance and long-term health outcomes. It is expected that additional international collaborations will evolve over time.

Conclusion

The APrON study has collected detailed information on nutrition and mental health of women during pregnancy and the development of their infants during the first few years of life. The data collected in this cohort will offer future researchers the opportunity to explore important questions related to maternal and child health. Canada's single payer (universal) health care system, much of which is frequently organized in large coordinated regions, creates a unique position for health care research, and particularly studies that require longitudinal population-based cohorts. This particular cohort offers an opportunity to follow a group of Canadian children as they develop and contribute to not only our understanding of the fetal origins of adult diseases, but also to helping policy makers and health professionals develop guidelines to optimize the fetal environment to ensure the health of future generations.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Contributions

BJK wrote the initial draft of the paper, along with GFG and BMYL. CJF and DD were co-leads with BJK in establishing the scientific team and the methodology. RCB, DPM, MO, VJP, NS, L Gagnon, FPB, LJM, LK, AF and MC were all responsible for a component of the initial methodology described in the paper. As Senior Project Manager, DWJ has been in charge of all procedural details described herein. The biostatisticians ME and L Goonewardene have provided direction for various statistical evaluations and modelling. LMC, NL and JWM each added a new component to the initial project, and joined in the writing of this paper. All co-authors participated in funding applications and in manuscript preparation.

Each co-author critically reviewed all sections of the text for important intellectual content.

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